ORAL CLEFTS

Describing and classifying sub-phenotypes and associated anomalies

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Colofon

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and associated anomalies

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en geassocieerde afwijkingen

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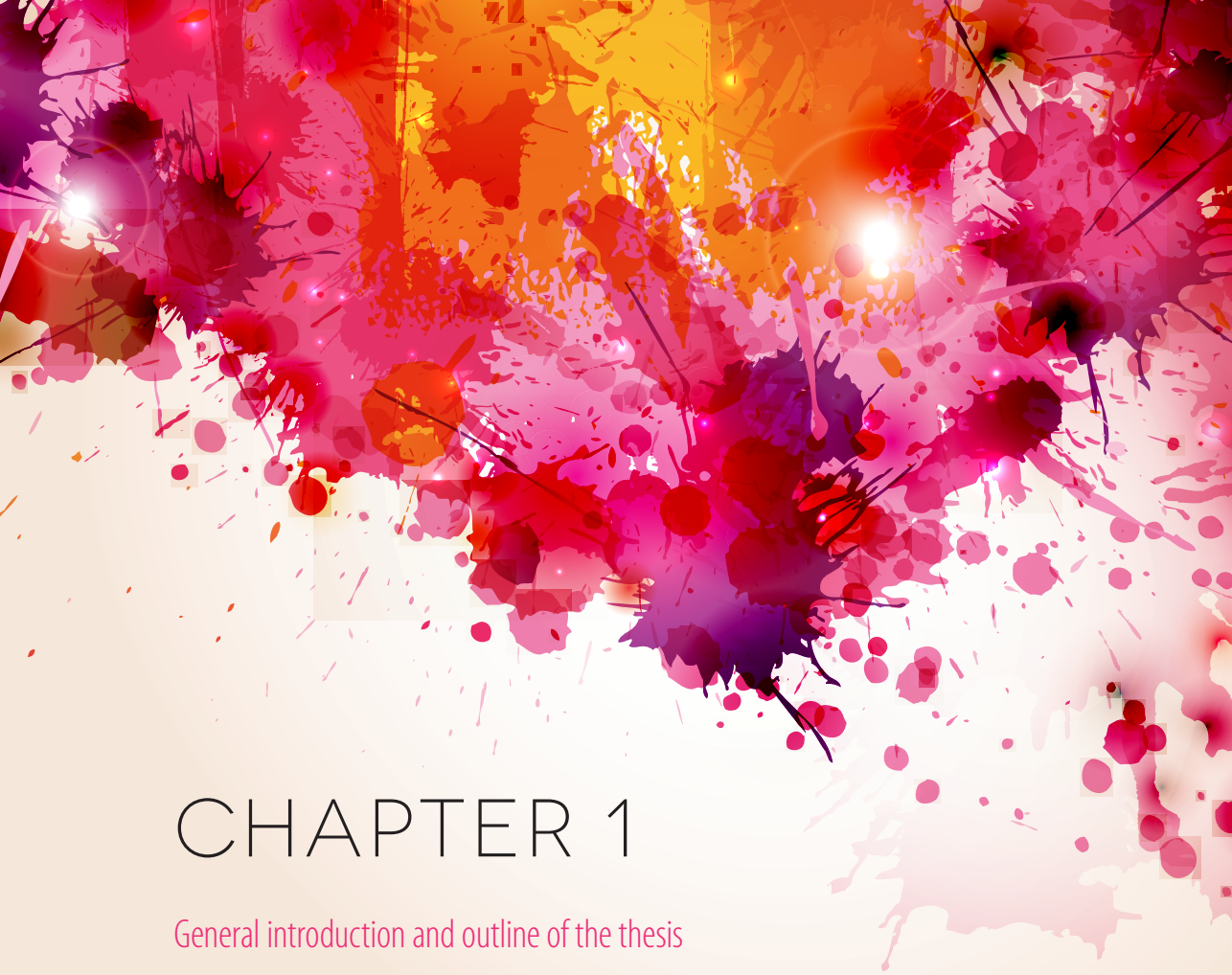

CHAPTER 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

The unexpected diagnosis of an oral cleft before or after birth is a shocking and traumatic experience for parents and their social environment, generating anxiety as well as numerous questions. What is an oral cleft? Why didn't our baby's mouth fully develop? How is it caused and can it be prevented? How many babies are born with clefts? What can be done to help our baby? What is the risk of other congenital anomalies? What will be the prognosis and outcome later in life?

Oral clefts

Oral clefts are heterogeneous often immediately recognizable congenital anomalies affecting the lip and oral cavity. They comprise a wide range of sub-phenotypes varying from mild types (subcutaneous or submucous clefts) to more severe incomplete or complete clefts of the lip, alveolus, hard palate, or soft palate including the uvula (Figure 1).[1-4] While median cleft lip and atypical facial clefts are regularly also included in the phenotypic spectrum of oral clefts, these anomalies should be considered as craniofacial anomalies because of their different pathogenesis and associated defects.[4-7] With regard to oral clefts, effects on feeding, speech, hearing, appearance, and cognition can lead to long-lasting adverse outcomes for health and social integration. Therefore, affected individuals need multidisciplinary care from birth until childhood, generally including surgery, dental treatment, speech and hearing therapy, genetic counseling, and psychosocial intervention.[8] Although rehabilitation is possible in developed countries with good quality care, children with oral clefts have higher morbidity and mortality throughout life than do unaffected infants. They are frequently affected with other congenital anomalies, often as part of Mendelian, chromosomal, or teratogenic syndromes.[1, 2, 8-10] If oral clefts occur as isolated entities with no other apparent structural anomalies, they are collectively termed as non-syndromic oral clefts.

Until the late 1990s, clefts were predominantly detected after birth, during the immediate postnatal period or—in case of a mild cleft palate—later in infancy. However, due to advances in ultrasound technology and the international introduction of routine prenatal screening at 18-22 weeks of gestation, structural congenital anomalies, including oral clefts, are diagnosed prenatally more frequently.[11-14] As a consequence, there is an increasing need for information to aid in prenatal counseling and optimize prenatal care. Decisions taken by parents, especially with regard to termination of the pregnancy, seem to be mainly affected by the long-term prognosis of the malformed fetus.[15] When informing parents about the prognosis of their child, accurate diagnosis of the cleft sub-phenotype as well as of associated congenital anomalies is vital. In particular the identification of an underlying chromosomal defect will influence counseling and management of the pregnancy significantly. However, the exact prevalence and risk factors for associated structural anomalies and chromosomal defects are not known as they vary significantly between studies.[16] This has led to lack of consensus on when to perform


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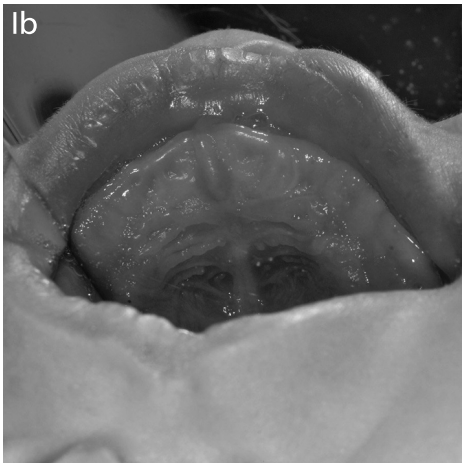
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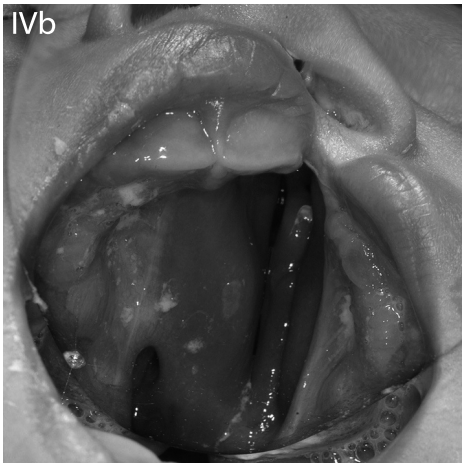
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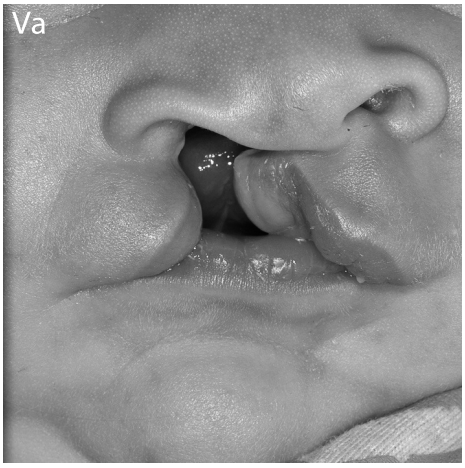
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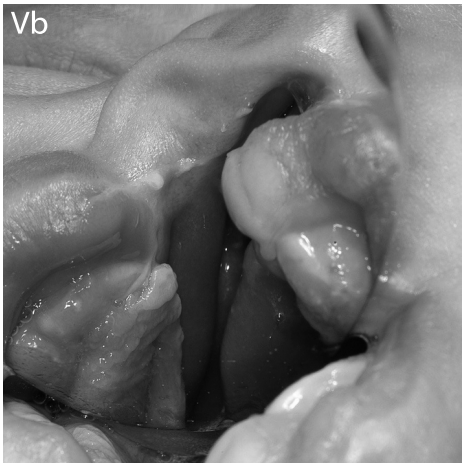
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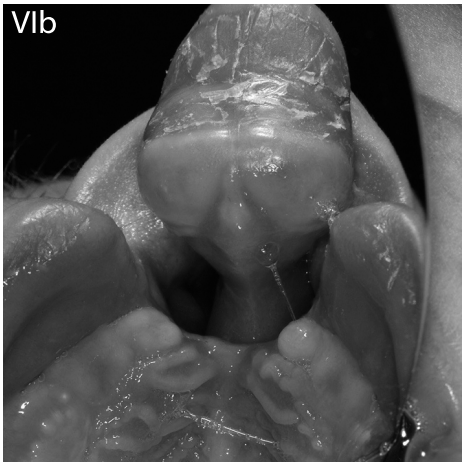
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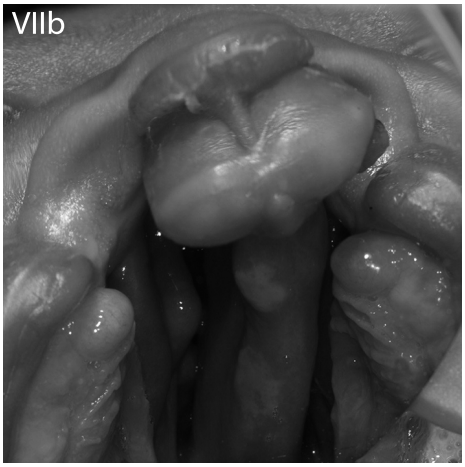
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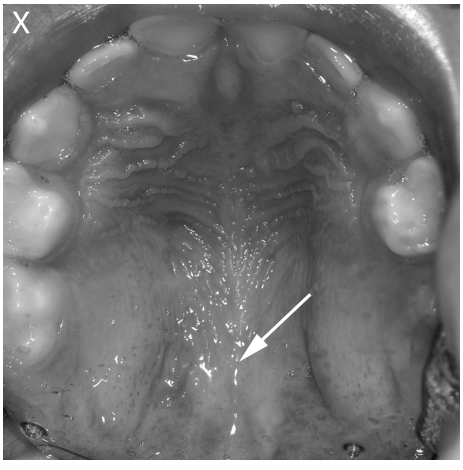
X

Figure 1. Wide variation of oral cleft sub-phenotypes.

I. Unilateral left submucous cleft lip (also known as forme fruste, congenital scar, and microform, subsurface, or subcutaneous cleft) showing a notch of the vermilion (a) with a normal alveolus (b).
II. Unilateral left incomplete cleft lip (a) with an incomplete cleft alveolus (b).
III. Unilateral left complete cleft lip (a) with a complete cleft alveolus (extending to the incisive foramen) (b).
IV. Unilateral left incomplete cleft lip (a) with a complete cleft alveolus and a complete cleft of the hard and soft palates (b). This sub-phenotype is also known as the Simonart's band.
V. Unilateral right complete cleft lip with a complete cleft alveolus (a) and a complete cleft of the hard and soft palates (b).
VI. Bilateral complete cleft lip with a complete cleft alveolus (a,b).
VII. Bilateral complete cleft lip with a complete cleft alveolus (a) and a complete cleft of the hard and soft palates (b).
VIII. Complete cleft of the hard and soft palates (a,b).
IX. Incomplete cleft of the hard palate with a complete cleft of the soft palate.
X. Submucous cleft of the soft palate showing a median cleft of the palatal muscles with intact mucosa (arrow).

invasive prenatal diagnostics to identify these underlying chromosomal abnormalities.[17] Should these invasive tests be advised in all prenatally identified cleft cases, or should they be limited to specific phenotypes and associated anomalies? In order to allow well-informed decisions on these invasive diagnostics and be able to optimize prenatal counseling and care as well as improve the prevention, prognosis, and outcome of oral clefts, one should understand the underlying embryology, etiology, and epidemiology of these conditions.

Development and etiology

Oral clefts result from a failure of normal embryonic processes that lead to the formation of the nose and oral cavity. Understanding of these processes explains how a particular sub-phenotype arises and why clefts occur in certain patterns. Normal development of the primary palate (presumptive lip and alveolus) and secondary palate (presumptive hard and soft palates including the uvula) entails a complex series of embryonic events that require close coordination of cell proliferation, apoptosis (programmed cell death), and cell differentiation,[6, 7, 18-21] regulated by many different genes during different time frames.[1, 22] In short, palatogenesis can be subdivided into an early (4-7 weeks postconception) and late (7-12 weeks postconception) embryonic period.[6, 7, 19, 22] During the first period, the primary nasal cavity and primary palate are formed in an occipito-frontal direction by subsequent outgrowth, adherence, and fusion of the three facial swellings around each nasal placode at both sides of the face (Figure 2:1-3).[6, 7, 18-22] After the fusion process of the primary palate, the late embryonic period starts with differentiation of the primary palate and formation of the secondary palate. The lip and alveolus are formed by merging, outgrowth, and differentiation (into bone and musculature) of the mesenchymal cores of the fused swellings. Simultaneously, the secondary palate is formed in a *fronto-occipital* direction by subsequent outgrowth, elevation, adherence, and fusion of

the two palatine shelves. These shelves fuse with each other, the primary palate, and the nasal septum, and their mesenchyme differentiates into bone and musculature (Figure 2:3-6).[6, 7, 19-22]

Derailments in any of these tightly regulated processes during different developmental periods may result in various cleft sub-phenotypes. Disturbing factors impacting on developmental events during the early embryonic period can cause failure of fusion between the facial swellings, resulting in defects such as *complete clefts of the lip/alveolus*. By contrast, disturbances during the late embryonic period may lead to failure of fusion between the palatal shelves causing *complete or incomplete clefts of the palate*.[6, 7, 19, 22] Subsequent disruption of differentiation of the primary or secondary palates may result in *incomplete or submucous clefts of the lip/alveolus* or *submucous clefts of the palate*, respectively.[7, 19] However, factors leading to these developmental disturbances are complex and largely unknown. Although there has been marked progress in identifying genetic and environmental triggers for syndromic clefts,[2, 8-10] the etiopathogenesis of the more common non-syndromic forms remains poorly characterized. They are thought to result from a complex interplay of genetic and environmental factors. Findings of a variety of genetic approaches (including mouse models, linkage and association studies, cytogenetics, and gene-expression analyses in human and mouse embryonic tissue) have suggested various candidate genes and pathways implicated in oral clefts.[1, 2, 9, 10, 22] However, results remain inconsistent owing to the considerable genetic heterogeneity.

With regard to environmental factors, maternal smoking during pregnancy has been linked with an increased cleft risk,[2, 8] especially in interaction with certain genes,[23, 24] such as MSX1.[25] The role of maternal alcohol use is less certain, although some positive associations have been reported.[2, 8] In addition, there is increasing evidence that nutritional factors and their related genes are involved.[8, 22] Research in this field has been mainly focused on the role of folic acid and multivitamins in the prevention of clefts. However, while numerous observational studies have suggested a beneficial role of periconceptional folic acid or multivitamin supplement use in decreasing cleft risk, the evidence remains largely inconclusive and their role in cleft etiology remains unresolved.[26-28] Other factors that have been associated with increased cleft risk are specific teratogens including maternal anticonvulsant drugs, corticosteroids, organic solvents, and agricultural chemicals.[8]

One of the main problems hampering the identification of genetic and environmental causal factors might be that oral clefts are generally defined as qualitative traits (that is, affected or unaffected).[1, 2] Given the wide range of phenotypic expressions of clefts, this too simplistic approach could potentially result in important information being lost. The phenotyping spectrum is more complex than previously realized and includes a variety of—less evident—subclinical features, such as *subcutaneous cleft lip* (also known as microform cleft or forme fruste lip),[29-31] *submucous cleft palate*, *hypoplastic palate*, or *bifid uvula*.[2, 3, 32] As these are often treated as 'unaffected', these individuals may represent genetic or environmental features that are currently overlooked. Another important aspect is that the power to detect effects is weakened when heterogeneous groups are treated as a single entity. Therefore, accurate

phenotyping—including subclinical features—and subsequent adequate classification is crucial not only for improving overall cleft management and prognosis, but also for furthering our understanding of the etiopathogenesis of clefts.[1, 2, 29-31] To facilitate the ongoing search for risk factors, detailed description and registration of sub-phenotypes with standardized protocols and data-sharing between cleft centers are needed.[1]

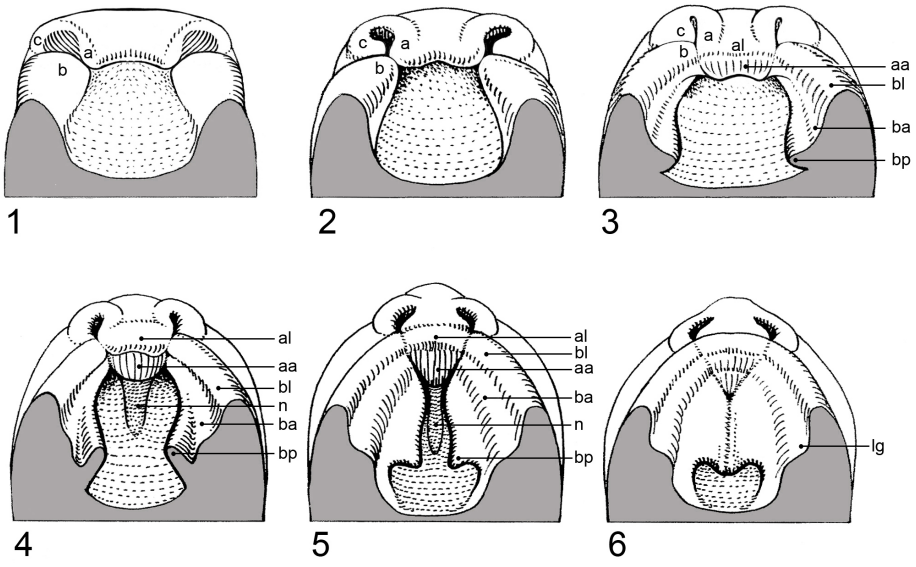

Figure 2. Embryonic development in successive stages viewed from the oral side: the fusion processes of the primary palate (1-3) and secondary palate (3-6), and differentiation of the lip and alveolus (3-6).

1. The nasal groove surrounded by the facial swellings (a-c) at five weeks;
2. Outgrowth and fusion of two (a-b) of the three facial swellings in occipito-frontal direction forming the nasal tubes at six weeks;
3. Further outgrowth and fusion of the three swellings (a-c), resulting in the formation of the primary palate at about seven weeks and the beginning of development of the lip (al + bl), alveolus (aa +ba) and the shelves of the secondary palate (bp);
4. Outgrowth of the nasal septum (n) and palatal shelves in vertical direction, and outgrowth of the lip and alveolus in caudal direction forming the presumptive labial groove at eight weeks;
5. Elevation and outgrowth of the palatal shelves in horizontal position and start of the fusion of the shelves with the primary palate at eight to nine weeks;
6. Completed fusion of the shelves in fronto-occipital direction with the primary palate and nasal septum, as well as with each other, and completion of the lip, alveolus, and labial groove (lg) at 10-12 weeks.

Abbreviations: a,b,c = facial swellings; al = lip developed from a; bl = lip developed from b; aa = alveolus (premaxillae) developed from a; ba = alveolus (maxillae) developed from b; bp = palatal shelves developed from b; n = nasal septum; lg = labial groove.

International epidemiology and registration

Although oral clefts are among the most widely known and common congenital anomalies, their prevalence is not known in every part of the world and varies widely across geographic origin, racial and ethnic groups, as well as socioeconomic status.[8, 33-38] Our current knowledge on the birth prevalence and figures of clefts around the world reveals not only the apparent variation, but also significant differences in methods of data collection and birth defect registration.[8, 16, 34, 38]

Overall, oral clefts affect approximately 1 in 700 live births,[34] but reported rates from different registries vary considerably from 4.8 to 28.6 per 10,000 live births and stillbirths (with or without terminations of pregnancy, TOP).[39] Generally, the highest prevalence has been found in Asian and Native American populations, while European populations have intermediate rates and African-derived populations the lowest rates.[2, 34] Within Europe, higher rates are reported from northern than southern countries.[8, 34] The prevalence also differs by gender and laterality: clefts involving the lip/alveolus are most frequently seen in males and have a left-sided dominance, while clefts of the palate only are most typical in females. These sex ratios vary with factors such as cleft severity and presence of associated anomalies.[8, 34, 40] Besides the cleft prevalence, the frequency and type of associated congenital anomalies vary also significantly between studies.[16] However, in general, further defects seem to be more frequent for individuals with clefts of the palate only than for those with clefts involving the lip/alveolus.[8] The most commonly reported anomalies include eye, brain, heart, limb, and neural tube defects as well as developmental retardation and deafness.[16] Finally, compared with other congenital anomalies, oral clefts have a relatively high rate of familial recurrence, especially for those of the palate alone.[1] Altogether, these differences suggest a stronger genetic basis and different etiopathogenesis for clefts of the palate alone than for clefts involving the lip/alveolus. Consistent with these epidemiological patterns as well as with the distinct developmental origins, oral clefts are traditionally divided into two categories: cleft lip with or without cleft palate (CL±P) and cleft palate only (CP). However, recent epidemiological studies have emphasized further subdivision of CL±P into clefts involving the lip only (CL) and clefts involving both the lip and palate (CLP) because of their suggested unique etiologic features, including different strong genetic associations[2, 41] as well as different associations with risk factors and additional congenital anomalies.[40, 42]

The epidemiological approach to congenital anomalies has been the backbone of research into their causes and prevention. Hypotheses about possible causative agents may arise from many different sources, but epidemiological analyses are necessary to test these hypotheses. To enable such activities, adequate description and registration of the type and number of congenital anomalies and their related factors is needed. Worldwide, various registers for congenital anomalies—including oral clefts—were established after the thalidomide "epidemic" in the 1960s.[37, 43] Most of them use coding systems based on the "International Statistical Classification of Diseases and Related Health Problems" (ICD) of the World Health Organization[44]

or its extensions, such as the "British Pediatric Association Classification of Diseases" (BPA).[34, 43] Using these classifications, oral clefts are not described, but interpreted and directly coded according to clinical diagnosis. Moreover, data are often presented for just two (CL±P and CP)[37, 43] or three (CL, CLP, and CP)[35, 36, 39] categories. As a consequence, important anatomical and morphological details are being lost. As interpretations of congenital anomalies change by increasing knowledge about their development and etiology, adjustment of previously recorded data to new insights—such as a new classification—is often impossible. Therefore, more specific systems have been developed to adequately describe and classify the more frequent cleft variations according to their anatomical[45-49] as well as morphological appearance.[50-53] However, infrequent or subclinical features are often not included and none of them has been fully based on the embryological processes underlying oral clefts, thereby lacking information needed to gain more insight into their causes and prevention.

Epidemiology and registration in the Netherlands

Virtually all surviving children with oral clefts who reside in the Netherlands are treated by one of the fourteen Dutch cleft palate teams.[3] These teams—housed within university as well as non-academic hospitals—offer multidisciplinary care and are united in the "Dutch Association for Cleft Palate and Craniofacial Anomalies" (NVSCA).

Until the late 1990s, the precise prevalence of oral clefts in the Netherlands was not known. One of the first reports found a prevalence of 13.8-17.7 per 10,000 live births and stillbirths through evaluation of children born in Dutch hospitals during 1982 and 1983.[54] A few years later, a prevalence of 17.3-19.9 per 10,000 live births was reported, estimated on the basis of questionnaires and medical records at the request of the National Health Department to aid in the planning of healthcare programs for Dutch children with clefts.[55, 56] While national figures were scarcely available during that period, regional registration of oral clefts had already been established. The local register of Eurocat in the Northern Netherlands started in 1981. This population-based registry records congenital anomalies—including oral clefts—among live births and stillbirths (including TOP) using ICD/BPA codes,[43, 57] and rates have mainly been provided for CL±P and CP.[37, 58, 59] In addition, the combined National Obstetric and Neonatal registries (LVR/LNR) have recorded diagnoses of congenital anomalies as part of information on pregnancy, delivery, and neonatal care since 1996. However, national data regarding CL±P and CP were firstly published in 2001 and appeared not to have complete regional and national coverage.[60-62]

Therefore, a new registry for oral clefts and craniofacial anomalies started in 1997 on behalf of the NVSCA with the following aims: a) to gain insight into the frequency and distribution of all categories and subgroups of oral clefts and craniofacial anomalies in the Netherlands; b) to detect changes in their frequency and distribution, thereby detecting and eliminating their influencing factors; c) to evaluate the effectiveness of prevention strategies; d) to aid in planning and quality surveillance of health services; and e) to facilitate research related to the

causes and prevention these anomalies as well as the treatment and care of affected children.[63, 64] The registry has been designed and coordinated by the Registration working group, housed in the Department of Plastic and Reconstructive Surgery, Erasmus MC. This group developed a unique descriptive recording form based on the embryology of the head and neck area to anticipate all conceivable craniofacial anomalies.[3, 19] With regard to oral clefts, all individual anomalies that form the cleft can be described by recording the affected anatomical structure (lip, alveolus, hard palate, soft palate including uvula), morphology (complete, incomplete, submucous), and side (left, right, median). The rationale behind this approach is that clefts are not classified or coded, but described in detail, thereby allowing NVSCA data to be fitted into any existing classification and to be compared with other studies. Additionally, infant and parental characteristics as well as diagnoses of associated congenital anomalies can be recorded. The NVSCA is not an ongoing registry and no data from other sources are included.

Since its establishment, the Dutch cleft palate teams have registered their anonymous live-born patients with clefts (no age limit) during the first visit to the team, prior to cleft surgery. This has resulted in an extensive database with unique descriptive data on a wide range of cleft sub-phenotypes and craniofacial anomalies.[3, 19, 63, 64] In order to provide a solid basis for research and clinical purposes and thus achieve the objectives of the NVSCA, it is crucial that the data provided by the database are accurate and complete. Previously, it was shown that the NVSCA's case ascertainment is of rather high quality.[62] However, it is unknown whether the individual case and cleft characteristics have also been adequately recorded in this system.

AIMS AND OUTLINE OF THE THESIS

This thesis is aimed at defining an approach and providing a solid basis to further understand the etiopathogenesis of oral clefts and optimize their prenatal and postnatal outcome and prognosis.

The specific objectives are:

I. To validate the Dutch Oral Cleft Registry (NVSCA) investigating whether this system is complete and feasible in clinical practice and whether its data are reliable for further fundamental and clinical studies.
II. To investigate the prevalence of oral clefts in the Netherlands, including its differences between regions and registries, as well as its influencing factors.
III. To test and further develop a new postnatal embryological classification of oral clefts providing subgroups related to specific time periods and underlying embryological processes in development.
IV. To assess the effects of periconceptional folic acid supplement use on the risk of oral clefts in the Netherlands.

V. To investigate the type and prevalence of associated structural anomalies and chromosomal defects in prenatal and postnatal oral cleft populations and develop a new prenatal ultrasound classification of clefts aiding in prenatal counseling and care.

Chapter 2 describes the study design and first results of a national validation project evaluating the quality of NVSCA registry data. Oral cleft data were evaluated broadly according to the three cleft categories that are most frequently used to study oral clefts (CL, CLP, and CP), thereby investigating the value and suitability of NVSCA data for comparison with other registries and studies. In addition, information on associated infant and parental characteristics was analyzed.

In **Chapter 3** the quality of NVSCA data was further evaluated by analyzing whether the specific features (topography and morphology) of the various sub-phenotypes are adequately recorded in clinical practice, thereby investigating the feasibility of this system and its additional value—compared to other systems—for fundamental and clinical research.

In **Chapter 4** NVSCA data on congenital anomalies, syndromes, and chromosomal defects associated with oral clefts were validated. Through two-phased medical data review, we investigated whether these anomalies are accurately diagnosed and recorded during the first consultations with the cleft palate teams and whether reregistration at a later age is needed. The type and frequencies of associated anomalies are presented, and the sources of underreporting as well as their implications are discussed.

Chapter 5 presents the prevalence of oral cleft live births in the Netherlands from 1997 to 2006. As time-trend analyses showed a decrease in prevalence, trends were stratified into CL±P and CP in order to gain more insight into their possible influencing factors. For example, we investigated whether the higher periconceptional use of folic acid supplements in the Netherlands[65, 66] and the greater prenatal detection of oral clefts and their associated anomalies[11] might have affected the live-birth prevalence of oral clefts during this period.

In **Chapter 6** the prevalence of oral cleft live births from three Dutch registries (NVSCA, LVR/LNR, and Eurocat) are described and compared to confirm the declining prevalence in the NVSCA and rule out underreporting as a cause of this decline. As previous studies have shown a wide variation in oral cleft prevalence not only between, but also within countries,[8, 33] and the region Northern Netherlands seems to have a relatively high prevalence compared to other European regions,[36, 58] we also investigated whether the prevalence differs regionally within the Netherlands.

For furthering our knowledge of causal factors and understand why oral cleft anomalies occur in certain epidemiological patterns, it is essential to subdivide them according to their specific time periods and underlying processes in development, allowing to link them to specific genes and environmental factors that are expressed during these periods. However, none of the previously published oral cleft classifications have been fully based on human embryology of the nose and oral cavity.[44-53] Therefore, a new postnatal classification based on the pathoembryology of the primary and secondary palates was proposed.[19] In **Chapter 7**

this new approach and its embryological basis are described and tested on all sub-phenotypes among Dutch newborns registered in the NVSCA.

While the embryogenesis of the secondary palate has extensively been investigated resulting in general consensus on this topic,[8] the developmental processes of the primary palate are complex and have been rather underexposed. As a consequence, several questions remain unanswered. First, several studies have proposed further morphological grading of incomplete clefts of the lip,[50-53, 67, 68] but its clinical and embryological relevance is unknown. More specifically, it has not been described whether these morphological grades (severity) are related to those of the alveolus and thus have a predictive value. Second, it is indefinite which part of the alveolus—that formed by the premaxilla or maxilla—is deficient in alveolar deformities. In **Chapter 8** the completeness and feasibility of our new embryological classification for all sub-phenotypes of the primary palate is investigated using adult unoperated patients from Indonesia. In addition, the questions regarding the clinical and embryological value of additional morphological grading, as well as regarding the deficient part in alveolar deformities are addressed.

After analyzing the feasibility and completeness of our new classification, its subgroups were applied in **Chapter 9** to assess the effects of periconceptional folic acid supplements on the risk of oral clefts relative to other non-folate related congenital anomalies. By combining complementary data from the NVSCA and Eurocat databases, we performed a population-based case-control study in the Northern Netherlands analyzing the type, timing, and duration of supplement use in relation to timing and processes underlying cleft development—an approach missing in earlier studies.[26, 28]

The prenatal characteristics of oral clefts differ significantly to those of postnatal cleft populations. Although the quality of prenatal detection of anatomical and morphological cleft characteristics is increasing,[69-73] the prenatal identification of subclinical features and involvement of the palate can still be challenging.[13, 17] Furthermore, before the recent advances in ultrasound diagnostics, the cases that were more likely to be detected prenatally tended to be the more severe cases with associated anomalies, intrauterine growth retardation, or other prenatal complications,[13] resulting in a different prognosis and outcome. As increasing numbers of isolated clefts are identified in utero nowadays, the epidemiological figures of previous prenatal studies are not representative of the current prenatal populations. To aid in optimal management of the pregnancy in terms of decisions on invasive diagnostics, antenatal counseling and care, referral for birth to required specialist level, and planning for the postnatal treatment of the child, adequate and up-to-date information is needed. Therefore, **Chapter 10** gives an overview of literature and complementary NVSCA data on the type and frequency of associated structural and chromosomal anomalies related to oral cleft category in prenatal and postnatal populations. In addition, an algorithm for prenatal invasive testing is given. Finally **Chapter 11** presents a new prenatal ultrasound classification of oral and craniofacial

clefts—designed for modern ultrasound technologies—subdividing clefts according to their patho-embryological processes, epidemiology, and associated congenital anomalies.

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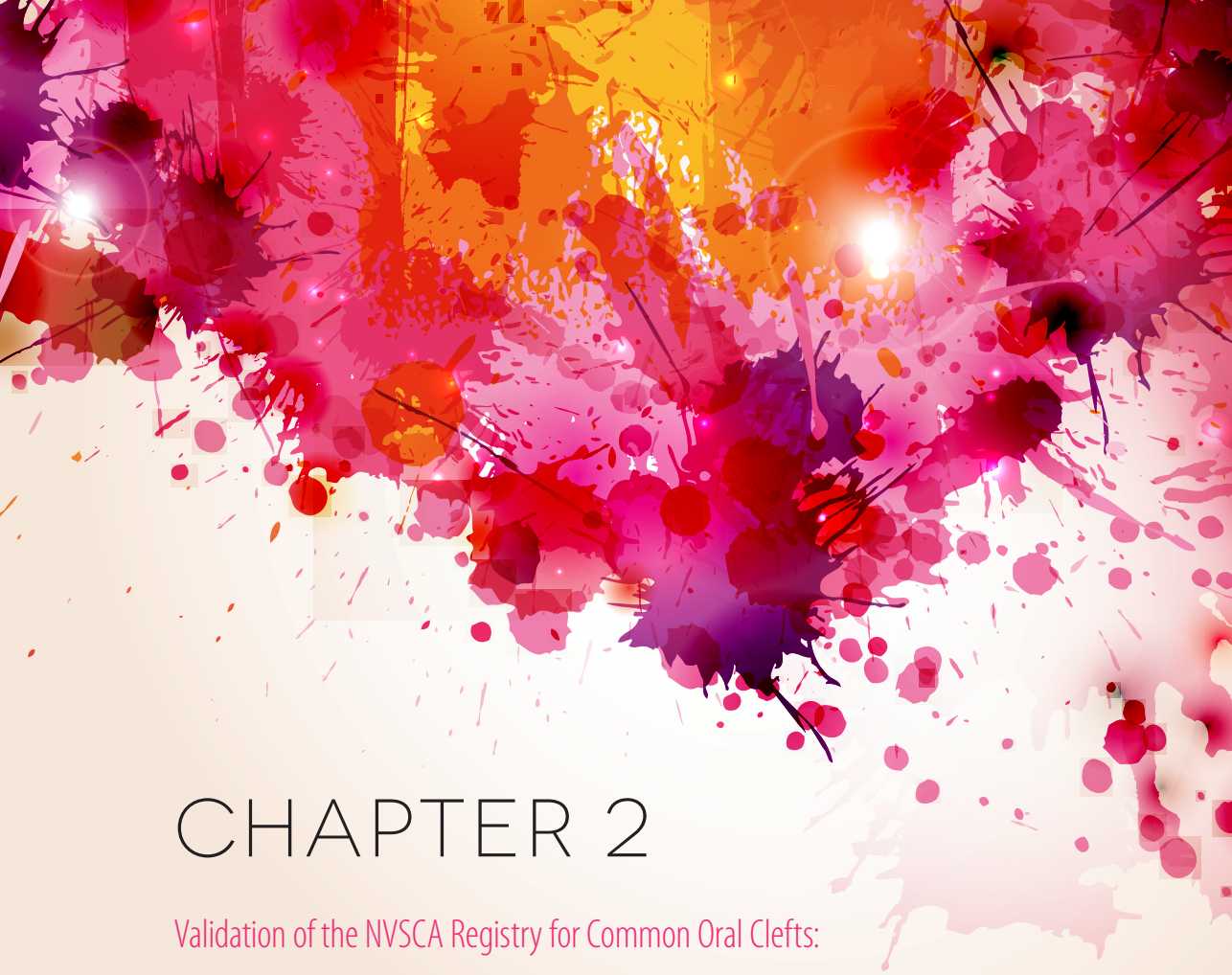
PART I

Descriptive registration and validation



CHAPTER 2

Validation of the NVSCA Registry for Common Oral Clefts: study design and first results

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ABSTRACT

Objective: Since 1997 the Dutch Association for Cleft Palate and Craniofacial Anomalies (NVSCA) has maintained a national registry of congenital craniofacial anomalies. This study validates data on three common oral cleft categories (cleft lip/alveolus = CL/A; cleft lip/alveolus and palate = CL/AP; and cleft palate = CP) and general items.
Design: Retrospective observational study.
Setting: All 15 Dutch cleft palate teams registered presurgery patients with common oral clefts (n = 2553) from 1997 to 2003.
Patients: A random sample of 250 cases was used; 13 cases were excluded.
Main Outcome Measures: The corresponding medical data were reviewed; these medical data served to validate the NVSCA registry data. Prevalence comparisons, 2 x 2 tables and validity measures were performed.
Results: The cleft categories most accurately recorded were CL/A and CP. Both categories had an observed agreement of 98%, kappa of 0.94, and a sensitivity and specificity of 97%. Cleft lip/alveolus and palate had an observed agreement of 95%, kappa of 0.89, a sensitivity of 90%, and a specificity of 99%. Regarding the general items, observed agreement and kappa were highest for adoption/foster child (99%; 0.76) and lowest for remarks about pregnancy (63%; 0.20). Sensitivity ranged from 25% (consanguinity) to 97% (white mother) and specificity was high for all items (> 93%) except for white father and mother (approximately 35%).
Conclusions: The NVSCA registry is a valuable tool for quality improvement and research because validity on all three common oral cleft categories is very good. Validity on the general items is reasonable to satisfying and appears to be related to the type of information.

INTRODUCTION

International registries

Oral clefts are one of the most common congenital anomalies in humans. Worldwide, the prevalence of oral clefts varies between 4.8 and 28.6 per 10,000 live births and stillbirths (with or without termination of pregnancy)[1] with considerable variations associated with sex, ethnicity, socioeconomic status, and geographic region.[2-6]

In many studies on oral clefts, median cleft lip and atypical facial clefts are included. However, these clefts should be considered as different craniofacial anomalies because of their different pathogenesis.[7, 8] Therefore, the term *common oral clefts* (OCs), which comprises cleft lip/alveolus and/or cleft palate, is introduced in this paper. Common oral clefts are very complex and heterogeneous birth defects. During embryonic development of the head and neck area, many different cell biological mechanisms and genes are involved, related to different time frames. [7, 9] Disturbance of this complex developmental process can result in many different types of OCs with variable degree of severity on clinical presentation.[8] Classically, OCs are divided into two categories: cleft lip with or without cleft palate (CL±P) and cleft palate only (CP) because of their embryologic and epidemiologic differences.[3] However, some studies have recently emphasized grouping cleft lip only (CL) and cleft lip with cleft palate (CLP) into different conditions, because of differences concerning their prevalence, relation to sex, relation to consanguinity and laterality, and different associations with other congenital anomalies. [10, 11] Although the etiopathogenesis has been widely studied, it is still poorly understood for all three categories of clefts. When considered as single defects, many genetic and environmental factors, such as nutrition and smoking, have been suggested.[3, 9, 12, 13] To facilitate further genetic and etiopathological studies and to improve prevention, diagnostics, and treatment, detailed descriptions of OCs and other anomalies of the head and neck area are needed.

The importance of registering the type and number of congenital anomalies is long recognized. Worldwide, several congenital anomaly registries were established after the thalidomide "epidemic" in the 1960s. [5, 14-19] Most registries use a coding system based on the International Statistical Classification of Diseases and Related Health Problems (ICD) published by the World Health Organization. Because the ICD is not sufficiently detailed for more specialized purposes some registries use extensions of its codes, for example the British Pediatric Association Classification of Diseases (BPA).[4, 5, 15, 20] The ICD (10th revision) has a section entitled "Cleft lip and cleft palate" (Q35-37) to record oral clefts (median cleft lip included).[21] These codes can give some information regarding the morphology and topography of the oral cleft, but not in great detail. Therefore, many registries do not supply the detailed information required for OCs as well as for other craniofacial congenital anomalies.

National registries

In the Netherlands, theoretically all surviving children with OCs who stay in the country are treated by one of the 15 cleft palate teams.[22] These teams offer multidisciplinary treatment to patients with OCs according to the team protocols. Members of the teams belong to the Dutch Association for Cleft Palate and Craniofacial Anomalies (NVSCA). Important goals of this association are: (1) description of the frequency and distribution of all categories and subgroups of OCs and other craniofacial anomalies, (2) promotion of clinically-related research on etiology, prevention, diagnostics, and treatment of oral clefts and other craniofacial anomalies, and (3) planning and quality surveillance.[23, 24] In order to fulfill these goals, a new descriptive recording form was developed based on the embryology of the head and neck area, describing the morphology and topography of the anomalies.[7, 25, 26] Since 1997, the NVSCA has maintained a national registry of congenital craniofacial anomalies, including OCs. Reporting is done for all new presurgery patients with OCs by the cleft palate teams through the standard NVSCA recording form (Figure 1).[22]

Before 1997, precise national prevalence of OCs was not known in the Netherlands. Felix-Schollaart et al.[27] described an oral cleft prevalence of 13.8 to 17.7 per 10,000 live and stillbirths among children born in Dutch hospitals during 1982 and 1983. Hoeksma et al.[28] reported in 1989 an estimated oral cleft prevalence of 17.3 to 18.9 per 10,000 live births for a 1-year period, based on questionnaires and medical records. Since 1981, Eurocat Northern Netherlands (NNL) has maintained a congenital anomaly registry for the region Northern Netherlands. Recording is based on ICD/BPA codes and regional prevalence rates of CL±P and CP among live and stillbirths (including termination of pregnancy) are provided.[2, 20] In addition, the National Obstetric and Neonatal Registries (LVR/LNR) record diagnoses of several congenital anomalies among live births and stillbirths from 16 weeks of gestation and have provide data regarding CL±P and CP from 1996 that were first published in an annual report in 2001. [29] In 2005, the prevalence of OCs in the Netherlands was estimated based on the NVSCA registry and LVR/LNR. The estimated national prevalence was 19.2 per 10,000 live births and the ascertainment—the proportion of cases recorded in at least one of the two registries—of OCs in live births appeared to be high (96%).[30]

Validation of registries

Worldwide, medical information is routinely collected and ICD coded in a variety of medical registries. In the past two decades, these registry data have been widely used for health research.[31] Because registry data can in practice only be used for research purposes when registries provide reasonably valid information, many registries have been validated. [18, 31-34] Therefore, a validation project of the NVSCA registry that evaluates data quality is essential to avoid invalid conclusions.

The NVSCA registry has a unique recording method, which is not based on a coding system but on the detailed description of the morphology and topography of each anatomic structure

of the anomalies of the head and neck area, e.g., lip, alveolus, and hard and soft palates including the uvula.[22, 26] These detailed recording data are collapsible to more general diagnoses or codes and allow classifying oral clefts in many different ways. For instance, NVSCA data can be compared with those of other Dutch registries, which include ICD/BPA codes (Eurocat NNL), or the categories CL±P and CP (LVR/LNR). Vice versa, data of these registries cannot be converted into the detailed information of the NVSCA registry. Even when the quality is good, data of these registries do not reflect the severity and specific characteristics of OCs; for example, no distinction is made between cleft lip and cleft alveolus.[20, 29] Therefore, medical records were used as our gold standard to validate the detailed NVSCA data.

The aim of this study was to provide a comprehensive profile on the validity of the NVSCA registry data for common oral clefts in the Netherlands. In view of the huge amount of data available, the present study describes the study design and results after evaluation of the first part of the NVSCA recording form; i.e., the general items and the three common oral cleft categories. The validity of more specific features (side, topography, and morphology) of the oral clefts and the associated additional congenital anomalies will be reported in future papers.

METHODS

NVSCA recording form and registry

The NVSCA registry is an anonymous prospective case registry that is formally fixed in accordance with the Dutch privacy law. All Dutch cleft palate teams record their live born presurgery patients on the standard NVSCA form, after careful examination by one of their consulting physicians. The form is subdivided into a general section (including infant/parental characteristics, e.g., sex, consanguinity, and birth weight), a section for craniofacial anomalies including OCs, and a section for congenital anomalies of other parts of the body (Figure 1). All individual anomalies of the head and neck area can be fully described by checking options regarding side, topography, and morphology. In addition, the form gives space for verbatim descriptions of (1) craniofacial anomalies not appropriate by checking options, (2) (preliminary) diagnosis of craniofacial anomalies, and (3) congenital anomalies of other organ systems.[22, 25, 26] Furthermore, a manual is included (Figure 2). The form is usually completed in the postnatal period. When patients are adopted or the oral cleft is detected later in infancy, the form is completed (before surgery) at a later age.[22] The completed forms are sent to the NVSCA registry, the working group "Registration" checks the forms, and the recorded information is subsequently transferred to the NVSCA registry database. At the end of each year, the cleft palate teams perform case-ascertainment activities. Note that the NVSCA is not an ongoing registry and that no data from other sources are included.

Registration of clefts and craniofacial malformations | **Hospital** | Dutch Association for Cleft Palate and Craniofacial Anomalies | **Please fill in white boxes**

1. GENERAL

Date of this registration _____
Patient identification number _____
Date of birth _____
Gender ◯ Male ◯ Female ◯ Unknown
Name of physician _____
Clinical genetics consulted ◯ Yes ◯ No ◯ Unknown
If affirmative, please specify center _____
Adoption or foster child ◯ Yes ◯ No ◯ Unknown

Caucasian father ◯ Yes ◯ No ◯ Unknown
Caucasian mother ◯ Yes ◯ No ◯ Unknown
Consanguinity ◯ Yes ◯ No ◯ Unknown
If affirmative, please specify _____
Congenital abnormalities among relatives ◯ Yes ◯ No ◯ Unknown
If affirmative, please specify _____
Birth weight (grams) _____
Gestational age (weeks) _____
Remarks about pregnancy ◯ Yes ◯ No ◯ Unknown
If affirmative, please specify _____

2. ABNORMALITIES OF THE HEAD AND NECK AREA

L = left R = right M = median	Mouth							Ala nasi	Septum nasi	Calvaria / facial skull									Orbita	Eyes	Eye-lids	Ears	Soft tissue*
	Lip	Pre./ max.*	Pre.*	Pal. dur.*	Pal. mol.*	Uvula	Ton.*			Par.*	Occ.*	Tem.*	Fro.*	Nas.*	Zyg.*	Max.*	Man.*	I.o.d.*					
Cleft									M											L R			
Complete	L R M	L R		L R M		M	M										M						
Incomplete	L R M	L R		L R M		M	M										M						
Submucous	L R M	L R		L R M		M	M										M						
Agenesis[1]	L R M		L R	L R	L R	L R	M	L R	M	L R	M	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R M
Aplasia[2]	L R M		L R	L R	L R	L R	L R M	L R	M	L R	M	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R M
Protruding																				L R	L R		
Adherent							L R													L R	L R		
Appendages																				L R	L R	L R M	
Hypoplasia[3]	L R M		L R	L R	L R	L R	L R	L R	M	L R	M	L R	L R	L R	L R	L R		L R	L R	L R	L R	L R M	
Hyperplasia[4]	L R M		L R	L R	L R	L R	L R	L R	M	L R	M	L R	L R	L R	L R	L R		L R	L R	L R	L R	L R M	
Synostosis										L R M	L R	L R	L R M	L R	L R	L R	L R M						
Non synostosis										L R M	L R	L R	L R M	L R	L R	L R	L R M						

Other abnormalities of the head and neck area, not appropriate above ◯ Yes ◯ No ◯ Unknown _____

■ **(Preliminary) diagnosis** ◯ **Yes** ◯ **No** ◯ **Unknown** _____

3. OTHER ABNORMALITIES

Circulatory system ◯ Yes ◯ No ◯ Unknown _____
Respiratory system ◯ Yes ◯ No ◯ Unknown _____
Digestive system ◯ Yes ◯ No ◯ Unknown _____
Urogenital system ◯ Yes ◯ No ◯ Unknown _____
Central nervous system ◯ Yes ◯ No ◯ Unknown _____
Vertebral column ◯ Yes ◯ No ◯ Unknown _____
Body wall ◯ Yes ◯ No ◯ Unknown _____
Skin ◯ Yes ◯ No ◯ Unknown _____
Upper limbs ◯ Yes ◯ No ◯ Unknown _____
Lower limbs ◯ Yes ◯ No ◯ Unknown _____
(Preliminary) common diagnosis ◯ Yes ◯ No ◯ Unknown _____

1 = absent; 2 = present, wrong shape; 3 = right shape, too small; 4 = right shape, too large
* Pre./max. = premaxilla - maxilla; Pre. = premaxilla; Pal.dur. = palatum durum; Pal.mol. = palatum molle; Ton. = tongue; Par. = os parietale; Occ. = os occipitale; Temp. = os temporale; Fro. = os frontale; Nas. = os nasale; Zyg. = zygoma; Max. = maxilla; Man. = mandible; I.o.d. = interorbital distance; Soft tissue = soft tissue of the head and neck area

Please send form to Mw Dr Chr. Vermeij-Keers; Research Unit Department of Plastic and Reconstructive Surgery; Room EE 1591; Erasmus MC - University Medical Center Rotterdam; P.O. Box 2040; 3000 CA Rotterdam; The Netherlands

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Figure 1. Recording form of the NVSCA registry.

Manual for the NVSCA-registration form

- One registration form for each un-operated patient.
- Please only use ballpoint to mark the white boxes and to fill the text boxes.
- The bold terms in this manual refer to the terms in the registration form.

Ad **2. ABNORMALITIES IN HEAD AND NECK AREA**

- The registration is based on aberrant embryonic development of the face and skull. Roughly, embryonic development can be distinguished in fusion of the facial/palatine swellings and differentiation of the calvarian and facial bones and soft tissue. Only fusion and differentiation defects of the primary palate (right and/or left) and the secondary palate (left or right or median) are registered as **cleft**. All other defects of bones and soft tissue, including clefts, are registered on the basis of their absence or presence and shape (**agenesis** or **aplasia**, **hypoplasia**, and **hyperplasia**), except for colobomas of the eyeball (see below). The definition of **agenesis, aplasia**, **hypoplasia**, and **hyperplasia** and the explanation of the abbreviations are described in the footnotes of the registration form.
- More abnormalities can be filled in for the same patient. If an abnormality can not be registered, this abnormality should be scored in the box **other abnormalities of the head and neck area, not appropriate above**, **yes**, and it should be specified.
- Cysts and fistulas of the tongue in the median are scored as **aplasia** of the **tongue** in the **median** plane.
- Hypotelorism and hypertelorism may be accompanied with an aberrant septum nasi in the median plane. For example, hypotelorism could be accompanied with **agenesis**, **aplasia** or **hypoplasia** of the **septum nasi**, and hypertelorism could be accompanied with **cleft** (= bifid), **aplasia** or **hypoplasia** of the **septum nasi**. Furthermore, the aberrant interorbital distance (**i.o.d.**) is registered.
- **Non synostosis** concerns a skull shape comparable with synostosis, but the sutures are open. Synostosis of sutures is registered as **synostosis** of the involved bones. **Synostosis** of both frontal bones, or both parietal bones are registered in the **median**.
- Colobomas of the eyeball concern fusion defects of the fissure, and these are scored as **cleft** of the **eyes**.
- Entropion and ectropion should be registered as **protruding eyelids**. Ptosis and phimosis of the eyelids, and epicanthal folds are scored as **aplasia** of the **eyelids**. Microblepharon is registered as **hypoplasia** of the **eyelids**.
- Colobomas of the eyelids, ears and ala nasi, are scored as **aplasia** of the **eyelids**, **ears**, and **ala nasi**.
- Aberrant position of the ears, such as low set or tilted ears, is filled in as **miscellaneous ears**.
- If a diagnosis of the *head and neck area* has been established, **(preliminary) diagnosis** should be filled in (**yes**) and should be specified. Moreover, all abnormalities should be registered in this box.

Ad **3. OTHER ABNORMALITIES**

- **Body wall** concerns thoracic and abdominal wall.
- Abnormalities of the shoulder and pelvis are filled in as abnormalities of **upper** and **lower limbs**, respectively.
- If the abnormalities are part of a syndrome, **(preliminary) common diagnosis** should be filled in (**yes**), and it should be specified.

Remarks about the registration form and this manual should be adressed to Mrs. Dr Vermeij-Keers, Research Unit Department of Plastic and Reconstructive Surgery, Room EE 1591, Erasmus MC - University Medical Center Rotterdam, P.O. Box 2040, 3000 DR Rotterdam, The Netherlands; Phone +31-10-7403292; fax +31-10-7044685; email c.vermeij-keers@erasmusmc.nl.

Figure 2. Manual for the NVSCA registration form.

Subjects

The validation project of registry data reported over a 7-year period was initiated and carried out in all 15 cleft palate teams. Each team gave written permission for the review of patients' medical data. Principles outlined in the Declaration of Helsinki were followed. Between January 1, 1997, and December 31, 2003, 2553 patients with OCs (median cleft lip and atypical facial clefts excluded) with or without associated congenital anomalies were recorded in the NVSCA registry and transferred to the NVSCA database. From this database a random sample of 250 cases was taken.

Data collection and verification

The cleft palate teams supplied medical information for all relevant disciplines (Plastic Surgery, Orthodontics, Pediatrics, Clinical Genetics, Maxillofacial Surgery and Otorhinolaryngology). A single investigator (AMR) obtained relevant data of 250 cases by making an anonymized copy by digital camera of medical records (including information about clinic visits, consultations, diagnostic tests, and hospitalizations), color photographs, panoramic radiographs, and dental casts. To be considered adequate, the information had to include at least one medical record. For 241 cases (96.4%), medical information was available for inspection, and this criterion was met. Preoperative and/or postoperative color photographs were obtained for 193 cases (77.2%). Panoramic radiographs and dental casts were retrieved for 26 cases (10.4%) and 91 cases (36.4%), respectively. Apart from the nine untraceable cases, one case with insufficient medical data and three cases that were operated on the oral cleft before registration were also excluded. Subsequently, a total of 237 cases remained in the study.

The same investigator, trained in recording principles and practice, performed data verification. The medical data were examined blindly and each of the 237 cases was reregistered with use of the standard NVSCA recording form (Figure 1). The criteria used to define the type of OCs were established in collaboration with a second investigator (CVK) and in accordance with existing literature.[7, 8] Guidance statements from the registry manual (Figure 2) were used to record present congenital anomalies. All cases with unclear clinical information were discussed with the second investigator. Subsequently, the recorded data were transferred to an independent reregister database. This database was checked for nonexistent, inappropriate, and invalid data and corrected when necessary.

Data analysis

In the present study the following variables were validated: the general information (clinical genetics consulted, adoption/foster child, white father and mother, consanguinity, congenital abnormalities among relatives, common oral clefts among relatives, birth weight, gestational age, and remarks about pregnancy) and the three common oral cleft categories (cleft lip/alveolus = CL/A; cleft lip/alveolus and palate = CL/AP; and cleft palate = CP). These variables concern information at birth and obvious external defects, for which recording should be

virtually complete.[4] To validate these items as being accurate as possible, all available medical information (i.e., the medical record) was used for comparison, since this medical record was the most complete available reflection of the cases' characteristics. Consequently, the NVSCA database was compared with the reregister database for concordance of information. Note that one case could contribute to more than one difference between the databases. In case of disagreement between the NVSCA and medical record database on the common oral cleft category (n = 12), the second investigator reviewed the medical data blindly and recorded the oral cleft independently. Regarding all 12 cases, the findings of the second investigator agreed with those of the first investigator.

Statistical analysis

Characteristics of the study population are presented as percentages or means ± 1 SD for the NVSCA database and medical record database. Comparisons were performed using the chi-square test and Student's paired *t* test.

To assess whether the NVSCA database accurately reproduced what was recorded in the reregister database, the observed agreement was assessed for dichotomous variables using two by two tables. In addition, the kappa statistic (κ) was used to describe agreement beyond chance. Kappa avoids the assertion that the reregister database has to be considered as a reference standard, and it determines the extent to which the two databases identified the same cases, i.e., inter-database agreement. [32, 35] According to the criteria reported by Landis and Koch,[36] and described by Quan et al.,[31] a κ value ranging from 0 to 0.20 indicates poor agreement; 0.21 to 0.40, fair agreement; 0.41 to 0.60, moderate agreement; 0.61 to 0.80, substantial agreement; and 0.81 to 1, near-perfect agreement. Because the reregister database could be considered as the best reflection of the cases' conditions, this database was designated as the gold standard to calculate sensitivity (the number of positive cases in the NVSCA database confirmed in the reregister database divided by the total number of positive cases in the reregister database), specificity (the number of NVSCA negatives confirmed by the reregister divided by the total number of negatives in the reregister), positive predictive value (the number of NVSCA positives that are confirmed by the reregister, divided by the total number of NVSCA positives), and negative predictive value (the number of NVSCA negatives confirmed by the reregister divided by the total number of NVSCA negatives). For continuous variables the differences between the databases were presented as medians and ranges, and Pearson correlation coefficients were calculated.

For all outcome measures, 95% confidence intervals (95% CIs) were calculated by assuming a normal distribution around the point estimate.

RESULTS

Characteristics of the study population are shown in Table 1. Distribution of sex, adoption/foster child, consanguinity, birth weight, and common oral cleft categories were comparable between the databases. Although gestational age was similar on average between the databases, there appeared to be a significant difference on case level ($p = 0.043$). Clinical Genetics consulted, congenital abnormalities among relatives, common oral clefts among relatives, and remarks about pregnancy were more often recorded in the reregister database ($p < 0.05$), whereas white father and mother were more often recorded in the NVSCA database ($p < 0.001$).

Agreement between the NVSCA and reregister database on the dichotomous general variables is shown in Table 2. The highest observed agreement was found for adoption/foster child and consanguinity (over 97%), and the lowest observed agreement was found for remarks about pregnancy (62.6%). For the remaining variables the observed agreement ranged from 73.4% (Clinical Genetics consulted) to 84.8% (common oral clefts among relatives). The κ value

Table 1. Characteristics of the study population in the NVSCA database and reregister database

Characteristic	NVSCA		Reregister		Comparison*
	Valid Cases	*n*	*Valid Cases*	*n*	*p Value*
General Information					
Sex, % boys†	57.4	237	57.4	237	
Clinical Genetics consulted, % yes	26.2	237	51.1	237	**<0.001**
Adoption/foster child, % yes	3.0	237	2.5	237	0.779
White father, % yes	86.5	237	72.2	237	**<0.001**
White mother, % yes	88.6	237	71.7	237	**<0.001**
Consanguinity, % yes	1.7	237	1.7	237	1.0
Congenital abnormalities among relatives, % yes	23.2	237	40.5	237	**<0.001**
Common oral cleft among relatives, % yes	13.9	237	23.2	237	**0.009**
Birth weight in grams (mean ± SD)	3290 ± 718	218	3234 ± 699	208	0.600
Gestational age in weeks (mean ± SD)	39 ± 2.4	216	39 ± 2.3	197	**0.043**
Remarks about pregnancy, % yes‡	16.1	174	43.9	174	**<0.001**
Common oral cleft					
Cleft lip/alveolus, % yes	31.6	237	30.0	237	0.691
Cleft lip/alveolus and palate, % yes	37.6	237	40.9	237	0.452
Cleft palate, % yes	30.8	237	29.1	237	0.688

* Chi Square test for proportions; paired Student's *t* test for continuous variables.
† Gender information was given for medical data retrieval and therefore not compared between the databases.
‡ Introduced in 1999 on the NVSCA recording form and gradually filled in by the cleft palate teams.

Table 2. Agreement between the NVSCA database and reregister database (gold standard) on general items (n = 237)*

General Information	Agreement	κ Value		Sensitivity		Specificity		(+) Predictive Value		(-) Predictive Value	
	%		*(95% CI)*	%	*(95% CI)*	%	*(95% CI)*	%	*(95% CI)*	%	*(95% CI)*
Clinical Genetics consulted	73.4	0.47	(0.38-0.57)	49.6	(40.4-58.8)	98.3	(93.9-99.8)	96.8	(88.8-99.6)	65.1	(57.6-72.2)
Adoption/foster child	98.7	0.76	(0.50-1.00)	83.3	(35.9-99.6)	99.1	(96.9-99.9)	71.4	(29.0-96.3)	99.6	(97.6-100.0)
White father	78.9	0.38	(0.25-0.51)	95.3	(91.0-98.0)	36.4	(24.9-49.1)	79.5	(73.3-84.8)	75.0	(56.6-88.5)
White mother	78.1	0.34	(0.21-0.47)	96.5	(92.5-98.7)	31.3	(20.6-43.8)	78.1	(71.9-83.5)	77.8	(57.7-91.4)
Consanguinity	97.5	0.24	(–0.16-0.64)	25.0	(0.6-80.6)	98.7	(96.3-99.7)	25.0	(0.6-80.6)	98.7	(96.3-99.7)
Congenital abnormalities relatives	76.8	0.48	(0.37-0.59)	50.0	(39.6-60.4)	95.0	(90.0-98.0)	87.3	(75.5-94.7)	73.6	(66.6-79.9)
Common oral cleft relatives	84.8	0.50	(0.37-0.64)	47.3	(33.7-61.2)	96.2	(92.2-98.4)	78.8	(61.1-91.0)	85.8	(80.2-90.3)
Remarks about pregnancy†	62.6	0.20	(0.09-0.32)	26.6	(17.3-37.7)	92.6	(85.4-97.0)	75.0	(55.1-89.3)	60.3	(51.9-68.3)

* CI = confidence interval.

† Number of valid cases = 174.

ranged from 0.20 (remarks about pregnancy) to 0.76 (adoption/foster child); one item was at the level of poor agreement, three were at the level of fair agreement, three were at the level of moderate agreement, and one at the level of substantial agreement. Sensitivity ranged from 25.0% for consanguinity to 96.5% for white mother. Meanwhile, specificity was high for all items (over 92%), except for white father and mother (36.4% and 31.3%, respectively). Positive predictive value was low for consanguinity (25.0%), but ranged for the other variables from 71.4% (adoption/foster child) to 96.8% (Clinical Genetics consulted). Negative predictive value ranged from 60.3% for remarks about pregnancy to 99.6% for adoption/foster child.

Validation of birth weight and gestational age was based on 196 and 186 cases, respectively, because of missing values in the NVSCA and reregister database. Agreement on birth weight was observed for 151 cases. For the remaining 45 cases a median difference of 50 g was found with a range of 1 to 3010 g. The Pearson correlation coefficient was 0.93 (95% CI: 0.91 to 0.95). Gestational age corresponded for 143 cases between the databases and disagreed for 43 with a median difference of 1 week and a range of 1 to 10 weeks. A Pearson correlation coefficient of 0.89 (95% CI: 0.86 to 0.92) was found.

Table 3 shows the agreement on the common oral cleft categories between the databases. The observed agreement was high for all three categories: 97.5% for both CL/A and CP and 94.9% for CL/AP. The κ value was 0.94 for both CL/A and CP and 0.89 for CL/AP; all were at the level of near-perfect agreement. The sensitivity was 98.6% for both CL/A and CP, 89.7% for CL/AP, and the specificity was over 97% for all categories. The positive and negative predictive values were over 93% for all three categories.

DISCUSSION

Validity NVSCA registry

This study assessed the accuracy and completeness of a part of the recording data of the NVSCA registry on OCs. The general information and oral cleft categories were validated using a reregister database based on all available medical data for comparison. The oral cleft categories (CL/A, CL/AP, and CP) were recorded most accurately and completely in the NVSCA registry. All categories were identified perfectly with validity measures of more than 89% and near-perfect agreement (Table 3).

In contrast, regarding the general information data, quality varied by item (Table 2). Information on consultation of Clinical Genetics was missing for about 50% of the cases. This is related to the fact that generally the patient is recorded in the postnatal period before the clinical geneticists are consulted. Regarding data on adoption/foster child and consanguinity, the quality was good. For these two items a "high agreement but low kappa" was found, which can be explained by the low prevalence of these items. This phenomenon was described by Feinstein and Cicchetti.[35] They identified the following paradox: when the vertical and horizontal

Table 3. Agreement between the NVSCA database and reregister database (gold standard) on common oral cleft category (n = 237)*

Common Oral Cleft Category	Agreement	κ Value		Sensitivity		Specificity		(+) Predictive Value		(-) Predictive Value	
	%		*(95% CI)*	%	*(95% CI)*	%	*(95% CI)*	%	*(95% CI)*	%	*(95% CI)*
CL/A	97.5	0.94	(0.89-0.99)	98.6	(92.4-100.0)	97.0	(93.1-99.0)	93.3	(85.1-97.8)	99.4	(96.6-100.0)
CL/AP	94.9	0.89	(0.84-0.95)	89.7	(81.9-94.9)	98.6	(94.9-99.8)	97.8	(92.1-99.7)	93.2	(87.9-96.7)
CP	97.5	0.94	(0.89-0.99)	98.6	(92.2-100.0)	97.0	(93.2-99.0)	93.2	(84.7-97.7)	99.4	(96.6-100.0)

* CI = confidence interval; CL/A = cleft lip/alveolus; CL/AP = cleft lip/alveolus and palate; CP = cleft palate.

marginal totals of the 2 x 2 tables are symmetrically unbalanced, high observed agreement values can be associated with low κ values. The items white father and mother were considerably overestimated in the NVSCA database with more than 60% false positives. This may not be surprising because the distinction between the white race and other races may sometimes be too subtle for recorders. Regarding the occurrence of congenital abnormalities among relatives and common oral clefts among relatives there may be insufficient inquiry by the specialists, because approximately 50% of the information recorded in the reregister database was found in the NVSCA database. For the item remarks about pregnancy, 27% of the reregister information was recorded in the NVSCA registry. This could be explained by the fact that "remarks about pregnancy" is a not well-defined item. As a result, it is not clear for the recorder what has to be recorded at this item. Birth weight and gestational age were underreported on the NVSCA recording form as well as in the medical records. Since both are available at birth, the only explanation for the degree of underreporting and disagreement is insufficient and inaccurate reporting and documentation. Overall, validity on the general items was expected to be higher because most of this information could be directly transcribed at admission. However, incompleteness of data on certain registry key items (for example, gestational age) is also reported elsewhere.[37]

Publications on the evaluation of data quality of congenital anomaly registries are scarce,[16, 37] and few data are available on the validity of registration of oral clefts.[18, 19] However, numerous articles have described the operations and strategies for case ascertainment of congenital anomaly registries. These show that case ascertainment is often still a problem and varies by defect, region, and hospital.[14, 16, 17] For example, according to Boyd et al.,[17] in the United Kingdom the surveillance of congenital anomalies by the national register is currently inadequate. Nevertheless, the ascertainment for oral clefts appeared to be among the highest in this register, 83% for CL±P and 71% for CP. A Norwegian study[18] reported an ascertainment of 94% for CLP cases in a national birth registry and a lower ascertainment of 83% and 57% for CL and CP cases, respectively. In another Scandinavian study,[19] the ascertainment of oral clefts was 78% (CL: 74%, CLP: 84%, and CP: 75%) for the Swedish Birth Defects Registry. As mentioned before, Anthony et al.[30] estimated the total number of live birth cases with OCs during 2002 in the Netherlands based on two Dutch registries: the NVSCA and LVR/LNR. Eighty-seven percent of the total number of cases found in this study appeared to be reported to the NVSCA registry, which is rather comparable to the ascertainment of the studies already mentioned. Because cases with severe additional anomalies resulting in neonatal deaths may not reach the cleft palate teams, these are most often not included in the NVSCA registry. This might explain why the ascertainment was not 100%.

Problems with registry data

In general, problems with quality of registry data can be caused by incorrect data entry, lack of entry of available information, or the original information may be correctly entered into

the database but may not reflect the true condition or characteristics of the case.[33] The latter can arise as a result of physicians' misdiagnoses, incomplete documentation, or incomplete or incorrect recording of a condition.[31]

As many congenital anomaly registries are based on (ICD) codes, they are affected by specific problems inherent in coding systems. Certainly, coding is essential for data management and retrieval in birth defects surveillance programs because they process large numbers of cases.[4, 5, 15] Furthermore, coding allows aggregation of similar cases. Thus, when collecting data on a large scale, the use of standard coding systems is necessary; however, it is also known that it brings structural limitations. Codes reduce the amount of clinical detail, and coders will differ with respect to definitions and their application.[4, 14, 15, 32, 34] Moreover, coding is generally based on written medical data, and thus correct recording of a condition also depends on the quality of this information.[34]

The NVSCA recording form is designed to prevent recording errors as much as possible. However, accurate and complete recording still depends on the knowledge and the willingness of physicians to record accurately. To prevent problems with interpretation of the recording form as much as possible, the NVSCA provides a registry manual (Figure 2).

Recently a digital NVSCA recording form was developed to make recording easier and to promote accurate and complete recording.[24] This has many advantages: no paperwork has to be sent by mail, and it cannot be lost; data do not need to be transferred from a paper form to a digital database; and obligatory fields are used for items such as birth weight and gestational age.

Strengths and limitations

One of the strengths of this study is the national distribution of the sampling frame, including cleft palate teams of large urban teaching and specialist hospitals as well as of small regional ones. During the last decade the diagnostic strategy and management of patients with OCs have undergone important changes. For example, most cleft palate teams now use imaging procedures and digitalization, which is needed for multidisciplinary treatment and favors this retrospective study. The retrieval of medical records was successful; for almost all cases at least one medical record (96.4%) was obtained along with pre-/postoperative color photographs, panoramic radiographs, and/or dental casts (82.8%).

Based on the strengths described above, the medical data were considered as the best available reflection of the cases' conditions, and therefore information extracted from these data was accepted as the gold standard. However, the use of medical data to validate registry data also has limitations. Medical data can never be equal to the presentation of patients in the outpatient clinical setting. In the present study, the amount and quality of medical information varied between the teams. For example, for some teams dental casts were lacking, and in some cases less extensive medical information was caused by death of the patient or change of the treating cleft palate team. Nevertheless, no systematic pattern regarding the quality of

medical data was found when analyzing case characteristics and the oral cleft categories could be recorded successfully for all 237 cases. Another limitation is that although review of medical data was particularly thorough, errors that occur when clinical information is documented in the medical records cannot be captured.[31, 34] On the other hand, an advantage of our method is that practice activity was examined retrospectively, so staff were not alerted to the study beforehand and had no opportunity to change recording behavior.

CONCLUSIONS

Despite the limitations and challenges described, this study provides useful information on the quality of the NVSCA registry data, which varies by type of information. Validity appears to be very good for the three common oral cleft categories and reasonable to satisfying for the general items. As a result of this study and other data quality measures,[30] the quality level of the NVSCA registry appears to be high. To attain the goals of the NVSCA optimally, it is important to get more insight in the detailed data. Therefore, further analysis will be carried out of the specific common oral cleft features (side, topography, and morphology) and associated additional congenital anomalies.

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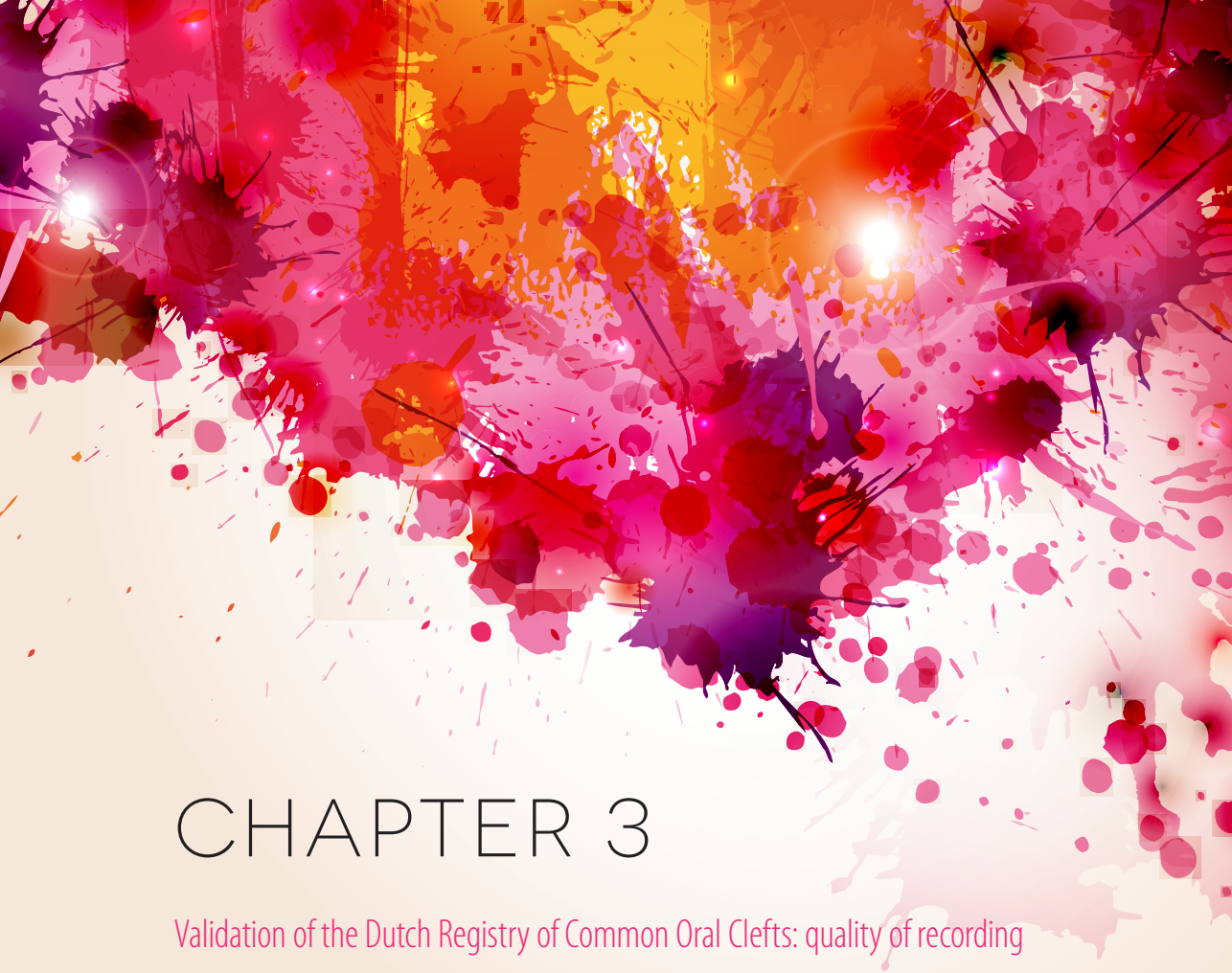

CHAPTER 3

Validation of the Dutch Registry of Common Oral Clefts: quality of recording specific oral cleft features

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ABSTRACT

Objective: Since 1997, common oral clefts in the Netherlands have been recorded in the national oral cleft registry using a unique descriptive recording system. This study validates data on the topographic-anatomical structure, morphology, and side of individual anomalies of the primary palate and secondary palate that form the oral cleft.

Design: Validation study.

Setting: All 15 Dutch cleft palate teams reporting presurgery oral cleft patients to the national registry.

Patients: A random sample of 250 cases registered in the national database with oral clefts from 1997 through 2003; of these, 13 cases were excluded.

Main Outcome Measures: By linking registry data with clinical data, we identified differential recording rates by comparing the prevalence, and we measured the degree of agreement by computing validity and reliability statistics.

Results: The topographic-anatomical structures (lip, alveolus, and hard and soft palates) of the anomalies had near-perfect inter-database agreement with a sensitivity of 88% to 99%. However, when analyzing the individual anomalies in detail (morphology and side), validity decreased and depended on morphological severity. This association was most evident for anomalies of the secondary palate. For example, sensitivity was higher for "complete cleft hard palate" (92%) than for "submucous cleft hard/soft palate" (69%).

Conclusions: Overall, the validity of Dutch registry data on oral clefts is good, supporting the feasibility of this unique recording system. However, when analyzing oral cleft data in detail, the quality appears to be related to anatomical location and morphological severity. This might have implications for etiologic research based on registry data and for guidelines on neonatal examination.

INTRODUCTION

Common oral clefts (OCs) are very complex and heterogeneous birth defects affecting the lip, alveolus, hard palate, soft palate, and uvula. In the embryonic development of the primary palate (the presumptive lip and alveolus) and secondary palate (the presumptive hard palate, soft palate, and uvula), many different cell-biological mechanisms and genes are involved, related to different time frames. During the formation of the primary and secondary palates, complex embryological processes—including outgrowth, fusion, and differentiation (into bone and musculature) of the facial swellings and of the palatine processes—take place.[1-3] Disturbance of these developmental processes can result in many different cleft types with variable degrees of severity on clinical presentation.[3-5]

Although the etiopathogenesis of OCs has been widely studied, it is still poorly understood.[6] To facilitate further genetic and etiopathological studies and to improve prevention, diagnostics, and treatment, it is of paramount importance that details of all OC types are described and recorded. Worldwide, many registration systems have been developed in order to record congenital anomalies, including OCs.[7-12] These registries classify OCs according to the International Classification of Diseases (ICD) or its extensions, thereby providing some information about topography, but not always complete information about morphological severity (e.g., completeness or incompleteness of the cleft).[7, 10, 13] Because different cleft types, which have specific topographic and morphologic features, originate from different time frames and are related to specific genes and cell-biological mechanisms, detailed information on the topography and morphology is essential for fundamental research on OCs. Therefore, a unique detailed recording system for OCs and other craniofacial anomalies has been developed on behalf of the Dutch Association for Cleft Palate and Craniofacial Anomalies (NVSCA). This unique NVSCA system is based on the embryology of the head and neck area and records all the individual anomalies of the primary and/or secondary palate that form the OC. Besides the topographic-anatomical structure and side, the morphology of each anomaly can be described to anticipate all conceivable anomalies. Since its establishment in 1997, virtually all new live-born presurgery patients with OCs in the Netherlands—an average of 351 patients per year—have been reported to the national NVSCA registry.[5]

The main purpose of the NVSCA registry is to provide a solid basis for epidemiological, clinical, and fundamental research. To serve this purpose, it is crucial to ensure that the data provided by the registry are of high quality. Sound description and complete reporting of OCs and their specific features are necessary to maintain high standards of data quality. Previously, it was shown that the case-ascertainment of OCs in the NVSCA registry is high.[14] In addition, we found recently that the NVSCA registry has high-quality data on the three OC categories: cleft lip/alveolus; cleft lip/alveolus and palate; and cleft palate.[15] As described by Luijsterburg and Vermeij-Keers),[5] these three categories manifest very heterogeneous cleft types, composed of individual anomalies of the primary and/or secondary palate having specific features regarding

topographic-anatomical structure, morphology, and side. However, it is unknown whether the individual anomalies in OCs have been recorded completely and accurately in the NVSCA registry.

The aim of this study was to investigate the quality of the NVSCA data on the individual anomalies of the primary palate and secondary palate in OCs by validating the registry data on the specific features of the anomalies: topographic-anatomical structure, morphology, and side. By linking the NVSCA database with a new independent reregister database derived from medical data review, we were able to identify differential recording rates by comparing the prevalence and to measure the degree of agreement by computing validity and reliability statistics.

METHODS

NVSCA Registry

The methodology of the NVSCA registry is described in detail elsewhere[5, 15] and is summarized here. The NVSCA registry is an anonymous registry that was formally established in accordance with Dutch privacy law. All Dutch cleft palate teams report their new live born patients with OCs—before these patients have an oral cleft operation—using the NVSCA recording form. This form is composed of three parts: a general section (infant/parent characteristics), a section for craniofacial anomalies including OCs, and a section for congenital anomalies of other organ systems; a manual is available.[5, 15] The section for OCs consists of a two-dimensional table, in which the specific features of the individual anomalies that form the OC can be described. As shown in Figure 1, the x-axis shows the topographic-anatomical structures: lip, alveolus (embryologically developed from the premaxillae and maxillae), hard palate (palatum durum), soft palate (palatum molle), and uvula. The y-axis depicts the morphology (complete, incomplete, and submucous) and the check boxes represent the side (left, right, and median).

The recording form is completed by the consulting physician during the first visit of the patient to the cleft palate team, and subsequently the form is sent to the NVSCA registry. The Registration working group checks the recorded data before these are transferred to the NVSCA database. In addition, the cleft palate teams perform case-ascertainment activities annually. Note that the NVSCA does not have active follow-up of patients and that no data from other sources are included.

Subjects

This validation study was initiated and carried out in the 15 Dutch cleft palate teams; all gave written permission for review of patients' medical data. Principles outlined in the Declaration of Helsinki were followed. During a 7-year period (1997 to 2003), a total of 2553 patients were registered in the national NVSCA database with an OC. Patients with median cleft lip/alveolus

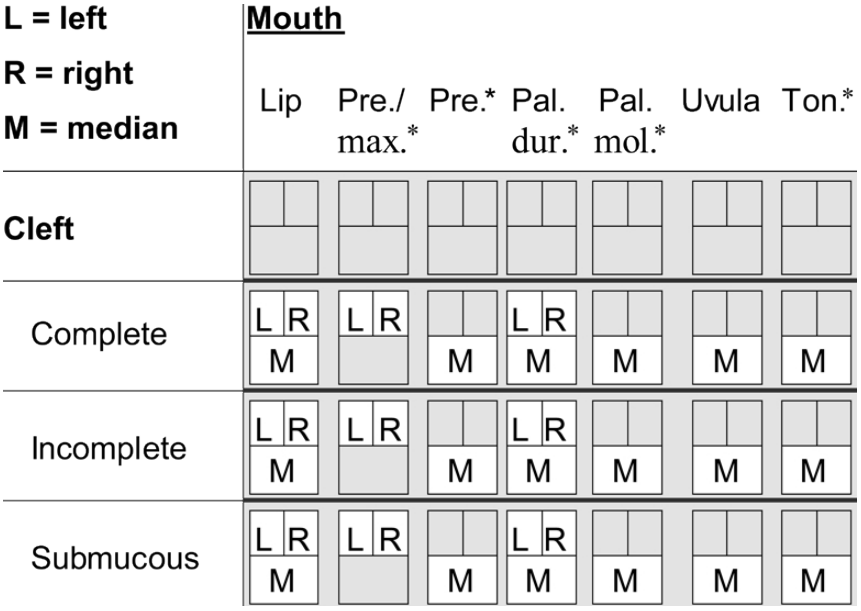

Figure 1. Section of the NVSCA recording form for common oral clefts in which the specific features of the individual anomalies that form the oral cleft can be described. The X-axis shows the topographic-anatomical structures: lip, alveolus (embryologically developing from the premaxillae and maxillae), hard palate (palatum durum), soft palate (palatum molle), and uvula; the Y-axis depicts the morphology: complete, incomplete, and submucous; and the checking boxes represent the side: left, right, and median. Abbreviations: Pre./Max. = premaxilla – maxilla; Pre. = premaxilla; Pal.dur. = palatum durum; Pal.mol. = palatum molle**.**

or atypical facial clefts were excluded due to their different pathogenesis.[1,4] From this database, a study population of 250 cases was selected using a standard random-sampling technique in Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL).

Data Collection and Verification

We used medical data to validate the NVSCA data on the specific features of the individual anomalies in OCs. The methods of medical data collection and verification were described in a previous paper by Rozendaal et al.[15] and are summarized here.

The relevant medical information, including medical records, color photographs, panoramic radiographs, and dental casts, was supplied by the cleft palate teams. For 241 of the 250 cases (96.4%), the minimum criterion for inclusion—the availability of at least one medical record—was met. Apart from the nine untraceable cases, we excluded one case that had insufficient medical information to record the cleft, and three patients who had undergone oral cleft surgery before registration. This resulted in a total of 237 cases that remained in the study.

Using the medical information that was created before as well as after completion of the original NVSCA recording forms, a single investigator (AMR) recorded each case blindly on the

standard NVSCA form.[15] The criteria used to define the type of OC were established in accordance with existing literature.[1] If the medical information was insufficient to record a specific feature, for example, the morphology or side of the hard palate, the investigator noted this on the form. This was done to allow exclusion of the case at a later stage from the specific feature's analysis. All the recorded data were then transferred to an independent reregister database, and finally, this database was checked for nonexistent, inappropriate, and invalid data.

Statistical Analysis

To get complete insight into the quality of the detailed registry data on the individual anomalies of the primary palate and secondary palate, their specific features were validated step by step. First, we analyzed the topographic-anatomic structures (lip, alveolus, hard palate, and soft palate including the uvula), then the morphology of the topographic-anatomic structures (e.g., complete cleft lip), then the side of the topographic-anatomical structures (e.g., left cleft lip), and finally the morphology and side of the topographic anatomical structures (e.g., left complete cleft lip), that is, the complete reflection of the individual anomaly. Note that the side of the soft palate including the uvula was not analyzed because clefts of the soft palate and uvula always develop in the median.

The prevalence of the specific features in the individual OC anomalies was calculated for both the NVSCA and the reregister. In addition, the NVSCA database was compared with the reregister database for concordance of individual patient data. Note that one case may contribute to more than one difference between the databases. In case of disagreement between the databases on a specific feature (n = 99), a second investigator (CVK) reviewed the medical data blindly and recorded the OC independently on a new NVSCA recording form. If the two investigators disagreed, there was discussion until consensus was reached (n = 21).

It is known that the "disease prevalence" can affect reliability and validity statistics,[16-18] and that the confidence intervals in reliability and validity statistics reflect the precision of the outcome measures. We validated, therefore, only those anomalies individually that had (1) a prevalence of n ^{3} 10 in the NVSCA database; and (2) a sufficiently small 95% confidence interval (CI) for all reliability and validity measures (distance between the upper and lower limits of 95% CI < 0.50 for kappa values and < 50% for sensitivity, specificity, positive predictive value, and negative predictive value). The anomalies not meeting these two criteria were grouped together with their embryologically related anomalies according to the classification of fusion and differentiation defects. The concept of this classification was described in detail previously[3] and is briefly explained here. This classification is based on the normal and abnormal development of the primary and secondary palates. During the formation of these structures, fusion and differentiation processes are regulated in time and place. Disturbances of these complex processes can give rise to fusion and/or differentiation defects of the lip, alveolus, hard palate, and soft palate including the uvula. Theoretically, all individual anomalies of the primary palate

and secondary palate that form the OC can be classified as a fusion or differentiation defect. The template for deciding which anomaly is a fusion or differentiation defect is listed in Table 1.[3]

When analyzing the morphology and/or side of the topographic-anatomical structures, the following anomalies were grouped together. We grouped "submucous cleft lip" together with "incomplete cleft lip", because both are differentiation defects of the lip. The differentiation defect "submucous cleft alveolus" was grouped together with "incomplete cleft alveolus", which is—in combination with an "incomplete/submucous cleft lip"—also a differentiation defect of the alveolus. We grouped "submucous cleft hard palate" together with "submucous cleft soft palate" because both anomalies are late differentiation defects of the secondary palate. The new group "submucous cleft hard/soft palate", which still had a 95% CI that was too wide, was not grouped further because other differentiation defects of the secondary palate do not exist. "Incomplete cleft soft palate" and "complete cleft soft palate" were grouped together, because both are fusion defects of the soft palate. The anomaly "right cleft hard palate" was grouped together with "left cleft hard palate" because both are unilateral fusion defects of the hard palate. Because the anomaly "right submucous cleft alveolus" had not been recorded in the NVSCA database, it was not validated. Finally, because practically all incomplete and submucous clefts of the hard palate present in the NVSCA database were median clefts, the side was not validated for these anomalies.

Table 1. Classification of the individual cleft anomalies of the primary palate and secondary palate according to fusion and differentiation defects. Any combination of anomalies of the lip, alveolus, hard palate, and soft palate is allowed.

Fusion defects	Primary palate	Complete cleft lip
		Complete cleft alveolus
		Incomplete cleft alveolus **(only if the lip is normal or has a complete cleft)**
	Secondary palate	Complete cleft hard palate
		Incomplete cleft hard palate
		Complete cleft soft palate including uvula
		Incomplete cleft soft palate including uvula
Differentiation defects	Primary palate	Incomplete cleft lip
		Submucous cleft lip
		Incomplete cleft alveolus **(only if the lip has an incompleet or submucous cleft)**
		Submucous cleft alveolus
		Hypoplastic lip/alveolus
	Secondary palate	Submucous cleft hard palate
		Submucous cleft soft palate including uvula

The prevalence data were presented as numbers and percentages. Prevalence comparisons between the databases were performed using the chi-square test. All p values of < 0.05 were considered statistically significant.

Although the comparison of prevalence rates indicates the extent to which the two databases detected the specific features of the individual anomalies, it does not indicate whether they have identified the same patients, and whether the NVSCA database accurately reproduced what was recorded in the reregister. We therefore determined the extent to which the two databases identified the same cases (i.e., the inter-database agreement) by calculating the kappa value (κ), which describes the agreement beyond chance and avoids the assertion that the reregister database has to be considered as a reference standard.[16, 19] According to criteria reported by Landis and Koch[20] and described by Quan et al.,[21] a kappa value ranging from 0 to 0.20 indicates poor agreement; 0.21 to 0.40, fair agreement; 0.41 to 0.60, moderate agreement; 0.61 to 0.80, substantial agreement; and 0.81 to 1, near-perfect agreement.

To assess whether the NVSCA database accurately reproduced what was recorded in the reregister, we used the reregister—the best available reflection of the cases' conditions—as the criterion standard to calculate the sensitivity (number of NVSCA positives confirmed by the reregister, divided by the total number of reregister positives), specificity (number of NVSCA negatives confirmed by the reregister, divided by the total number of reregister negatives), positive predictive value (number of NVSCA positives confirmed by the reregister, divided by the total number of NVSCA positives), and negative predictive value (number of NVSCA negatives confirmed by the reregister, divided by the total number of NVSCA negatives). For all outcome measures, 95% CIs were calculated, assuming a normal distribution around the point estimate. Statistics were performed using two software packages (SPSS version 17.0 and Stata version 10.0, StataCorp L.P., College Station, TX).

RESULTS

Prevalence of Specific Features of Individual Anomalies in Common Oral Clefts

Table 2 presents the prevalence of the specific features of the individual anomalies of the primary and secondary palates by database. The prevalence of the four topographic-anatomical structures (lip, alveolus, hard palate, and soft palate including the uvula) in the NVSCA database was similar to that in the reregister database. For the two structures of the primary palate (lip and alveolus), the distribution of the morphology, of the side, and of the morphology and side in the NVSCA was similar to that in the reregister. For one structure of the secondary palate (hard palate), however, three anomalies were underreported significantly in the NVSCA (incomplete cleft hard palate: $p = 0.007$; median cleft hard palate: $p = 0.009$; and median incomplete cleft hard palate: $p = 0.006$). Only one anomaly (left complete cleft hard palate) was significantly less frequent in the reregister than in the NVSCA (4.8% vs. 11.0%, $p = 0.015$).

Table 2. Prevalence of specific features of individual anomalies of the primary palate and secondary palate in common oral clefts (n = 237)

Specific Feature of Individual Anomaly	NVSCA		Reregister		Cases with Information*	
	n	%	*n*	%	*n*	*p Value*†
Topographic-anatomical structure						
Primary palate						
Cleft lip	164	69.2	164	69.2	237	1.000
Cleft alveolus	126	53.4	139	58.9	236	0.228
Secondary palate						
Cleft hard palate	117	50.0	128	54.7	234	0.309
Cleft soft palate	160	67.5	166	70.0	237	0.552
Morphology of topographic-anatomical structure						
Primary palate						
Complete cleft lip	83	35.2	67	28.4	236	0.114
Incomplete cleft lip	85	36.0	102	43.2	236	0.110
Submucous cleft lip	5	2.1	13	5.5	236	0.055
Complete cleft alveolus	80	34.2	83	35.5	234	0.771
Incomplete cleft alveolus	46	19.7	57	24.4	234	0.220
Submucous cleft alveolus	2	0.9	0	0.0	234	0.156
Secondary palate						
Complete cleft hard palate	94	40.9	83	36.1	230	0.292
Incomplete cleft hard palate	16	7.0	34	14.8	230	**0.007**
Submucous cleft hard palate	6	2.6	6	2.6	230	1.000
Complete cleft soft palate	151	64.8	154	66.1	233	0.770
Incomplete cleft soft palate	7	3.0	6	2.6	233	0.778
Submucous cleft soft palate	7	3.0	12	5.2	233	0.242
Side of topographic-anatomical structure						
Primary palate						
Left cleft lip	120	50.6	122	51.5	237	0.854
Right cleft lip	79	33.3	77	32.5	237	0.845
Left cleft alveolus	96	40.9	102	43.4	235	0.575
Right cleft alveolus	58	24.7	70	29.8	235	0.214
Secondary palate‡						
Left cleft hard palate	24	10.6	13	5.7	227	0.059
Right cleft hard palate	15	6.6	7	3.1	227	0.080
Median cleft hard palate	73	32.2	100	44.1	227	**0.009**

Table 2. *(Continued)*

Specific Feature of Individual Anomaly	NVSCA		Reregister		Cases with Information*	
	n	%	*n*	%	*n*	*p Value*†
Morphology and side of topographic-anatomical structure						
Primary palate						
Left complete cleft lip	57	24.2	47	19.9	236	0.267
Right complete cleft lip	45	19.1	37	15.7	236	0.331
Left incomplete cleft lip	60	25.4	70	29.7	236	0.303
Right incomplete cleft lip	32	13.6	38	16.1	236	0.437
Left submucous cleft lip	3	1.3	8	3.4	236	0.127
Right submucous cleft lip	2	0.8	5	2.1	236	0.253
Left complete cleft alveolus	60	25.6	56	23.9	234	0.668
Right complete cleft alveolus	43	18.4	46	19.7	234	0.724
Left incomplete cleft alveolus	34	14.5	42	17.9	234	0.316
Right incomplete cleft alveolus	14	6.0	19	8.1	234	0.367
Left submucous cleft alveolus	2	0.9	0	0.0	234	0.156
Right submucous cleft alveolus	0	0.0	0	0.0	234	1.000
Secondary palate‡						
Left complete cleft hard palate	25	11.0	11	4.8	228	**0.015**
Right complete cleft hard palate	15	6.6	9	3.9	228	0.134
Median complete cleft hard palate	54	23.7	61	26.8	228	0.387
Left incomplete cleft hard palate	0	0.0	1	0.4	228	0.317
Right incomplete cleft hard palate	1	0.4	0	0.0	228	0.317
Median incomplete cleft hard palate	15	6.6	33	14.5	228	**0.006**
Left submucous cleft hard palate	0	0.0	0	0.0	228	1.000
Right submucous cleft hard palate	0	0.0	0	0.0	228	1.000
Median submucous cleft hard palate	6	2.6	6	2.6	228	1.000

* Number of cases that had sufficient information to record the topographic-anatomical structure, morphology and/or side of the individual anomalies.

† *p* value presents statistical significance level between the NVSCA and reregister database in prevalence of feature/anomaly; $p < 0.05$ is used to determine statistical significance and is presented in bold format.

‡ Side of the soft palate was not analyzed because clefts of the soft palate always develop in the median.

Agreement on Specific Features of Individual Anomalies in Common Oral Clefts

Table 3 shows the degree of agreement between the databases for the specific features of the individual anomalies of the primary and secondary palates. When analyzing the morphology and/or side of the topographic-anatomical structures, several anomalies did not meet the criteria for validation (i.e., they had a prevalence of $n < 10$ in the NVSCA database and/or 95% CIs for reliability and validity measures that were too wide). These anomalies were therefore grouped together with their embryologically related anomalies as described in the "Methods" section.

Topographic-anatomical Structure

All four topographic-anatomical structures had near-perfect inter-database agreement (κ value: 0.82 to 0.98) with a sensitivity of 87.8% or more, a specificity and positive predictive value of more than 95%, and a negative predictive of 84.5% or more.

Morphology of Topographic-Anatomical Structure

After regrouping the anomalies, four anomalies of the primary palate remained. Table 3 shows that the κ values ranged from 0.67 to 0.84; one anomaly (incomplete/submucous cleft alveolus) was at the level of substantial agreement, and three were at near-perfect agreement. Sensitivity ranged from 68.4% for incomplete/submucous cleft alveolus to 97.0% for complete cleft lip. Positive predictive values ranged from 78.3% for complete cleft lip to 97.7% for incomplete/submucous cleft lip. The specificity and negative predictive values were more than 87% for all four anomalies.

For the remaining four anomalies of the secondary palate, the κ value ranged from 0.43 to 0.91; one anomaly (incomplete cleft hard palate) was at the level of moderate agreement, two were at substantial agreement, and one (complete/incomplete cleft soft palate) was at near-perfect agreement. Sensitivity was 35.3% for incomplete cleft hard palate, 69.2% for submucous cleft hard/soft palate, and more than 91% for the other two anomalies. Positive predictive values ranged from 75.0% for incomplete cleft hard palate to 98.7% for complete/incomplete cleft soft palate. The specificity and negative predictive values were more than 87% for all four anomalies.

Side of Topographic-Anatomical Structure

Table 3 shows that all four anomalies of the primary palate had near-perfect inter-database agreement (κ value: 0.84 to 0.95), with a sensitivity of 81.4% or more and a specificity, positive predictive value, and negative predictive value of more than 91%.

For the secondary palate, there were two remaining anomalies after regrouping. One anomaly (left/right cleft hard palate) had a κ value of 0.42 (moderate agreement), sensitivity of 73.7%, positive predictive value of 35.9%, and specificity and negative predictive value of 88.0% and over. The other anomaly (median cleft hard palate) had a κ value of 0.62 (substantial agreement), sensitivity of 66.0%, specificity and positive predictive value of more than 90%, and negative predictive value of 77.9%.

Morphology and Side of Topographic-Anatomical Structure

For the eight anomalies of the primary palate that remained after regrouping, the κ values ranged from 0.64 for right incomplete cleft alveolus to 0.88 for right complete cleft alveolus; five anomalies were at the level of substantial agreement and three at near-perfect agreement. Sensitivity ranged from 57.9% for right incomplete cleft alveolus to 93.6% for left complete cleft lip. Positive predictive values ranged from 75.6% for right complete cleft lip to 95.2% for left

Table 3. Agreement between the NVSCA database and reregister database (gold standard) on specific features of individual anomalies of the primary palate and secondary palate in common oral clefts (n = 237)*

Specific Feature of Individual Anomaly	κ Value		Sensitivity		Specificity		(+) Predictive Value		(-) Predictive Value		Cases†
		(95% CI)	*%*	*(95% CI)*	*%*	*(95% CI)*	*%*	*(95% CI)*	*%*	*(95% CI)*	*n*
Topographic-anatomical structure											
Primary palate											
Cleft lip	0.98	(0.95-1.00)	99.4	(96.6-100.0)	98.6	(92.6-100.0)	99.4	(96.6-100.0)	98.6	(92.6-100.0)	237
Cleft alveolus	0.82	(0.75-0.89)	87.8	(81.1-92.7)	95.9	(89.8-98.9)	96.8	(92.1-99.1)	84.5	(76.4-90.7)	236
Secondary palate											
Cleft hard palate	0.84	(0.77-0.91)	88.3	(81.4-93.3)	96.2	(90.6-99.0)	96.6	(91.5-99.1)	87.2	(79.7-92.6)	234
Cleft soft palate	0.92	(0.87-0.98)	95.8	(91.5-98.3)	98.6	(92.4-100.0)	99.4	(96.6-100.0)	90.9	(82.2-96.3)	237
Morphology of topographic-anatomical structure											
Primary palate											
Complete cleft lip	0.81	(0.73-0.89)	97.0	(89.6-99.6)	89.3	(83.7-93.6)	78.3	(67.9-86.6)	98.7	(95.4-99.8)	236
Incomplete/submucous cleft lip	0.82	(0.74-0.89)	81.9	(73.2-88.7)	98.5	(94.6-99.8)	97.7	(92.0-99.7)	87.2	(80.7-92.1)	236
Complete cleft alveolus	0.84	(0.77-0.91)	88.0	(79.0-94.1)	95.4	(90.7-98.1)	91.3	(82.8-96.4)	93.5	(88.4-96.8)	234
Incomplete/submucous cleft alveolus	0.67	(0.56-0.78)	68.4	(54.8-80.1)	94.9	(90.6-97.6)	81.3	(67.4-91.1)	90.3	(85.1-94.2)	234
Secondary palate											
Complete cleft hard palate	0.77	(0.69-0.86)	91.6	(83.4-96.5)	87.8	(81.3-92.6)	80.9	(71.4-88.2)	94.9	(89.7-97.9)	230
Incomplete cleft hard palate	0.43	(0.25-0.60)	35.3	(19.7-53.5)	98.0	(94.9-99.4)	75.0	(47.6-92.7)	89.7	(84.8-93.4)	230

Table 3. *(Continued)*

Specific Feature of Individual Anomaly	κ Value		Sensitivity		Specificity		(+) Predictive Value		(-) Predictive Value		Cases†
		(95% CI)	*%*	*(95% CI)*	*%*	*(95% CI)*	*%*	*(95% CI)*	*%*	*(95% CI)*	*n*
Submucous cleft hard/soft palate‡	0.77	(0.58-0.97)	69.2	(38.6-90.9)	99.5	(97.5-100.0)	90.0	(55.5-99.7)	98.2	(95.4-99.5)	230
Complete/incomplete cleft soft palate	0.91	(0.86-0.97)	95.5	(91.0-98.2)	97.4	(90.8-99.7)	98.7	(95.3-99.8)	91.4	(83.0-96.5)	233
Side of topographic-anatomical structure											
Primary palate											
Left cleft lip	0.95	(0.91-0.99)	96.7	(91.8-99.1)	98.3	(93.9-99.8)	98.3	(94.1-99.8)	96.6	(91.5-99.1)	237
Right cleft lip	0.94	(0.90-0.99)	97.4	(90.9-99.7)	97.5	(93.7-99.3)	94.9	(87.5-98.6)	98.7	(95.5-99.8)	237
Left cleft alveolus	0.84	(0.73-0.91)	88.2	(80.4-93.8)	95.5	(90.4-98.3)	93.8	(86.9-97.7)	91.4	(85.4-95.5)	235
Right cleft alveolus	0.85	(0.78-0.93)	81.4	(70.3-89.7)	99.4	(96.7-100.0)	98.3	(90.8-100.0)	92.7	(87.8-96.0)	235
Secondary palate§											
Left/right cleft hard palate	0.42	(0.25-0.58)	73.7	(48.8-90.9)	88.0	(82.8-92.1)	35.9	(21.2-52.8)	97.3	(93.9-99.1)	227
Median cleft hard palate	0.62	(0.52-0.72)	66.0	(55.8-75.2)	94.5	(89.0-97.8)	90.4	(81.2-96.1)	77.9	(70.5-84.2)	227
Morphology and side of topographic-anatomical structure											
Primary palate											
Left complete cleft lip	0.80	(0.71-0.90)	93.6	(82.5-98.7)	93.1	(88.5-96.3)	77.2	(64.2-87.3)	98.3	(95.2-99.7)	236
Right complete cleft lip	0.79	(0.69-0.90)	91.9	(78.1-98.3)	94.5	(90.3-97.2)	75.6	(60.5-87.1)	98.4	(95.5-99.7)	236
Left incomplete/ submucous cleft lip	0.81	(0.73-0.90)	79.7	(68.8-88.2)	98.1	(94.7-99.6)	95.2	(86.5-99.0)	91.4	(86.2-95.1)	236
Right incomplete/ submucous cleft lip	0.79	(0.68-0.90)	76.9	(60.7-88.9)	98.0	(94.9-99.4)	88.2	(72.5-96.7)	95.5	(91.7-97.9)	236
Left complete cleft alveolus	0.82	(0.73-0.90)	89.3	(78.1-96.0)	94.4	(89.9-97.3)	83.3	(71.5-91.7)	96.6	(92.6-98.7)	234

Table 3. *(Continued)*

Specific Feature of Individual Anomaly	κ Value		Sensitivity		Specificity		(+) Predictive Value		(-) Predictive Value		Cases†
		(95% CI)	*%*	*(95% CI)*	*%*	*(95% CI)*	*%*	*(95% CI)*	*%*	*(95% CI)*	*n*
Right complete cleft alveolus	0.88	(0.80-0.96)	87.0	(73.7-95.1)	98.4	(95.4-99.7)	93.0	(80.9-98.5)	96.9	(93.3-98.8)	234
Left incomplete cleft alveolus	0.72	(0.60-0.84)	69.0	(52.9-82.4)	97.4	(94.0-99.1)	85.3	(68.9-95.0)	93.5	(89.1-96.5)	234
Right incomplete cleft alveolus	0.64	(0.45-0.84)	57.9	(33.5-79.7)	98.6	(96.0-99.7)	78.6	(49.2-95.3)	96.4	(93.0-98.4)	234
Secondary palate§											
Left/right complete cleft hard palate	0.45	(0.28-0.61)	78.9	(54.4-93.9)	88.0	(82.8-92.1)	37.5	(22.7-54.2)	97.9	(94.6-99.4)	228
Median complete cleft hard palate	0.49	(0.36-0.62)	57.4	(44.1-70.0)	89.2	(83.4-93.4)	66.0	(51.7-78.5)	85.1	(78.9-90.0)	228

* Abbreviation: 95% CI = 95% confidence interval.

† Number of cases that had sufficient information to record the topographic-anatomical structure, morphology and/or side of the individual anomalies.

‡ This group had a distance of >50% between the upper and lower limit of the 95% CI for the sensitivity but was not grouped further, because other embryologically related anomalies do not exist.

§ The side of the soft palate was not analyzed because clefts of the soft palate always develop in the median.

incomplete/submucous cleft lip. Meanwhile, specificity and negative predictive values were high for all eight anomalies (more than 91%).

For the secondary palate, two anomalies remained for validation. Left/right complete cleft hard palate had a κ value of 0.45 (moderate agreement), sensitivity of 78.9%, positive predictive value of 37.5%, and specificity and negative predictive value of 88.0% and more. The anomaly median complete cleft hard palate had a κ value of 0.49 (moderate agreement), sensitivity of 57.4%, positive predictive value of 66.0%, and specificity and negative predictive value of more than 85%.

DISCUSSION

This continuation of the NVSCA validation study shows that the quality of the NVSCA data on the specific features of the individual anomalies in OCs varies by type of anomaly. By linking the NVSCA database with a new independent reregister database derived from medical data review, we found that validity of the registry data is related to anatomical location and morphological severity of the individual anomalies.

The following results illustrate the pattern of recording in the NVSCA. The topographic-anatomical structures of the individual anomalies of the primary palate (lip and alveolus) and of the secondary palate (hard and soft palates) were identified perfectly in the NVSCA and had high validity measures (85% to 99%) with near-perfect inter-database agreement. However, when analyzing the anomalies more in detail (i.e., analyzing the morphology and/or side) the validity decreased and appeared to be related to the type of anomaly. First, anomalies of the primary palate were recorded better than anomalies of the secondary palate; the inter-database agreement was near-perfect for most primary palate anomalies, whereas, it was moderate to substantial for most secondary palate anomalies. This suggests better registration of externally visible anomalies (such as cleft lip/alveolus) than anomalies that require a diagnostic procedure (such as opening the mouth for inspection and palpating the palate). In addition, validity was related to morphological severity, given that severe anomalies were generally recorded better than mild anomalies. This association applied to both the primary and secondary palates, but was most evident for the secondary palate. For example, 35% of the incomplete cleft hard palates and 69% of the submucous cleft hard/soft palates present in the reregister were also present in the NVSCA, compared with more than 91% of the complete cleft hard palates and complete/incomplete cleft soft palates.

Although many registries record OCs, studies on the validity of OC data are scarce. There are some studies, however, that describe the case-ascertainment of OCs in medical registries.[9, 11, 12, 22] In one study, that of Kubon et al.,[9] this was done in relation to the various cleft types within the three main OC categories. Similar to our study, they found that registration in the Norwegian medical birth registry was more complete for clefts of the primary palate than for

clefts of the secondary palate. They suggested that this could be explained by the delayed diagnoses of clefts of the hard/soft palate and thus incomplete routine examination of newborns, which was also reported in other studies.[22-24] Different from registries that receive information from birth admissions or hospital discharge records, the NVSCA receives the OC data directly from the cleft palate teams, which are expected to be focused on OCs and to examine patients carefully.[15] Still, part of our findings may be explained by incomplete examination, because the number of patients—and probably the experience and routine of diagnostics—varies strongly among the 15 Dutch cleft palate teams.

Delayed diagnosis of cleft palate might have several clinical implications. For example, the presence of a cleft palate is often associated with additional congenital anomalies and syndromes.[6] and the diagnosis of a cleft palate should therefore generate an even more extensive examination of the newborn.

Additionally, our findings that the quality of recording increased with the morphological severity of the anomalies and that this association was most evident for the secondary palate are also consistent with the findings of Kubon et al.[9] Perhaps more unexpectedly, both studies showed that besides morphologically mild clefts of the secondary palate, those of the primary palate, which are clearly visible and require surgery, also tended to be underreported. A possible explanation for these findings is that greater morphological severity of an anomaly might be a factor that encourages doctors to report better.

The under-representation of morphologically mild anomalies may have consequences for research on registry data. These anomalies develop during stages in embryological development and can be related to cell-biological mechanisms and genes other than morphologically severe anomalies.[1-3] Consequently, studies based on registry data examining environmental factors or genes that are associated with morphologically mild clefts might underestimate the importance of such factors and genes.

The strength of this study is that all cleft palate teams gave permission to collect the medical data. The sampling frame thus had a national distribution, including cleft palate teams of large urban teaching and specialist hospitals as well as of small regional ones. Most of these treatment centers have carried out high-quality documentation needed for modern multidisciplinary treatment, which favors our retrospective detailed evaluation. However, the use of medical data to validate registry data also has its limitation. It can never be equal to the presentation of the patient in the outpatient clinical setting, and therefore it is never 100% accurate.[17, 21] As we showed previously.[15] the amount and quality of the medical data varied by cleft palate team. For some cases, the collected medical information was insufficient to evaluate certain specific features of the individual anomalies, and therefore these cases had to be excluded from the features' analysis in this study.

Another limitation is that, although we grouped anomalies having a sample prevalence of $n < 10$ together with their embryologically related anomalies, there were still considerable differences in the prevalence rates of the evaluated anomalies; morphologically mild anomalies

were, for example, less prevalent in the study sample than morphologically severe anomalies. Because it is known that disease prevalence can affect the reliability (kappa) or validity statistics (sensitivity, specificity, and positive and negative predictive values)[16-18] we used to measure the degree of agreement between the NVSCA and reregister, the differences in validity of registry data on morphologically severe and mild anomalies might partially be explained by the differences in prevalence.

Finally, the study sample was not large enough to examine all anomalies of the primary and secondary palates individually. Nevertheless, we were able to analyze most of the individual anomalies in OCs recorded over a 7-year period, thereby evaluating the feasibility of the unique descriptive NVSCA recording system for OCs.

CONCLUSIONS

Our study is the first that validates descriptive registry data on OCs. The unique NVSCA system records the individual anomalies of the primary palate and secondary palate that form the OC by describing the specific features (topographic-anatomical structure, morphology, and side) of each anomaly. This study shows that the quality of the NVSCA data on the specific features of the individual anomalies in OCs varies by type of anomaly and is related to anatomical location and morphological severity. Greater morphological severity of an anomaly might be a factor that encourages doctors to report better, but underreporting might also partly be explained by incomplete examination of the oral cleft. These factors might have implications for genetic and etiologic research based on registry data, for example, and for guidelines on neonatal examination by the cleft palate teams.

Despite the limitations and challenges described, this study shows together with other quality studies[14, 15] that, overall, the data quality of the NVSCA registry on OCs is high, supporting the feasibility of the unique NVSCA recording system. However, data on morphologically severe clefts can be interpreted with higher confidence than those on morphologically mild clefts. In contrast to ICD-based registries, the NVSCA registry has valid detailed OC data that are collapsible to more general diagnoses or codes, which allows classifying OCs in many different ways. This makes the NVSCA registry a very valuable tool for epidemiological, clinical, and fundamental research and for the improvement of OC care.

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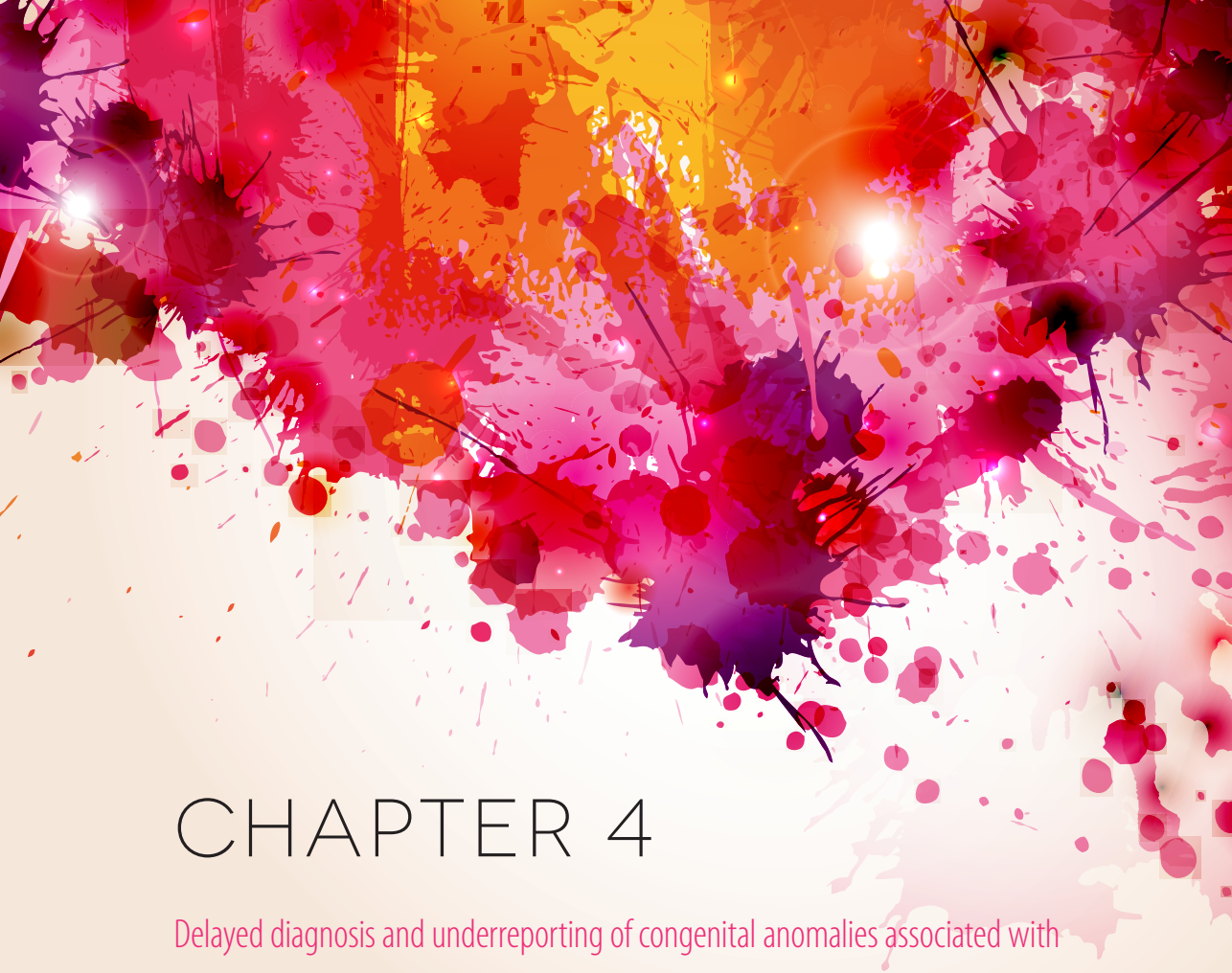

CHAPTER 4

Delayed diagnosis and underreporting of congenital anomalies associated with oral clefts in the Netherlands: a national validation study

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ABSTRACT

Objective: Since 1997, the 15 Dutch cleft palate teams have reported their patients with oral clefts to the national oral cleft registry (NVSCA). During the first visit of the patient to the team—which is usually within the first year of life—the oral cleft and associated congenital anomalies are recorded through a unique recording form by a plastic surgeon/orthodontist/pediatrician. In this study, we evaluated the quality of data on congenital anomalies associated with clefts.

Methods: We drew a random sample of 250 cases registered in the national database with oral clefts from 1997 through 2003; of these, 13 were excluded. Using two independent reregisters derived from two-phased medical data review, we analyzed whether associated anomalies were correctly diagnosed and recorded.

Results: The agreement on associated anomalies between the NVSCA and medical data ranged from moderate to poor (kappa 0.59 to 0). Seventy-seven percent of the craniofacial anomalies were underreported in the NVSCA: 30% due to delayed diagnosis and 47% due to deficient recording. Additionally, 80% of the associated anomalies of other organ systems were underreported: 52% due to delayed diagnosis and 28% due to deficient recording. The reporting of final diagnoses was somewhat better; however, 54% were still underreported (24% delayed diagnosis and 30% deficient recording). The rate of overreporting was 1.6% or lower.

Conclusion: Congenital anomalies associated with clefts are underreported in the NVSCA because they are under diagnosed and deficiently recorded during the first consultations with the cleft palate teams. Our results emphasize the need of routine and thorough examination of patients with clefts. Team members should be more focused on co-occurring anomalies, and early genetic counseling seems warranted in most cases. Additionally, our findings underline the need for postnatal follow-up and ongoing registration of associated anomalies; reregistration in the NVSCA at a later age is recommended.

INTRODUCTION

Oral clefts—one of the most common birth defects in humans—range from mild types to complete clefts affecting the lip/alveolus/palate. Although many genetic and environmental factors (e.g., smoking and nutrition) have been found to contribute to their development,[1, 2] the etiopathogenesis of oral clefts is still poorly understood. Oral clefts are frequently associated with other congenital anomalies, often as part of a syndrome or chromosomal defect. However, the proportion of individuals with additional anomalies varies greatly between studies (3% to 63%) and appears to be related to time of registration and how data have been collected.[3] It has also not conclusively been established whether oral clefts are related to specific types of associated anomalies and there are differences among reports concerning which organ systems are most commonly affected.[4, 5] Given that children with oral clefts associated with other congenital anomalies have much higher morbidity and mortality throughout life than do individuals with isolated clefts,[6-8] early and sound diagnosis of co-occurring anomalies is of paramount importance. Furthermore, complete and accurate data on oral clefts and their associated anomalies are needed to facilitate further genetic and etiopathological studies and prevention of clefts.[1]

Since 1997, the 15 multidisciplinary cleft palate teams in the Netherlands have reported their new presurgery patients with clefts to the national oral cleft registry, which is maintained by the Dutch Association for Cleft Palate and Craniofacial Anomalies (NVSCA). These teams treat virtually all surviving children with clefts who reside in the Netherlands. Using a unique detailed recording form based on the embryology of the head and neck area, oral clefts and their associated congenital anomalies are recorded.[9, 10] Depending on the team, the form is completed by a plastic surgeon (9 teams), orthodontist (3 teams), or pediatrician (3 teams) during the first visit of the patient to the team, which is usually within the first year of life.

As the main purposes of the NVSCA are to optimize diagnostics, treatment, and prevention of oral clefts and to provide a solid basis for clinical, epidemiological, and fundamental research, it is crucial that data provided by this registry are of high quality. Early and sound diagnosis and complete reporting of oral clefts and their associated anomalies are essential to maintain high standards of cleft care and data quality. Previously, it was shown that oral clefts—especially those types that are readily diagnosed at birth—are recorded completely and accurately in the NVSCA.[10-12] It is not known, however, whether associated anomalies are also correctly recorded by the 15 cleft palate teams. Because not all associated anomalies are detectable at birth or in the neonatal period,[3, 13] these anomalies might be underreported due to delayed diagnosis. Another factor that might cause underreporting of associated anomalies is deficient (incomplete/incorrect) recording by the consulting physicians.[14]

In this paper, the last of three articles validating the NVSCA registry,[10, 11] we evaluated the quality of data on congenital anomalies associated with clefts by validating I) additional anomalies of the head and neck area, II) additional anomalies of other organ systems, and

III) final diagnoses (including syndromes and chromosomal defects). Using two independent reregisters derived from two-phased medical data review, we investigated whether these anomalies were diagnosed and recorded correctly during the first consultations with the Dutch cleft palate teams.

MATERIAL AND METHODS

NVSCA registry

The methodology by which the NVSCA registry was established is described in detail elsewhere[9-11] and is summarized here. The NVSCA is an anonymous registry. All Dutch cleft palate teams report their new patients—before they have an oral cleft operation—through a standard NVSCA form; a manual is available.[9, 10] The recording form is composed of three parts. The first is a general section for infant/parental characteristics. The second part consists of a two-dimensional table based on the embryology of the head and neck area, in which oral clefts and any associated craniofacial anomaly (e.g., mandibular hypoplasia or congenital ear anomalies) can be recorded in detail. As shown in Figure 1, the X-axis depicts the topographic-anatomical structures, the Y-axis the morphologic features, and the checking boxes represent the side. The last (third) part gives space for verbatim descriptions of both major and minor congenital anomalies of other parts of the body and final diagnoses, including syndromes and chromosomal defects.

Note that the NVSCA does not have active follow-up of patients and that no data from other sources are included. To optimize data quality, recorded data are verified on a case-by-case basis and the cleft palate teams perform case-ascertainment activities annually.

Subjects

This quality study was initiated and carried out in the 15 Dutch cleft palate teams; all gave written permission for review of patients' medical data. During 1997-2003, the NVSCA database included 2553 patients with oral clefts with or without associated anomalies. Median cleft lip/alveolus and atypical facial clefts were excluded due to their different pathogenesis.[15, 16] From this database, we selected a study population of 250 cases using a standard random-sampling technique in SPSS version 17.0 (SPSS Inc., Chicago, IL).

Data collection and verification

To validate NVSCA data on associated anomalies, we used medical information provided by the cleft palate teams. The methods of medical data collection and verification have been described in detail elsewhere.[10] Medical records with or without color photographs, panoramic radiographs, and dental casts were obtained for 241 of the 250 cases (96%); nine cases were untraceable (medical data were missing in different hospitals, reasons unknown due to

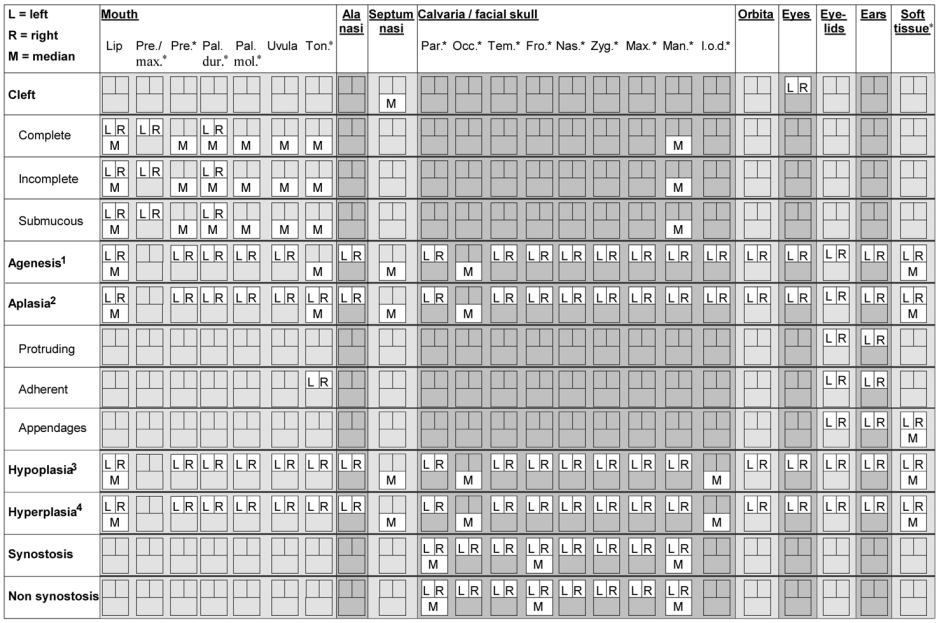

Figure 1. Section of the NVSCA recording form in which craniofacial congenital anomalies, including oral clefts, can be described in detail. The X-axis depicts the topographic-anatomical structures (mouth, ala nasi, septum nasi, calvaria/facial skull, orbita, eyes, eyelids, ears, and soft tissue), the Y-axis shows the morphology (cleft, agenesis, aplasia, hypoplasia, hyperplasia, synostosis, and non synostosis), and the checking boxes represent the side (left, right, and median).
[1] = absent; [2] = present, wrong shape; [3] = right shape, too small; [4] = right shape, too large.
* Pre./Max. = premaxilla – maxilla; Pre. = premaxilla; Pal.dur. = palatum durum; Pal.mol. = palatum molle; Ton. = tongue; Par. = os parietale; Occ. = os occipitale; Temp. = os temporale; Fro. = os frontale; Nas. = os nasale; Zyg. = zygoma; Max. = maxilla; Mand. = mandible; I.o.d. = interorbital distance; Soft tissue = soft tissue of the head and neck area.

confidentiality constraints). We also excluded one case that had insufficient medical data and three cases that had been operated before registration. This resulted in a total of 237 cases that remained in the study.

The obtained medical data were reviewed in two steps by a single investigator (AMR). Firstly, the investigator reviewed only medical data created before and during the first visit of the patients to the teams in order to identify the associated anomalies that were diagnosed before and during those first consultations. The median age at referral was 0.6 months [interquartile range 0.3-1.8 months]. For each case, a standard NVSCA form[10] was blindly completed, thereby creating reregister 1. Secondly, the investigator analyzed all medical data to identify the true types and frequencies of associated anomalies. The median period of follow-up was 5 years [interquartile range 3-7 years]. Again, an NVSCA form was blindly completed for each case, thereby creating reregister 2. The criteria used to define the type of anomaly were established in accordance with existing literature[15-18] and the NVSCA recording manual.[10] Note that any

associated congenital anomaly was recorded, including minimal/minor defects (e.g., epicanthic folds or fetal pads), as they may be recognizable components of specific syndromes or chromosomal defects.[3, 17-19] All recorded data were then transferred to two independent databases, which were subsequently checked for nonexistent, inappropriate, and invalid data.

Statistical analysis

To get complete insight into the quality of NVSCA data, validation was performed in two steps. First, we compared the NVSCA with reregister 2 (based on all medical data) to analyze whether associated anomalies were recorded completely and accurately in the NVSCA. For both databases, we calculated the prevalence of associated anomalies of the head and neck area, associated anomalies of other organ systems, and final diagnoses. Prevalence data were presented as numbers and percentages, and prevalence comparisons were performed using the chi-square test. All *p* values of 0.05 were considered statistically significant.

Although comparison of prevalence rates indicates the extent to which the two databases detected the anomalies, it does not indicate whether they have identified the same cases and whether the NVSCA accurately reproduced what was recorded in reregister 2. Therefore, the concordance of individual case data was also calculated. We calculated kappa statistics to determine the extent to which the two databases identified the same cases (i.e., inter-database agreement). Kappa describes the agreement beyond chance and avoids the assertion that reregister 2 has to be considered as a reference standard.[20] To analyze whether the NVSCA accurately reproduced what was recorded in reregister 2, we calculated rates of sensitivity and specificity, assuming reregister 2 (i.e., the best available reflection of the cases' conditions) to be the criterion standard. Sensitivity ("true positives") was defined as the proportion of cases with a given anomaly according to reregister 2 that were correctly identified as having the anomaly by the NVSCA. Specificity ("true negatives") was defined as the proportion of cases with no anomaly according to reregister 2 that were correctly identified as having no anomaly by the NVSCA. Note that the proportions underreporting and overreporting correspond to 1–sensitivity and 1–specificity, respectively.

During the second step of validation, we compared reregister 1 (based on medical data created before and during the first consultations with the teams) with reregister 2 to analyze whether anomalies were underreported due to delayed diagnosis or due to deficient recording. Underreporting due to delayed diagnosis was defined as the proportion of cases with a given anomaly according to reregister 2 that were not identified as having the anomaly by reregister 1 as well as by the NVSCA. Underreporting due to deficient recording was defined as the proportion of cases with a given anomaly according to reregister 2 that were identified as having the anomaly by reregister 1, but not by the NVSCA. Statistics were performed using SPSS version 17.0 and Stata version 10.0 (StataCorp L.P., Texas, USA).

RESULTS

Prevalence and type of associated anomalies

Table 1 shows the prevalence and type of associated anomalies in cases with oral clefts for the NVSCA and reregister 2. The latter showed that 61% of the 237 cases had one or more associated anomalies, while the NVSCA showed a significant lower prevalence (19%, $p = 0.000$).

For 47% of the 237 cases, reregister 2 showed one or more associated craniofacial anomalies. Defects of the facial skull (hypoplastic mandible or maxilla), eyelids (e.g., epicanthic folds or upslanting /downslanting palpebral fissures), and ears (e.g., misshapen or low set/rotated ears) were most frequently reported, while the NVSCA showed a significantly lower prevalence for all craniofacial structures, except for the interorbital distance.

For 39% of the 237 cases, reregister 2 showed one or more associated anomalies of other organ systems. Anomalies of the central nervous system were most frequently recorded, followed by anomalies of the skin, upper limbs, and lower limbs. The NVSCA, however, showed a significantly lower prevalence (8.4%, $p = 0.000$). Anomalies that were less frequently reported in the NVSCA included anomalies of the respiratory system, urogenital system, central nervous system, body wall, skin, upper limbs, and lower limbs.

Table 1. Prevalence of associated congenital anomalies in cases with oral clefts (n = 237)[a]

	NVSCA Data		Medical Data		*p* Value[b]
Associated anomaly	46	(19%)[c]	144	(61%)[c]	**0.000**
Head and Neck	28	(12%)[c]	112	(47%)[c]	**0.000**
Mouth	2	(0.8%)	9	(3.8%)	**0.033**
Tongue anomalies	1		8		
Miscellaneous	1		1		
Septum nasi	0	(0%)	7	(3.0%)	**0.008**
Hypoplasia	0		7		
Calvaria	0	(0%)	18	(7.6%)	**0.000**
Aplasia	0		3		
Hypoplasia (microcephaly)	0		3		
Craniosynostosis/non synostosis[d]	0		9		
Miscellaneous	0		3		
Facial skull	15	(6.3%)	54	(23%)[c]	**0.000**
Hypoplastic maxilla	0		9		
Hypoplastic mandible	15		46		
Interorbital distance	2	(0.8%)	5	(2.1%)	0.253
Hyperplasia	0		4		
Miscellaneous	2		1		

Table 1. *(Continued)*

	NVSCA Data		Medical Data		*p* Value[b]
Eyes	0	(0%)	9	(3.8%)[c]	**0.002**
Coloboma	0		3		
Miscellaneous	0		9		
Eyelids	3	(1.3%)[c]	52	(22%)[c]	**0.000**
Aplasia	4		48		
Hypoplasia	0		6		
Miscellaneous	0		1		
Ears	7	(3.0%)[c]	43	(18%)[c]	**0.000**
Aplasia	9		32		
Miscellaneous	2		27		
Soft tissue	0	(0.0%)	6	(2.5%)	**0.014**
Aplasia	0		6		
Other craniofacial anomalies[e]	2	(0.8%)	13	(5.5%)	**0.004**
Other organ systems	20	(8.4%)[c]	92	(39%)[c]	**0.000**
Circulatory system	9	(3.8%)[c]	19	(8.0%)[c]	0.051
Atrial or ventricular septum defects	6		17		
Cardiac valve anomalies	1		4		
Persistent ductus arteriosus	0		4		
Vessel anomalies	3		8		
Miscellaneous	1		2		
Respiratory system	1	(0.4%)	7	(3.0%)	**0.032**
Infant Respiratory Distress Syndrome	1		3		
Miscellaneous	0		4		
Digestive system	2	(0.8%)	7	(3.0%)	0.092
Pyloric stenosis	0		3		
Miscellaneous	2		4		
Urogenital system	3	(1.3%)	19	(8.0%)[c]	**0.000**
Renal hypoplasia	1		4		
Undescendent or retractile testis	1		4		
Hypospadia	1		5		
Miscellaneous	0		15		
Central nervous system	3	(1.3%)	32	(14%)[c]	**0.000**
Ventriculomegaly	0		4		
Epilepsy	0		7		
Mental or psychomotor retardation	0		20		
Hypotonia or hypertonia	0		14		
Miscellaneous	4		14		
Vertebral column	4	(1.7%)	10	(4.2%)[c]	0.104
Sacral dimple and/or spina bifida occulta	3		5		

Table 1. *(Continued)*

	NVSCA Data		Medical Data		*p* Value[b]
Miscellaneous	1		6		
Body wall	2	(0.8%)	12	(5.1%)[c]	**0.007**
Chest wall anomalies	1		4		
Inguinal or umbilical hernias	1		5		
Miscellaneous	0		4		
Skin	2	(0.8%)	23	(9.7%)[c]	**0.000**
Hemangiomas and vascular malformations	1		12		
Hypopigmentation or depigmentation	0		3		
Café au lait spots	0		3		
Hypertrichosis	0		3		
Miscellaneous	1		4		
Upper limb	3	(1.3%)[c]	22	(9.3%)[c]	**0.000**
Hypoplasia or agenesis	3		4		
Clinodactyly	0		4		
Abnormal palmar crease	0		10		
Miscellaneous	1		9		
Lower limb	3	(1.3%)	24	(10%)[c]	**0.000**
Hip dysplasia	0		5		
Clubfeet	1		6		
Syndactyly	0		7		
Miscellaneous	2		9		
Final diagnosis	21	(8.9%)	46	(19%)[c]	**0.001**
Chromosomal defect	6	(2.5%)	14	(5.9%)	0.068
Deletion 4p (Wolf Hirschhorn)	1		2		
Deletion 4q	1		1		
Partial trisomy 8	0		1		
Trisomy 13	0		1		
Trisomy 14	0		1		
Ring chromosome 18	0		1		
Trisomy 21 (Down)	1		1		
Deletion 22q11 (DiGeorge / VCF / Shprintzen)	1		1		
46,XX, t(2;4)	0		1		
46,XY,der(6)t(2;6)	0		1		
46,XX,add(14)(q?)	0		1		
46,X,t(X;15)	0		1		
46,XY,der(18)t(16;18)	1		1		
Nonchromosomal syndrome	15	(6.3%)	31	(13%)	**0.013**
Amniotic band	0		1		
Branchio-oculo-facial	1		1		

Table 1. *(Continued)*

	NVSCA Data		Medical Data		*p* Value[b]
CHARGE	1		1		
Ectrodactyly Cleft Palate	1		1		
Hay-Wells / AEC	0		1		
Pierre Robin sequence	11		24		
Van der Woude	1		1		
Waardenburg	0		1		
Other diagnosis	0	(0.0%)	2	(0.8%)	0.156
Neonatal Abstinence Syndrome	0		1		
Neurofibromatosis type I	0		1		

[a] Agenesis = absent; aplasia = present, wrong shape; hypoplasia = right shape, too small; and hyperplasia = right shape, too large. Morphological features are based on the terminology used in the recording system of the NVSCA (Dutch Association for Cleft Palate and Craniofacial Anomalies).[9-11]

[b] *p* value presents statistical significance level in prevalence of associated anomaly between NVSCA data and medical data; $p < 0.05$ is used to determine statistical significance and is presented in bold format.

[c] A patient can have more than one associated anomaly.

[d] This group included one case with a trigonocephaly associated with a chromosomal defect and one case with a metopic ridge associated with the Hay-Wells/AEC syndrome. The other seven patients showed a skull shape comparable with craniosynostosis but with open sutures (i.e., non synostosis).

[e] These anomalies included anomalies of the ala nasi, orbita, and neck.

Finally, 19% of the 237 cases had a chromosomal defect, non-chromosomal syndrome or other final diagnosis according to reregister 2. The prevalence of these final diagnoses was significantly lower in the NVSCA (8.9%, $p = 0.001$).

Agreement and under/overreporting of associated anomalies

The degree of agreement between the NVSCA and reregister 2 on associated anomalies is presented by craniofacial structure, organ system, and final diagnosis in Table 2.

By comparing reregister 1 with reregister 2, we analyzed whether anomalies were underreported in the NVSCA due to delayed diagnosis or due to deficient recording. Reregister 2 showed 112 cases with associated anomalies of the head and neck area. Of these cases, 23% (n = 26) were recorded in the NVSCA as having one or more craniofacial anomalies, and 77% were underreported: 30% (n = 33) due to delayed diagnosis and 47% (n = 53) due to deficient recording. Of the 92 cases with associated anomalies of other organ systems, 20% (n = 18) were recorded in the NVSCA as having one or more anomalies, and 80% were underreported: 52% (n = 48) due to delayed diagnosis and 28% (n = 26) due to deficient recording. Finally, 46 cases had a final diagnosis according to reregister 2. Of these cases, 46% (n = 21) were recorded correctly in the NVSCA, and 54% were underreported: 24% (n = 11) due to delayed diagnosis and 30% (n = 14) due to deficient recording. Figure 2 shows the quality of reporting by craniofacial structure, organ system, and final diagnosis.

Table 2. Agreement between the NVSCA and medical data (criterion standard) on congenital anomalies associated with oral clefts (n = 237)[a]

Congenital Anomaly	Observed Agreement	Kappa		Underreporting (1-Sensitivity)	Over-reporting (1-Specificity)
	%		*95% CI*	%	%
Head and Neck[b]	63	0.23	(0.14-0.31)	77	1.6
Mouth	96	0.17	(–0.13-0.47)	89	0.4
Septum nasi	97	0		100	0
Calvaria	92	0		100	0
Facial skull	84	0.37	(0.23-0.51)	72	0
Interorbital distance	98	0.28	(–0.16-0.72)	80	0.4
Eyes	96	0		100	0
Eyelids	78	0.05	(–0.03-0.13)	96	0.5
Ears	84	0.20	(0.06-0.34)	86	0.5
Soft tissue	97	0		100	0
Other craniofacial anomalies[c]	95	0.12	(–0.11-0.35)	92	0.4
Other organ systems[b]	68	0.21	(0.12-0.31)	80	1.4
Circulatory system	94	0.47	(0.24-0.70)	63	0.9
Respiratory system	97	0.21	(–0.15-0.57)	86	0.4
Digestive system	98	0.44	(0.03-0.84)	71	0
Urogenital system	93	0.26	(0.02-0.49)	84	0
Central nervous system	88	0.15	(0.00-0.31)	91	0
Vertebral column	97	0.56	(0.25-0.87)	60	0
Body wall	96	0.28	(–0.03-0.58)	83	0
Skin	90	0.01	(0.01-0.02)	100	0.5
Upper limb	92	0.22	(0.01-0.43)	86	0
Lower limb	91	0.20	(0.01-0.40)	88	0
Final diagnosis	89	0.58	(0.43-0.72)	54	0
Chromosomal defect	97	0.59	(0.33-0.84)	57	0
Nonchromosomal syndrome	92	0.57	(0.40-0.75)	55	0.5
Other diagnosis	99	0		100	0

[a] NVSCA = Dutch Association for Cleft Palate and Craniofacial Anomalies; 95% CI = 95% confidence interval. A patient may contribute to more than one difference between the databases.

[b] A structure or system can have one ore more associated congenital anomalies.

[c] These anomalies included defects of the ala nasi, orbita, and neck.

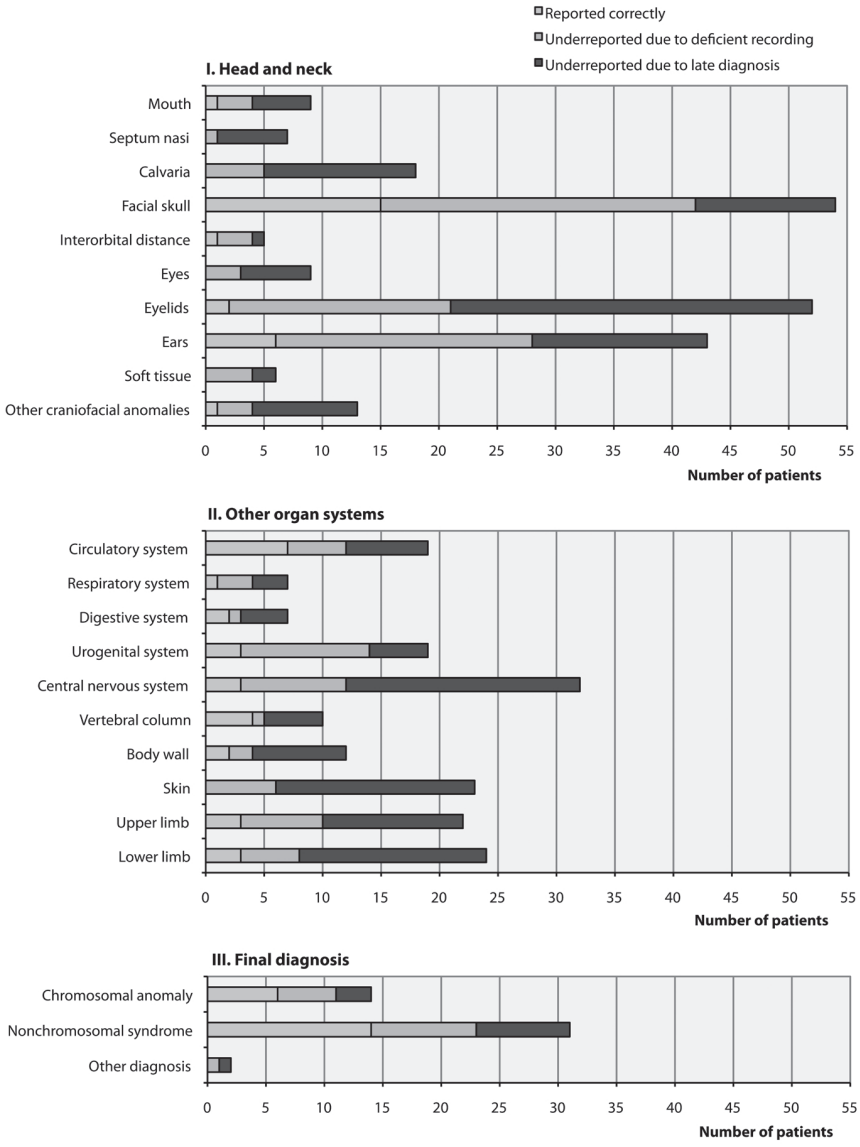

Figure 2. Distribution of cleft patients with associated anomalies (n = 144) according to quality of reporting of I) craniofacial structure, II) other organ systems, and III) final diagnoses. Note that a patient can have more than one associated anomaly.

DISCUSSION

This validation study showed that the quality of NVSCA data on congenital anomalies associated with oral clefts is moderate to poor and varies by type of anomaly. Associated anomalies are underreported because they are not diagnosed during the first consultations with the cleft palate teams or because they are deficiently recorded by the consulting physicians.

Using the classification system developed by Landis and Koch,[21] our kappa values showed moderate to poor agreement between the NVSCA and reregister 2. Seventy-seven percent of the defects in cases with associated craniofacial anomalies were underreported in the NVSCA (30% delayed diagnosis and 47% deficient recording). Additionally, 80% of the defects in cases with associated anomalies of other organ systems were underreported in the NVSCA (52% delayed diagnosis and 28% deficient recording). The reporting of final diagnoses was somewhat better; however, 54% were still underreported in the NVSCA (24% delayed diagnosis and 30% deficient recording). The rate of overreporting appeared to be negligible (just 1.6% or lower).

Strengths and weaknesses

The main strength of this study is that its sampling frame had a national distribution; all Dutch cleft palate teams participated, including teams of large urban teaching and specialist hospitals as well as of small regional ones. Another strength is its focus on detailed dysmorphology and syndromology. Besides major anomalies, we also evaluated minimal/minor defects, such as epicanthic folds and ear pits. In and of themselves, these anomalies do not cause increased morbidity. However, as they may be recognizable components of specific syndromes or chromosomal defects, characterization of both major and minor anomalies is essential to help arrive at correct diagnosis and improve clinical care and outcome of the patient.[3]

Additionally, the postnatal follow-up of our study allowed us to include anomalies detected later in infancy. Consequently, our study showed a relatively high proportion (61%) of cases with associated anomalies compared with other studies (3% to 63%).[3-6, 8] Anomalies of the head and neck area were most frequently diagnosed, followed by anomalies of the central nervous system, skin, upper limbs, and lower limbs. Unfortunately, comparison of our findings with those of others is restricted due to great differences in case definitions, inclusion/exclusion criteria, times of registration, ascertainment methods (active vs. passive), sample sizes, population characteristics, and ever-increasing knowledge of cleft syndromes.[3]

We realize that the postnatal follow-up in our study also had its limitation. Because our cases were born from 1991 (recorded in 1997) through 2003, the follow-up, and thus the chance of detecting anomalies, varied by case. Another limitation of this study is that the medical information used for comparison can never be 100% accurate. Medical data are not equal to the presentation of patients in the outpatient clinical setting, and errors that occur when clinical information is documented cannot be captured.[14] Furthermore, the amount and quality of data varied by cleft palate team.[10] On the other hand, all medical data were abstracted and

subsequently recorded by the same investigator, thereby minimizing the variability in selection of medical information in this study.

Possible explanations underreporting

As has previously been described,[3, 13] early registration might underestimate the true frequency of associated anomalies, especially of those that require specific diagnostic procedures (e.g., chromosomal defects) and those that can only be detected later in infancy (e.g., epilepsy and mental or psychomotor retardation). Therefore, our high rates of delayed diagnosis may partly be explained by the fact that most patients were registered during the first months of life. This is in line with several other studies reporting similar rates of underreporting of congenital anomalies during the neonatal period (37% to 86%),[13, 22-25] while studies evaluating longer periods of follow-up reported considerably lower rates (7% to 21%).[26-29] However, our study also showed that obvious external defects (e.g., craniofacial anomalies and defects of the upper/lower limbs) had also been missed during intake. This may be explained by the fact that cleft patients in the Netherlands are initially seen by plastic surgeons, orthodontists, or pediatricians, who are generally not fully trained in the principles of dysmorphology and syndromology. If patients are referred to a clinical geneticist, this usually takes place at a later stage. On the other hand, part of our findings—especially regarding minimal/minor defects, such as epicanthic folds—could also be explained in terms of greater vigilance in identifying anomalies by the investigator rather than through routine examination by any physician at the outpatient clinic.[30]

We also found that anomalies were deficiently recorded, which may be explained by the following factors. Given that incorrect recording would have caused both underreporting and overreporting in the NVSCA, our low rates of overreporting suggest that data were simply not entered. According to Mackeprang et al.,[22] this incomplete recording of anomalies may be a consequence of the desire to first confirm a diagnosis. Another explanation could be the lack of awareness, focus or willingness among physicians to record completely and accurately.

Implications and recommendations

From a clinical perspective, timely and correct identification of all associated malformations, including major and minor defects, is essential for accurate diagnosis, prognosis, and counseling and to develop policies of healthcare.[3] As a result of advances in ultrasound technology and its routine use in obstetric practice, oral clefts and their associated anomalies (including less obvious internal defects) are being diagnosed prenatally more frequently.[31] However, our study showed that external as well as less obvious internal defects are missed during intake. This emphasizes the need for more thorough evaluation of children with clefts. Team members, including plastic surgeons, orthodontists, and pediatricians, should be more aware of prenatal findings and should focus on postnatal detection of co-occurring congenital anomalies, especially regarding cardiovascular or urogenital anomalies and Pierre Robin sequence. These anomalies are often missed during intake, and early accurate diagnosis of these anomalies

will change treatment policy and thus possibly the outcome of the patient. Additionally, early genetic counseling seems warranted in most cases, particularly to detect anomalies that should be diagnosed as soon as possible given their implications for treatment and prognosis. For example, early diagnosis of deletion 22q11.2 is important given the need to identify hypothyroidism, to check calcium preoperatively as well as postoperatively, and to evaluate the immune system before administering live vaccines. Furthermore, cytomegalovirus-negative irradiated blood products should be used for infant surgeries.[32]

The underrepresentation of associated anomalies in the NVSCA restricts its use for research purposes, such as genetic/etiological studies, since clefts with accompanying defects have epidemiological and etiological features different from isolated clefts.[1, 8] On the other hand, as long as one remains cognizant of the limitations, NVSCA data can still be useful, for example in providing low-end estimates of rates of associated anomalies.[24] To improve data quality, recording physicians should be educated in identifying and recording associated anomalies, and optimally, each cleft patient should be examined by a clinical geneticist or dysmorphologist before registration.[1]

Finally, registration at birth is the most reliable way to capture the complete population of cleft patients. However, our findings highlight the importance of postnatal follow-up and the ongoing accurate reporting of birth defects. According to Van der Veen et al. and Wyszynski et al.,[3, 13] most associated anomalies are diagnosed before 4-6 years of age. Therefore, reregistration after 6 years of age seems adequate to obtain complete and accurate information on associated anomalies in patients with oral clefts.

CONCLUSIONS

This paper, the last of three articles validating the NVSCA registry,[10, 11] showed that congenital anomalies associated with oral clefts are underreported due to delayed diagnosis and deficient recording. It emphasizes the need of early routine and thorough examination of patients with clefts. Timely diagnosis of anomalies like Pierre Robin sequence and cardiovascular or urogenital anomalies will change treatment policy and thus the outcome of the patient. Health professionals involved in the management of oral clefts should therefore be more focused on such anomalies, and a clinical geneticist as well as an obstetric specialist should be included in each multidisciplinary cleft team to maximize the ascertainment of these anomalies.

Our findings underline the need for postnatal follow-up and ongoing reporting of congenital anomalies. Registration at birth is the most reliable way to capture the complete population of cleft patients, but to obtain complete and accurate data on associated anomalies these patients should be reregistered after the age of 4-6 years.

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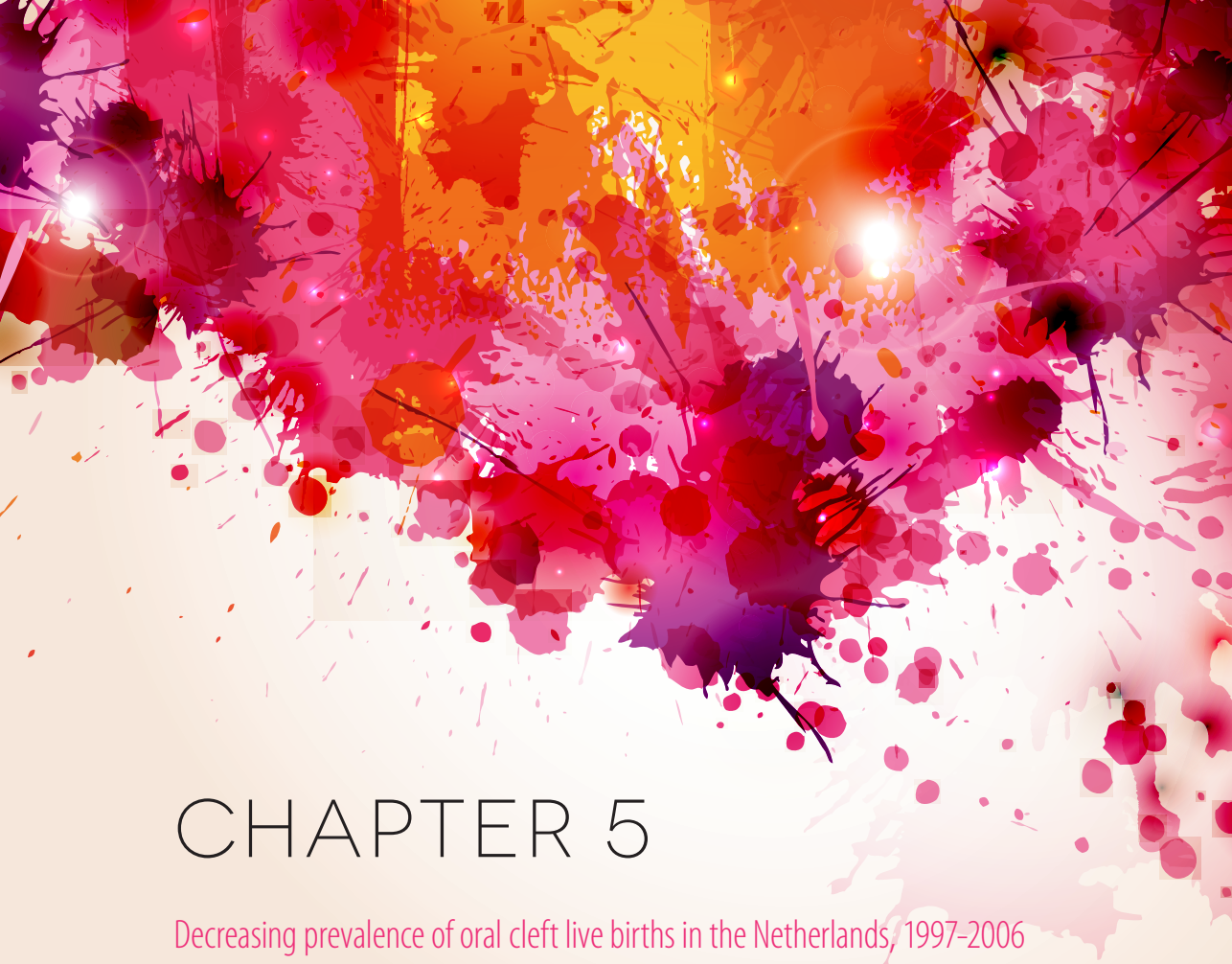
PART II

Prevalence in the Netherlands



CHAPTER 5

Decreasing prevalence of oral cleft live births in the Netherlands, 1997-2006

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ABSTRACT

Objectives: The number of new oral cleft patients has fallen in the Netherlands. This may be explained by two hypotheses: 1) greater prenatal detection of congenital anomalies has led to more pregnancy terminations, and 2) increased folic acid use has reduced the oral cleft risk. Both hypotheses would mainly apply to the category cleft lip/alveolus ± cleft palate (CL±P), since, unlike cleft palate only (CP), CL±P can be detected prenatally by 2D ultrasound and develops during the period recommended for folic acid use. We aimed to determine trends in prevalence over 1997-2006 and to evaluate the hypotheses by stratifying trends by cleft category.

Methods: This study was a time-trend analysis of infants born alive with oral clefts in the Netherlands during 1997-2006 and registered in the national oral cleft registry. We calculated prevalence rates and the estimated annual percentage change (EAPC) for all oral clefts and the two categories.

Results: In 1997-2006, 3308 infants out of 1,970,872 live births had oral clefts, an overall prevalence per 10,000 live births of 16.8 (CL±P: 11.3; CP: 5.5). Time-trend analysis showed that the prevalence of all oral clefts decreased (EAPC –1.8%; 95% CI –3.0% to –0.6%), as did the CL±P prevalence (EAPC –2.3%; 95% CI –3.8% to –0.9%). No significant trends were found for the CP prevalence.

Conclusions: Because the live-birth prevalence of CL±P decreased, that of all oral clefts decreased. These findings are in line with both hypotheses and may therefore have implications for prenatal counseling and folic acid policy.

INTRODUCTION

Oral clefts—one of the most common birth defects in humans—range from mild types to complete clefts affecting the lip/alveolus/palate. They may either be isolated or be associated with other congenital anomalies, syndromes, or chromosomal defects. Although the etiopathogenesis of non-syndromic clefts has been widely studied, it is still poorly understood. Many genetic and environmental factors, such as nutrition and medication, have been suggested to contribute to their development.[1-3]

Since 1997, the fifteen cleft palate teams in the Netherlands have reported their new live-born presurgery patients with oral clefts to the national oral cleft registry, which is maintained by the Dutch Association for Cleft Palate and Craniofacial Anomalies (NVSCA). These teams treat virtually all surviving children with clefts who reside in the Netherlands.[4, 5] The annual reports of this registry show that the number of cleft patients referred to the teams has fallen since 2003.[6] This decline may be explained by two hypotheses. Firstly, the performance of prenatal 2D ultrasound examinations has increased since the 1990s, resulting in greater prenatal detection of congenital anomalies, including oral clefts,[7] which has led to more terminations of affected pregnancies. Secondly, the government-sponsored mass media-campaign in 1995 and the proactive intervention by Dutch pharmacies in 2004 to promote the use of folic acid have increased the periconceptional use of this supplement,[8] thereby reducing the risk of oral clefts. To investigate these hypotheses, oral clefts have to be divided into two categories: cleft lip/alveolus with or without cleft palate (CL±P); and cleft palate only (CP). These categories differ embryologically and epidemiologically,[2] and, unlike CP, the category CL±P can be detected prenatally using 2D ultrasound.[9] This means that if pregnancies were terminated because of the presence of an oral cleft with or without associated anomalies, the CL±P prevalence would have been affected most. Additionally, most types of CL±P develop during weeks 4-7 post-conception,[3, 10] which is during the recommended period for folic acid use (four weeks before conception to eight weeks after it). CP, however, develops during weeks 8-12 postconception,[3, 10] and may therefore be less influenced by a higher use of folic acid during the recommended gestational period in the population.

To investigate whether the prevalence of oral clefts among live births in the Netherlands decreased over the 1997-2006 period, we used data from the NVSCA to establish the rates of oral clefts. By stratifying trends by cleft category, we were able to investigate whether the oral cleft prevalence may have been affected by the greater prenatal detection of CL±P with or without associated anomalies, and/or by the higher periconceptional use of folic acid.

METHODS

The methodology by which the NVSCA registry was established is described elsewhere [4, 11] and is summarized here.

The NVSCA registry includes anonymous presurgery patients with oral clefts (no age limit) reported by the Dutch cleft palate teams. Using a unique recording system, detailed information on the topography and morphology of each anatomical structure of the head and neck area (e.g. lip, alveolus, and hard/soft palate) is recorded. Additionally, general information concerning the infant/parents and diagnoses of associated anomalies are included. Note that the form is completed during the first visit of the patient to the team, which is usually in the immediate postnatal period, and that there is no active follow-up of patients. To optimize data quality, data are verified on a case-by-case basis and the cleft palate teams perform case-ascertainment activities. Furthermore, the registry has been systematically validated.[11]

The study population included all infants born alive in the Netherlands from 1997 to 2006 who had been registered in the NVSCA database with an oral cleft (with or without associated anomalies). We excluded median and atypical facial clefts because of their different pathogenesis.[12, 13]

The descriptive data of the study population (infant/maternal characteristics and information on associated anomalies) were presented as percentages, medians with interquartile range [IQR], or means ± 1 standard deviation (SD).

We performed time-trend analyses to estimate the change in live-birth prevalence of all clefts and of the two categories (CL±P and CP) over the period 1997-2006. The prevalence was determined annually as the number of registered live-born infants with a cleft per 10,000 live births in the Netherlands for the same year. The annual numbers of live births in the Netherlands were retrieved from Statistics Netherlands.[14]

We used Poisson regression, modeling counts against year, to calculate the estimated annual percentage change (EAPC) in prevalence; this method has been previously described by De Vries and colleagues.[15] The EAPC, presented with its 95% confidence interval [95% CI], represents an estimate of the year-to-year change in rate; negative values represent declining rates and positive values represent increasing rates. Statistics were performed using the SPSS v 17.0® software package.

RESULTS

During the study period, 3308 infants were born alive with an oral cleft and were registered in the national database. The descriptive data of the study population are presented in Table 1. The median age at cleft palate team admission was 0.6 months. Most infants were born to Caucasian parents (approximately 88%), after a normal gestational age of 39 weeks, and the mean

Table 1. Characteristics of the study population, the Netherlands 1997-2006.* Values are numbers (percentages) unless stated differently

No of patients:	*3308*	
General information:		
Boys	1935	(58.5)
Median referral age in months [IQR]	0.6	[0.3-1.8]
Caucasian father	2885	(87.2)
Caucasian mother	2930	(88.6)
Consanguinity	74	(2.2)
Mean gestational age in weeks ± SD	39	± 2.2
Mean birth weight in grams ± SD	3299	± 654
Congenital anomalies among relatives	915	(27.7)
Oral clefts among relatives	642	(19.4)
Oral clefts:		
Cleft lip/alveolus with or without cleft palate	2218	(67.0)
Cleft palate only	1090	(33.0)
Associated anomalies:		
Other craniofacial anomalies	352	(10.6)
Anomalies other organ systems	430	(13.0)
Syndrome and/or chromosomal defect	327	(9.9)

* Based on data of the NVSCA registry.

birth weight was 3299 grams. Almost 28% of the infants had a family history of congenital anomalies, about 11% had congenital anomalies of the head and neck area other than clefts, and 13% had accompanying defects of other organ systems. In 10%, the oral cleft was part of a syndrome and/or chromosomal defect.

Between 1997 and 2006, there were 1,970,872 live births in the Netherlands; the average oral cleft prevalence was 16.8 per 10,000 live births. Figure 1 displays the prevalence over time. The annual prevalence of all clefts ranged from 14.5 to 18.6 per 10,000 live births (IQR: 15.8-17.7). Time-trend analysis showed that over the 1997-2006 period, the oral cleft prevalence decreased significantly by –1.8% per year (95% CI: –3.0% to –0.6%).

Infants with CL±P accounted for 67% of the study population (2218 infants), corresponding to an average prevalence of 11.3 per 10,000 live births. The prevalence of CP was 5.5 per 10,000 live births. The annual CL±P prevalence ranged from 9.6 to 12.9 per 10,000 live births (IQR: 10.5-12.0), and the annual CP prevalence ranged from 4.7 to 6.3 per 10,000 live births (IQR: 5.0-6.2). Time-trend analysis for CL±P showed a trend similar to that observed for all clefts; per year, the prevalence decreased by a significant –2.3% (95% CI: –3.8% to –0.9%). By contrast, for CP there was no evidence of a significant trend over time (EAPC –0.8%; 95% CI: –2.9% to +1.2%).

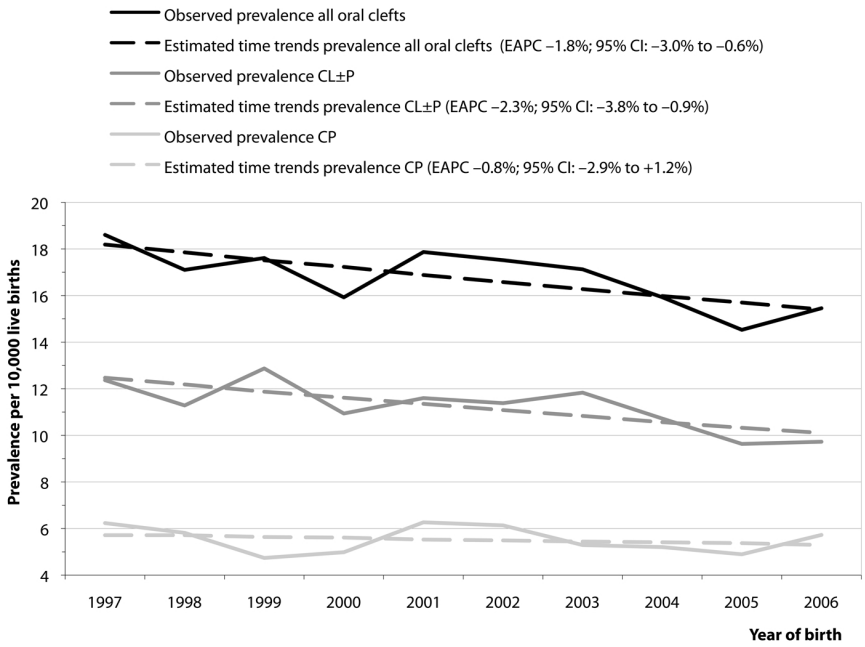

Figure 1. Time trends in prevalence of all oral clefts and the two categories (CL±P and CP) per 10,000 live births in the Netherlands from 1997 to 2006. Time trends estimated by Poison regression with year of birth as independent variable. Estimated Annual Percentage Change (EAPC) calculated by fitting the regression line to the natural logarithm of the prevalence rates.

DISCUSSION

Our study shows that the live-birth prevalence of oral clefts in the Netherlands decreased significantly during 1997-2006. By stratifying this trend by cleft category, we found a trend for CL±P similar to that observed for all clefts, while there was no significant trend for CP. This specific decrease in CL±P supports the hypothesis that the live-birth prevalence of oral clefts was affected by the greater prenatal detection of CL±P (with or without associated anomalies), and/or by the higher periconceptional use of folic acid.

Strengths and weaknesses

While the use of the NVSCA database was the main strength of our study—it allowed us to collect detailed national data on oral clefts among live births—it also had some limitations. The first is that registry databases are known to be prone to underreporting and misclassification. Our findings are nonetheless unlikely to be explained by underreporting or misclassification, because case-ascertainment is performed annually, and extra control activities were performed after the decline in newly recorded patients. Furthermore, quality studies showed that the

NVSCA registry has a high case-ascertainment and contains high quality data for both CL±P and CP.[5, 11]

Another limitation is that the national database records only patients who are treated by the cleft palate teams. Stillbirths are therefore not included, and infants with severe associated anomalies who die during the first weeks of life might also not be captured. A change in perinatal/neonatal mortality could thus have affected our rates. Although we cannot rule out the impact of this factor, we suspect it is of minor importance given that the Dutch perinatal/neonatal mortality decreased during the study period.[14] Besides, a change in these mortalities should have mainly affected the CP prevalence, since further anomalies are more frequently associated with CP than with CL±P.[1]

Finally, the database does not provide complete and reliable data on associated anomalies (Unpublished data, Rozendaal et al. 2010). The NVSCA has no active follow-up of patients, and therefore, associated anomalies detected later in infancy are often not included.[16, 17] For this reason, trends according to whether clefts were isolated or associated with other anomalies could not be given to further support that the increased prenatal detection accounted for the decline in prevalence.

Another source for Dutch oral cleft data is the EUROCAT registry of the Northern Netherlands.[18] However, cleft rates of the Northern Netherlands are significantly higher than those of the Netherlands and can therefore not be used in a national context.[19, 20] Additionally, international registries show large variations in cleft prevalence without consistent time trends [1, 21, 22]; the worldwide prevalence comes to 15.2 per 10,000 births.[21] Unfortunately, comparison of our findings with those of other studies is restricted, due particularly to the great differences between data sources, times of diagnosis, classifications, inclusion/exclusion criteria, time scales, sample sizes, and population characteristics.[1, 16, 23]

Possible explanations

Our first hypothesis—that greater prenatal detection of congenital anomalies, including oral clefts, has led to more terminations of affected pregnancies—is a plausible explanation for the decline in oral clefts among live births in the Netherlands. This hypothesis is supported by our findings and additional national data.[24]

Since 2007, 2D ultrasound screening in the Netherlands has been routinely performed at 18-20 weeks gestation. However, the use of prenatal ultrasounds started to increase as early as the 1990s, and, before 2007, over 90% of pregnant women residing in the Netherlands underwent one or more ultrasound scan.[7] As it did elsewhere,[9, 22, 25, 26] the subsequent rise in prenatally detected anomalies, including oral clefts,[7] may have led more affected pregnancies to be terminated in the Netherlands. The rates reported internationally for termination of pregnancy (TOP) on the basis of an isolated cleft range from 0 to 92%,[26] but TOPs are performed more frequently when the cleft is associated with other anomalies.[9] Unlike CP, the category

CL±P can be detected prenatally using 2D ultrasound,[9, 23] which may explain why we found a significant decreasing trend for CL±P and no trend for CP.

This hypothesis is also supported by national data on pregnancy terminations.[24] In the Netherlands, TOP is allowed only under the provision of the Termination of Pregnancy Act; after 24 weeks gestation it is prohibited.[27] Under the terms of this act, all Dutch hospitals or clinics licensed to perform TOPs are required to report information relevant to these TOPs. Indications are not included. The annual reports submitted under this act show that the number of second-trimester terminations, especially those performed by the hospitals, have increased since 2003. This implies that there has been a rise in the termination of pregnancies affected with congenital anomalies, since these are performed mainly by the hospitals.[24]

Our second hypothesis—that higher periconceptional use of folic acid has reduced the risk of oral clefts in the Netherlands—is also plausible. Several studies have reported a significant protective association between periconceptional folic acid and oral cleft risk,[28-32] but the evidence on the role of this supplement in cleft etiology is still inconclusive.[31, 32] In the Netherlands, women are recommended to take 400µg folic acid/day from four weeks before conception until eight weeks thereafter, and the frequency of expecting mothers correctly using additional folic acid increased gradually over the past decade.[8, 33] Our study shows a gradual decline in CL±P prevalence, but not in CP prevalence, during approximately the same time frame. These findings are consistent with two other studies that investigated the same supplementation period,[28, 30] while countries with compulsory fortification (United States and Canada) showed a decline in both CL±P and CP.[31]

A possible explanation for these findings is that the recommended period for folic acid supplementation does not cover the etiologically relevant time period for CP (8-12 weeks postconception).[3] This theory is supported by the study of Bakker and colleagues,[34] who showed that, after discontinuation of folic acid supplementation, the folate concentration (a general term for this B-vitamin) in serum immediately decreases and the plasma total homocysteine immediately increases. Given that there may be a dose-response relationship between folic acid and oral clefts, and that folate may be indirectly associated with clefts through its effects on homocysteine metabolism,[28, 31, 35] folic acid supplementation until 8 weeks postconception might be too short to prevent CP.

Finally, other environmental or lifestyle factors (e.g. dietary patterns) changing over time may also account for the decrease in cleft prevalence.[1] Specific data on these factors are not available for the oral cleft population, but data based on the general Dutch population show that maternal smoking and alcohol consumption decreased during the study period.[36] Since these factors are suggested to be associated with CL±P and CP risk,[1] they may play contributory roles regarding the decrease in prevalence.

Possible implications

Our findings may have several implications for healthcare and policy makers. Firstly, TOP after prenatal diagnosis of congenital anomalies raises moral and ethical dilemmas, since most of these anomalies are nonlethal (e.g. non-syndromic clefts). If oral clefts are identified prenatally, future parents should be counseled by a multidisciplinary cleft palate team that focuses on psychosocial support, genetic counseling, education on the management of clefts, and parents' options, TOP being one of them.[25, 26, 37, 38] The Netherlands does not yet have a uniform strategy, but is developing an evidence-based guideline to optimize prenatal counseling on oral clefts.

Secondly, if folic acid protects against oral clefts, increases in pregnant women's exposure to folic acid and extension of the recommended period to at least 12 weeks postconception ought to produce further reductions in prevalence. Since folic acid consumption has been increased by food fortification,[31, 33] the possible effect on oral clefts is also relevant to the ongoing discussions about fortification.

Population-based studies including live births and stillbirths could give more insight into the causes of the decrease in oral clefts, especially if prenatally diagnosed cases are included. Since national data on prenatally diagnosed anomalies and indications of TOP are still lacking, uniform registration is needed to evaluate the epidemiological impact of prenatal ultrasound screening. Additionally, to further investigate the possible preventive effect of folic acid, future studies should focus on timing, duration, dose, and intensity of use of folic acid as well as of folic-acid containing supplements.[32] Further studies should also differentiate between the various cleft types within CL±P and CP, since these are related to different time frames and cell biological mechanisms in embryonic development.[3, 10, 12] Therefore, a unique case-control study has been recently started in the Northern Netherlands, based on detailed data regarding the various cleft types and periconceptional folic acid/multivitamins from the NVSCA and Eurocat registries.

ACKNOWLEDGEMENTS

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CHAPTER 6

Regional variation in prevalence of oral cleft live births in the Netherlands 1997-2007: time-trend analysis of data from three Dutch registries

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ABSTRACT

The Eurocat registry Northern Netherlands (NNL) has been used in regional context, as well as in national/international context, to describe the epidemiology of oral clefts (OC). However, the region NNL seems to have prevalence data different from Dutch national registries and certain other European areas. This may be due to differences in registration methods or geographical variation. To investigate whether the prevalence of OC live births varies regionally in the Netherlands, we established time trends for NNL and the rest of the Netherlands over 1997-2007 using data from two national registries (the OC Registry and The Netherlands Perinatal Registry) and a regional registry (Eurocat NNL). We found that the overall live-birth prevalence—comprising cleft lip/alveolus ± cleft palate and cleft palate only—was significantly higher in NNL (15.1-21.4 per 10,000) than in the rest of the Netherlands (13.2-16.1 per 10,000). None of the registries showed significant trends for NNL, whereas both national registries showed that the live-birth prevalence of cleft lip/alveolus ± cleft palate decreased significantly in the rest of the Netherlands. Despite some differences in prevalence between the registries, they showed similar regional variation in prevalence and trends. In conclusion, the prevalence of OC live births varies significantly in the Netherlands, not only between but also within registries. This underlines that extrapolation of regional cleft data should be done with caution. To further investigate OC etiology and evaluate preventive strategies, future studies should consider geographical differences—between and within countries—regarding the various cleft subphenotypes among live births, stillbirths, and pregnancy terminations.

INTRODUCTION

Oral clefts are one of the most common birth defects in humans. Worldwide, the reported prevalence varies from 4.8 to 28.6 per 10,000 live births and stillbirths (with or without termination of pregnancy)[1] with considerable variations associated with gender, ethnicity, socioeconomic status, and geographic region.[2-9] Oral clefts are very complex and heterogeneous birth defects, being microform/submucous, incomplete, or complete clefts affecting the individual structures: lip, alveolus, hard palate, and soft palate including the uvula.[10] Classically, they are subdivided into two categories: cleft lip/alveolus with or without cleft palate (CL±P) and cleft palate only (CP), because of their differences in embryologic development and epidemiology.[8, 11] Oral clefts may either be isolated or be associated with other congenital anomalies, syndromes, or chromosomal defects. Although the etiopathogenesis of non-syndromic oral clefts has been widely studied, it is still poorly understood. Many genetic and environmental factors, such as smoking, alcohol, and nutrition, have been suggested to contribute to their development (OMIM 119530; 119540).[8, 11-14] Registration of oral clefts—including their associated anomalies—and combined epidemiological, fundamental, and clinical approaches may enhance our understanding of the causes and pathogenesis of oral clefts.[3]

Oral clefts in the Netherlands are registered in three registries: 1) National Registry of Common Oral Clefts (NVSCA);[15, 16] 2) The Netherlands Perinatal Registry (LVR/LNR);[4] and 3) Eurocat Northern Netherlands (NNL).[17] Internationally, data of Eurocat NNL have been frequently used to describe the epidemiology of oral clefts in the region NNL,[2, 5, 9, 18] and occasionally, these data have been extrapolated to the rest of the Netherlands or used in a European context.[7, 19] It is doubtful, however, whether the region NNL contains a representative sample of the Dutch oral cleft population, as its oral cleft prevalence and trends seem to differ from those of Dutch national registries.[7, 20, 21] These discrepancies may partly be due to differences in registration methods, such as ascertainment procedures and inclusion/exclusion criteria. Another possible explanation is that the oral cleft prevalence varies within the Netherlands. Since it was found previously that the cleft prevalence in NNL was higher than that in certain other European regions,[2, 5] it might also be higher than that in the rest of the Netherlands. Analysis of regional differences in prevalence and time trends—between as well as within populations—is needed, as they may reflect differences in risk factors, preventive factors, or genetic predisposition.[7, 11]

In the present study, we used data from three Dutch registries (NVSCA, LVR/LNR, and Eurocat NNL) to establish the prevalence and time trends of oral cleft live births in the region NNL and in the rest of the Netherlands from 1997 to 2007, thereby investigating differences between and within registries in the Netherlands.

METHODS

Data sources

The recording methods of the three registries we used have been described elsewhere[4, 10, 15-17, 22] and are summarized here.

The NVSCA registry is an anonymous national registry, which has been maintained by the Dutch Association for Cleft Palate and Craniofacial Anomalies (NVSCA) since 1997. All fifteen multidisciplinary cleft palate teams in the Netherlands report their live-born patients with oral clefts—irrespective of age—before these patients have an oral cleft operation. These teams treat virtually all surviving children with oral clefts who reside in the Netherlands.[15, 23] The NVSCA uses a unique detailed recording system, which is based on the embryology of the head and neck area. All individual anomalies can be described by recording the topography and morphology of each anatomical structure (e.g. lip, alveolus, hard palate, and soft palate including the uvula). These detailed data can be collapsed to more general diagnoses or codes and can be classified in many different ways (e.g., CL±P and CP). Additionally, the NVSCA includes general information on infant and parents, and diagnoses of congenital anomalies of other organ systems. Note that the recording form is completed during the first visit of the patient to the team, which is usually in the postnatal period. No active follow-up of patients takes place. To optimize data quality, data are verified on a case-by-case basis, and the cleft palate teams perform case-ascertainment activities. Furthermore, the NVSCA registry has been systematically validated (Rozendaal et al. submitted).[10, 16]

The LVR/LNR is a linked database of three anonymous national registers, which has been maintained by The Netherlands Perinatal Registry since 1995: the National Register of Midwifes (LVR1), the National Register of Obstetricians (LVR2), and the National Neonatal Register (LNR). The LVR includes information on all pregnancies and births with at least 16 weeks of gestation, reported by midwives and obstetricians until the first week of life. The LNR includes information on all admissions and re-admissions of newborns to pediatric neonatal departments within the first 28 days of life for perinatal problems, reported by pediatricians and neonatologists. Besides a large amount of information on pregnancy, delivery, puerperium and neonatal care, the LVR/LNR contains information on diagnoses of several congenital anomalies. Oral clefts are recorded in the two categories CL±P and CP.[4]

Eurocat NNL is a population-based birth defect registry that covers three provinces: Groningen, Friesland, and Drenthe. This registry is part of a European network of regional and national registers, and a WHO Collaborating Centre for the Epidemiologic Surveillance of Congenital Anomalies.[24] Since 1981, children (until the age of 16 years at notification) and fetuses with congenital anomalies diagnosed before or after birth have been reported voluntarily by midwives, general practitioners, well-baby clinic doctors, specialists, and parents. In addition, various sources, like hospital registries, are searched to find children and pregnancies eligible for registration. There is no lower limit for gestational age, and both spontaneous and induced

abortions are included. Note that parental informed consent is needed for registration. Eurocat includes detailed information on congenital anomalies and risk factors (e.g., medication use). Oral clefts (and other congenital anomalies) are coded according to the "International Classification of Diseases" (ICD 9th and 10th revision) and subsequently classified into the two categories CL±P and CP.[17]

Study population

The population under study included all infants born alive in the Netherlands from 1997 through 2007 who had been registered in the NVSCA, LVR/LNR, or Eurocat with an oral cleft (with or without additional congenital anomalies). We excluded median and atypical facial clefts because of their different pathogenesis.[25, 26]

Data analysis

To analyze population characteristics of the region NNL and the rest of the Netherlands, the NVSCA data on infant/maternal characteristics and additional congenital anomalies were used. These data were presented as percentages, medians with interquartile range [IQR], or means ± 1 standard deviation (SD). Comparisons between the two regions were performed using the Chi-square test for categorical variables, the independent t-test for continuous variables having a normal distribution, and the Mann-Whitney test for continuous variables having a skewed distribution.

We performed time-trend analyses to estimate the change in live-birth prevalence of all oral clefts and of the two cleft categories (CL±P and CP) in the region NNL and in the rest of the Netherlands from 1997 to 2007. To analyze the prevalence in NNL, data from all three registries were used: a) NVSCA data reported by the cleft palate teams in NNL (extracted by team); b) LVR/LNR data of patients born in NNL (extracted by zip code); and c) Eurocat NNL data. The prevalence in the rest of the Netherlands was analyzed using data from the two national registries (NVSCA and LVR/LNR) that remained after extraction of the NNL data. For each data source and region, the annual prevalence was determined as the number of registered live-born infants with an oral cleft per 10,000 live births in the same region and year. The information on the annual numbers of live births in NNL and the rest of the Netherlands was retrieved from Statistics Netherlands.[27]

We calculated 95% confidence intervals (CI) for the average prevalence, and we used the prevalence proportion ratio (PPR) and Chi-square test for analyzing differences between registries and regional comparisons within registries. Trends in the annual prevalence were analyzed by Poisson regression, modeling counts against year. The logarithm of the population size of live births was used as an offset. Subsequently, we calculated the estimated annual percentage change (EAPC) by fitting a regression line to the natural logarithm of the annual prevalence, using calendar year as a regressor variable, i.e., $y = b + mx$, where y = ln(annual prevalence) and x = calendar year**.** The EAPC was then estimated as 100 * (e^m – 1). Testing that the EAPC is 0%

is equivalent to testing the null hypothesis that the slope of the line in the above equation is equal to zero. This was tested by comparing *m*/SE(m) with a t-distribution with k – 2 degrees of freedom, where k is the length of the period. The standard error of *m*, SE(*m*), was generated from the fit of the regression. The calculation assumed that the logarithm of the annual prevalence changed at a constant rate over the entire period. The EAPC represents an estimate of the year-to-year change in prevalence; negative values represent a declining prevalence and positive values represent an increasing prevalence. Statistical significance level for α was set at 0.05. Statistics were performed using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL).

Table 1. Characteristics of the study population of the Northern Netherlands and the rest of the Netherlands during 1997-2007, based on data of the NVSCA[a]

Characteristic	Northern Netherlands (n = 456)		Rest of the Netherlands (n = 3118)		*p* Value[b]
General information					
Boys	258	(56.6)	1841	(59.0)	0.318
Mean birth weight in grams ± SD[c]	3330	± 647	3300	± 655	0.373
Mean gestational age in weeks ± SD[d]	39	± 2.1	39	± 2.2	0.566
Median referral age in months [IQR]	1.9	[1.2-3.0]	0.53	[0.23-1.37]	**0.000**
Caucasian father	425	(93.2)	2696	(86.5)	**0.000**
Caucasian mother	433	(95.0)	2731	(87.6)	**0.000**
Consanguinity	5	(1.1)	76	(2.4)	0.072
Congenital anomalies among relatives	133	(29.2)	840	(26.9)	0.318
Oral clefts among relatives	81	(17.8)	561	(18.0)	0.905
Oral clefts					
CL±P	313	(68.6)	2094	(67.2)	0.528
CP	143	(31.4)	1024	(32.8)	0.528
Additional congenital anomalies[e]					
Other craniofacial anomalies	66	(14.5)	208	(6.7)	**0.000**
Anomalies other organ systems	67	(14.7)	553	(17.7)	0.109
Syndrome or chromosomal defect	58	(12.7)	300	(9.6)	**0.040**

NVSCA = Dutch Association for Cleft Palate and Craniofacial Anomalies; n = number; CL±P = cleft lip/alveolus with or without cleft palate; CP = cleft palate only; IQR = interquartile range; SD = standard deviation.

[a] Values are numbers (percentages) unless stated differently.

[b] *p* value presents statistical significance level for differences between the two regions: Chi Square test for proportions, Mann-Whitney test or independent t-test for continuous variables. *p* value <0.05 is used to determine statistical significance and is presented in bold format.

[c] Valid cases: Northern Netherlands (n = 443) and rest of the Netherlands (n = 2982).

[d] Valid cases: Northern Netherlands (n = 450) and rest of the Netherlands (n = 2993).

[e] Note that the NVSCA has no active follow-up of patients and that associated anomalies detected later in infancy are often not included.

RESULTS

Study population characteristics

During 1997-2007, a total of 456 and 3118 infants were born alive with oral clefts in the region NNL and in the rest of the Netherlands, respectively, and registered in the NVSCA. The characteristics of the study population are presented in Table 1. The median age at cleft palate team admission was significantly higher in NNL than in the rest of the Netherlands (difference in months: 1.37). The percentage of Caucasian parents was also significantly higher (difference in percentage points (pp): 6.7 and 7.4 for Caucasian father and mother, respectively). Finally, oral clefts in NNL were more frequently associated with other anomalies of the head and neck area (difference in pp: 7.8) and with syndromes or chromosomal defects (difference in pp: 3.1) than those in the rest of the Netherlands.

Average prevalence

During 1997-2007, there were 213,209 live births in the region NNL; the average oral cleft prevalence of the NVSCA was 21.4 per 10,000 live births (Table 2). Infants with CL±P accounted for 68.6% of the study population, corresponding to a prevalence of 14.7 per 10,000 live births. The average prevalence of CP was 6.7 per 10,000 live births. The NVSCA identified 42% (PPR = 1.42) more oral clefts than did the LVR/LNR (PPR = 1.47 for CL±P, and PPR = 1.31 for CP). By contrast, the NVSCA prevalence was not significantly different from that of Eurocat: all clefts (PPR = 1.05), CL±P (PPR = 1.11), and CP (PPR = 0.93).

In the rest of the Netherlands (Table 2), there were 1,938,999 live births during the study period. In this region, the NVSCA also identified more oral clefts than did the LVR/LNR: 22% (PPR = 1.22) more oral clefts, 24% (PPR = 1.24) more CL±P, and 18% (PPR = 1.18) more CP. When comparing both regions within the NVSCA, we found that 33% (PPR = 1.33) more oral clefts were identified in NNL than in the rest of the Netherlands (PPR = 1.36 for CL±P, and PPR = 1.26 for CP)

Time trends

Figure 1A-C displays the annual live-birth prevalence in the region NNL from 1997 through 2007 for the NVSCA, LVR/LNR, and Eurocat. Although the oral cleft prevalence in NNL seemed to decrease in the NVSCA, time-trend analyses showed no significant trends: all clefts (EAPC –2.3%; 95% CI: –5.3% to +0.60%), CL±P (EAPC –1.5%; 95% CI: –5.1% to +2.0%), and CP (EAPC –4.0%; 95% CI: –9.3 to +1.3%) (Figure 1A). For the LVR/LNR, we observed a small, but not statistically significant, increase in live-birth prevalence over time: all clefts (EAPC +2.3; 95% CI: –1.2 to +5.8), CL±P (EAPC +1.8; 95% CI: –2.5 to +6.1), and CP (EAPC +3.1; 95% CI: –3.0 to +9.1) (Figure 1B). Like the two national registries, Eurocat showed no significant changes in prevalence over time: all clefts (EAPC –0.20%; 95% CI –3.2% to +2.9%), CL±P (EAPC –0.30%; 95% CI: –4.0% to +3.5%), and CP (EAPC 0%; 95% CI: –5.1% to +5.1%) (Figure 1C).

Table 2. Prevalence of oral clefts per 10,000 live births in the Northern Netherlands and the rest of the Netherlands in 1997-2007, based on the NVSCA, LVR/LNR, and Eurocat

Region	Cleft Category	NVSCA				LVR/LNR				Eurocat			
		n	*(%)*	*Prevalence (95% CI)*		*n*	*(%)*	*Prevalence (95% CI)*		*n*	*(%)*	*Prevalence (95% CI)*	
Northern Netherlands	CL±P	313	(68.6)	14.7	(13.1-16.3)	214	(66.5)	10.0	(8.7-11.4)	281	(64.7)	13.2	(11.6-14.7)
	CP	143	(31.4)	6.7	(5.6-7.8)	108	(33.5)	5.1	(4.1-6.0)	153	(35.3)	7.2	(6.0-8.3)
	Total	456	(100)	21.4	(19.4-23.3)	322	(100)	15.1	(13.5-16.8)	434	(100)	20.4	(18.4-22.3)
Rest of the Netherlands	CL±P	2094	(67.2)	10.8	(10.3-11.3)	1694	(65.9)	8.7	(8.3-9.2)				
	CP	1024	(32.8)	5.3	(5.0-5.6)	875	(34.1)	4.5	(4.2-4.8)				
	Total	3118	(100)	16.1	(15.5-16.6)	2569	(100)	13.2	(12.7-13.8)				

NVSCA = Dutch Association for Cleft Palate and Craniofacial Anomalies; LVR/LNR = The Netherlands Perinatal Registry; n = number; CI = confidence interval; CL±P = cleft lip/alveolus with or without cleft palate; CP = cleft palate only.

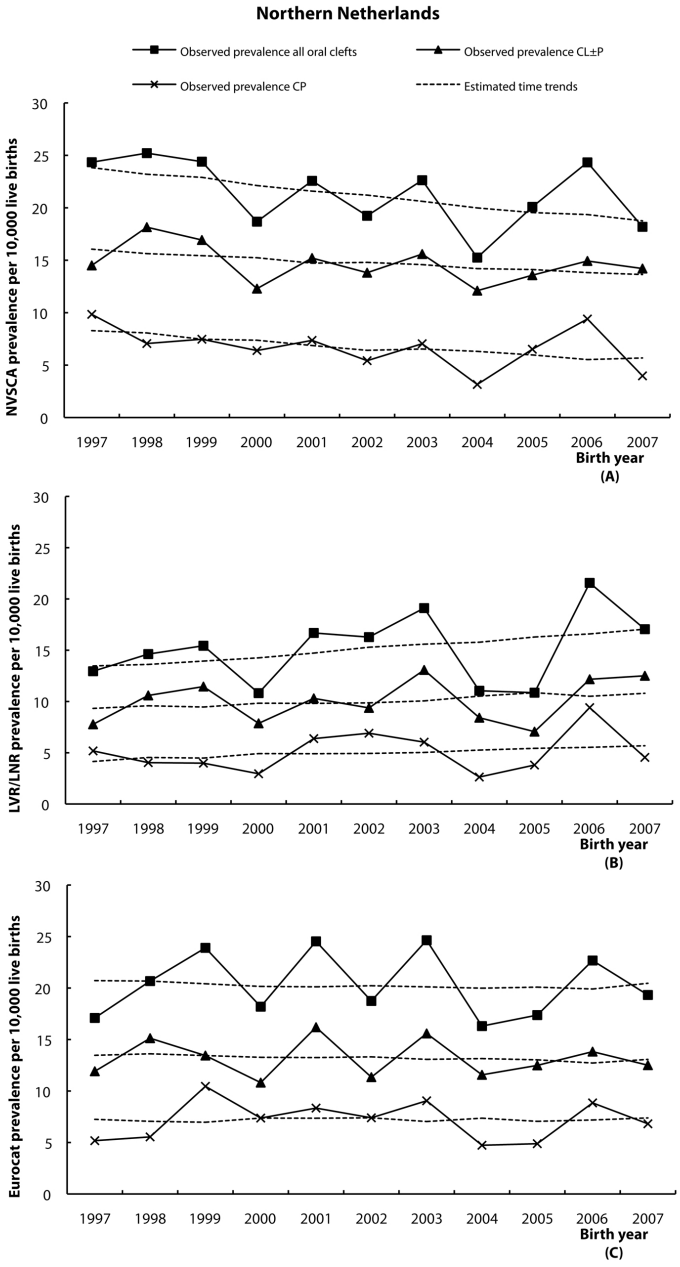

Figure 1. Regional time trends in prevalence of all clefts and the two categories (CL+/-P and CP) per 10,000 live births in the Netherlands, 1997-2007. Trends in the Northern Netherlands were based on the NVSCA (A), LVR/LNR (B), and Eurocat (C). CL+/-P = cleft lip/alveolus with or without cleft palate; CP = cleft palate only.

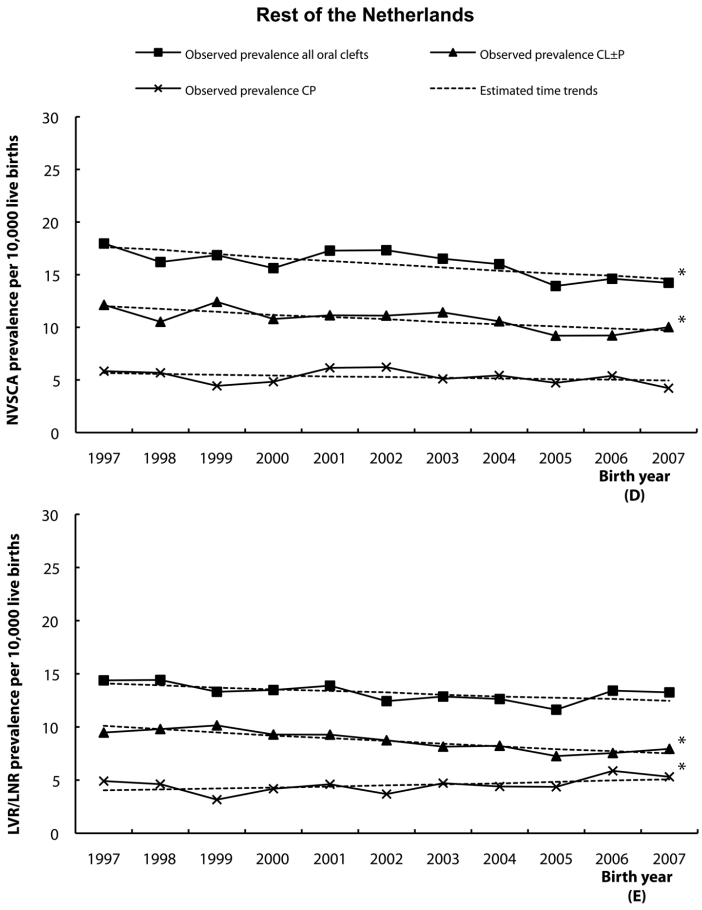

Figure 1. (continued) Trends in the rest of the Netherlands were based on the NVSCA (D) and LVR/LNR (E). CL+/-P = cleft lip/alveolus with or without cleft palate; CP = cleft palate only.

For the rest of the Netherlands, the annual live-birth prevalence of the NVSCA and LVR/LNR is shown in Figure 1D,E. Time-trend analysis of NVSCA data showed that over the 1997-2007 period, the live-birth prevalence of all clefts in the rest of the Netherlands decreased significantly by 1.9% per year (95% CI: –3.1% to –0.80%) (Figure 1D). The prevalence of CL±P showed a trend similar to that observed for all clefts; per year, it decreased by a significant 2.2% (95% CI: –3.6 to –0.80%). By contrast, no significant trend in CP prevalence was found (EAPC –1.4%; 95% CI: –3.4% to +0.60%). For the LVR/LNR, a significant decreasing trend in the rest of the Netherlands was detected for CL±P (EAPC –3.0%; 95% CI: –4.6% to –1.5%). However, no evidence for a significant trend in all clefts (EAPC –1.3%; 95% CI: –2.5% to 0%) was found. Surprisingly, the CP prevalence in the LVR/LNR increased significantly during the study period (EAPC +2.2; 95% CI: +0.10% to +4.3%) (Figure 1E).

DISCUSSION

This study shows that the live-birth prevalence of oral clefts varies significantly in the Netherlands, not only between registries but also within registries. Specifically, we found that the live-birth prevalence of all clefts and of both categories (CL±P and CP) is higher in NNL than in the rest of the Netherlands. Furthermore, a significant decreasing trend in CL±P prevalence was found for live births in the rest of the Netherlands, but not for those in the region NNL. By comparing the average prevalence between registries, we found for both regions that the prevalence from the NVSCA is significantly higher than that from the LVR/LNR, while it is similar to that of Eurocat.

Strengths and limitations

While the use of three registry databases gave our study its main strength—it allowed us to analyze complementary data on oral cleft live births—it also had some limitations. The registries have different aims and registration methods, such as ascertainment procedures and inclusion/exclusion criteria, that will always vary. So will the subjectivity that is inevitable among personnel who enter the information. For example, the primary aim of the LVR/LNR is to collect information on all pregnancies and not solely on congenital anomalies, and data are recorded by many different health care providers. As a result, this registry might have more underreporting and misclassification of clefts than do specific congenital anomaly registries.[23] This could partly explain its significantly lower prevalence for both regions. Another explanation for this lower prevalence is that not all health care providers participate in the LVR/LNR yet.[22] However, the coverage of the LVR/LNR increased during the study period,[22] which is probably reflected by the increasing CL±P and CP trends in this registry.

Despite various case-ascertainment and validation methods,[10, 16, 17] the NVSCA and Eurocat also differed somewhat in prevalence, most likely due to selection/survival bias. Eurocat needs parental informed consent to record its patients, which might explain its slightly lower oral cleft prevalence. Conversely, the NVSCA misses infants with severe associated anomalies who die after birth and do not reach the cleft palate teams (mostly being CP patients), which probably explains its slightly lower CP prevalence.

Another weakness of the databases we used is that cases could not be directly matched between the registries due to confidentiality constrains and limitations/differences in key information. Additionally, we were not able to give complete and reliable national trends according to whether clefts were isolated or associated with other anomalies. The NVSCA and LVR/LNR have no active follow-up of patients, and therefore, associated anomalies detected after registration are not included (Rozendaal et al. submitted).[4, 28] In preliminary analyses, even low-end estimates of isolated and non-isolated clefts did not provide more insight into the causes of regional variation in prevalence and trends (personal communication A.M.R.).

Finally, the databases do not provide complete and reliable national data on clefts among stillbirths and pregnancy terminations, which are essential to gain more insight into the causes of the decline in oral clefts. Hopefully, national information on these cases will be available in the future, as the Netherlands is developing a national uniform registry for the outcomes of prenatal screening, including prenatally diagnosed anomalies and outcomes of pregnancies.[29]

Possible explanations for regional variation in overall prevalence

Our results for the region NNL are in line with those of Cornel et al.,[2] who reported a similar prevalence for all clefts and both categories (CL±P and CP) for the period 1981-1988. Hence the high prevalence in NNL seems to have already existed for a long time and to be fairly constant. This is also consistent with combined international registry data, which showed that the prevalence of oral clefts is relatively high in the region NNL.[5, 6] Worldwide, the average prevalence of oral clefts is 15.2 per 10,000 births,[1] and international studies have shown a large variation in prevalence between and within countries, without consistent time trends.[3, 6, 7, 9, 11, 18] Unfortunately, comparison of our findings with those of other studies is restricted, due particularly to the great differences between data sources, times of diagnosis, classifications, inclusion/exclusion criteria, time scales, sample sizes, and population characteristics.[3, 6, 8, 9]

Regional differences in epidemiological patterns may be due to variations in genetic and environmental risk factors, and in gene-environment interactions as well.[4, 8, 12, 13] In this study, we found some regional variation in population characteristics, which may explain the differences in prevalence we found within the Netherlands. Firstly, the NNL population included relatively more infants with Caucasian parents than that of the rest of the Netherlands. Given that Anthony et al.[4] described previously that Dutch people have a higher oral cleft risk than other ethnic groups, the geographical variation in oral clefts in the Netherlands may be explained by ethnic differences. Additionally, the NNL population might have a higher risk on genetic predisposition, because many families have lived in this region since ages, and there is less immigration in this region than in the rest of the Netherlands.[27] This is supported by the relatively high cleft prevalence found in Northern European countries, especially Denmark, which have until recently homogenous populations and high quality registrations.[1, 5, 6, 9, 18] The fact that patients in NNL had more associated syndromes and chromosomal defects also suggests genetic variation between the two study populations, since these syndromic clefts are more frequently related to a genetic background than non-syndromic clefts.[8] The higher proportion of associated defects may on the other hand also be explained by the higher referral age we found for NNL patients, since many associated anomalies are diagnosed at a later age (Rozendaal et al. submitted).[28]

Possible explanations for differences in regional time trends

Our findings of a decline in oral cleft live births in the rest of the Netherlands may be explained by several factors that changed during the study period. The first might be the increase in

prenatal detection of congenital anomalies (including oral clefts) followed by termination of pregnancy (TOP). Abortion in the Netherlands is allowed until the 24th week of pregnancy, only under the provision of the Termination of Pregnancy Act (WAZ).[30] Since no specific indication is needed, TOP is allowed for clefts with associated anomalies as well as for isolated clefts. If pregnancies are terminated because of an oral cleft, the prevalence of CL±P will be affected most since CL can be detected prenatally using 2D ultrasound, while CP is often not being found.[3, 31]

Since the 1990s, the performance of prenatal 2D ultrasound examinations has increased in the Netherlands, resulting in greater prenatal detection of congenital anomalies, including CL±P, in both NNL and the rest of the Netherlands.[32, 33] As it did elsewhere,[3, 6, 9, 31] this may have led more affected pregnancies to be terminated in the Netherlands. However, our findings suggest that this happened only in the rest of the Netherlands, as we found no declining trends in NNL. This is supported by Eurocat data showing low and rather stable rates of TOP among clefts in NNL;[6] all of these were associated with other congenital anomalies, including chromosomal defects (personal communication M.K.B.). Additionally, this explanation is also supported by national data on TOPs; under the terms of the WAZ, all Dutch hospitals and clinics performing TOPs are required to report these terminations (indication not included).[34] Annual reports of the WAZ have shown that second-trimester terminations have hardly been performed in NNL, while they have increased in the rest of the Netherlands since 2003, especially those of 20-24 weeks of gestation performed by the hospitals. As stated in these reports, this increase implies that there has been a rise in termination of pregnancies affected with congenital anomalies, because these terminations are mainly performed by the hospitals.[34]

Another explanation for the decline in oral cleft live births in the rest of the Netherlands might be that increased periconceptional use of folic acid and/or multivitamins has reduced the risk of oral clefts. The preventive effects of folic acid/multivitamins have been frequently described, but the results are mixed in terms of estimated effects and whether CL±P or CP or both are affected.[35-37] In a meta-analysis including the most recent observational studies, Johnson and Little[35] estimated that the risk of CL±P decreased with 18% while using folic acid-containing supplements and with 23% while using multivitamins. They found no significant reduction in CP after supplementation, while compulsory folic acid fortification reduced the risk for both CL±P and CP.[35]

In the Netherlands, women are recommended to take 400 µg folic acid/day from 4 weeks before conception until 8 weeks thereafter. Although complete data on the use of folic acid in the Netherlands are lacking, several data have shown that the proportion of expecting mothers correctly using folic acid increased from 15-29% in 1996 to about 50% in 2003-2005.[38, 39] Given that most sub-phenotypes of CL±P (complete clefts of the lip/alveolus) develop during weeks 4-7 after conception, while incomplete CL±Ps and all sub-phenotypes of CP develop during weeks 8-12 after conception (Vermeij-Keers et al., submitted),[12] the increase in folic acid supplementation until 8 weeks postconception might have mainly affected the prevalence of

CL±P.[20] This would explain the absence of significant trends for CP in our study, which is in line with other studies reporting a reduced risk for only CL±P after the same supplementation period (4 weeks before conception until 8 weeks thereafter).[40-42] As our effect-measures for NNL also indicated decreasing trends, the absence of a significant decline of CL±P in this region could solely be due to sample size.

Finally, other environmental or lifestyle factors (e.g., dietary patterns) changing over time may also account for the decrease in prevalence.[8] Specific data on these factors are not available for the Dutch cleft population, but data on the general population have shown that smoking and alcohol consumption decreased among Dutch women of reproductive age during the study period.[43] Although these changes are certainly too small to account for the complete decline in oral clefts, they may have played contributory roles given the causal heterogeneity of clefts.[8]

CONCLUSIONS

In conclusion, this study presents evidence from three Dutch registries that the epidemiological pattern of oral clefts varies within the Netherlands. Although the registries showed some differences in prevalence, they showed similar results on the regional variation in prevalence as well as in time trends of oral cleft live births. Therefore, our findings are unlikely to be explained by methodological factors, such as ascertainment methods.

The findings that the oral cleft prevalence in NNL is not only higher than that in certain other European areas but also than that in the rest of the Netherlands may have several implications, both nationally and internationally. Although regional data have utility for health services, clinicians and researchers in that specific area and can be compared to global means and trends, our results underline that extrapolation to a whole country or larger area should be made with caution.[9] To further investigate environmental and genetic factors that influence the risk on oral clefts, future studies should consider the geographical differences in oral clefts—between and within countries—regarding the various cleft sub-phenotypes among live births, stillbirths, and spontaneous/induced abortions. Finally, our results suggest that the decline in live-birth prevalence of oral clefts may have been caused by increased prenatal detection followed by TOP and/or by increased periconceptional use of folic acid in the Netherlands. This would have implications for healthcare and policy makers, as evidence-based guidelines can optimize prenatal counseling, and increases in folic acid exposure, including extension of the supplementation period, may produce further reductions in prevalence.

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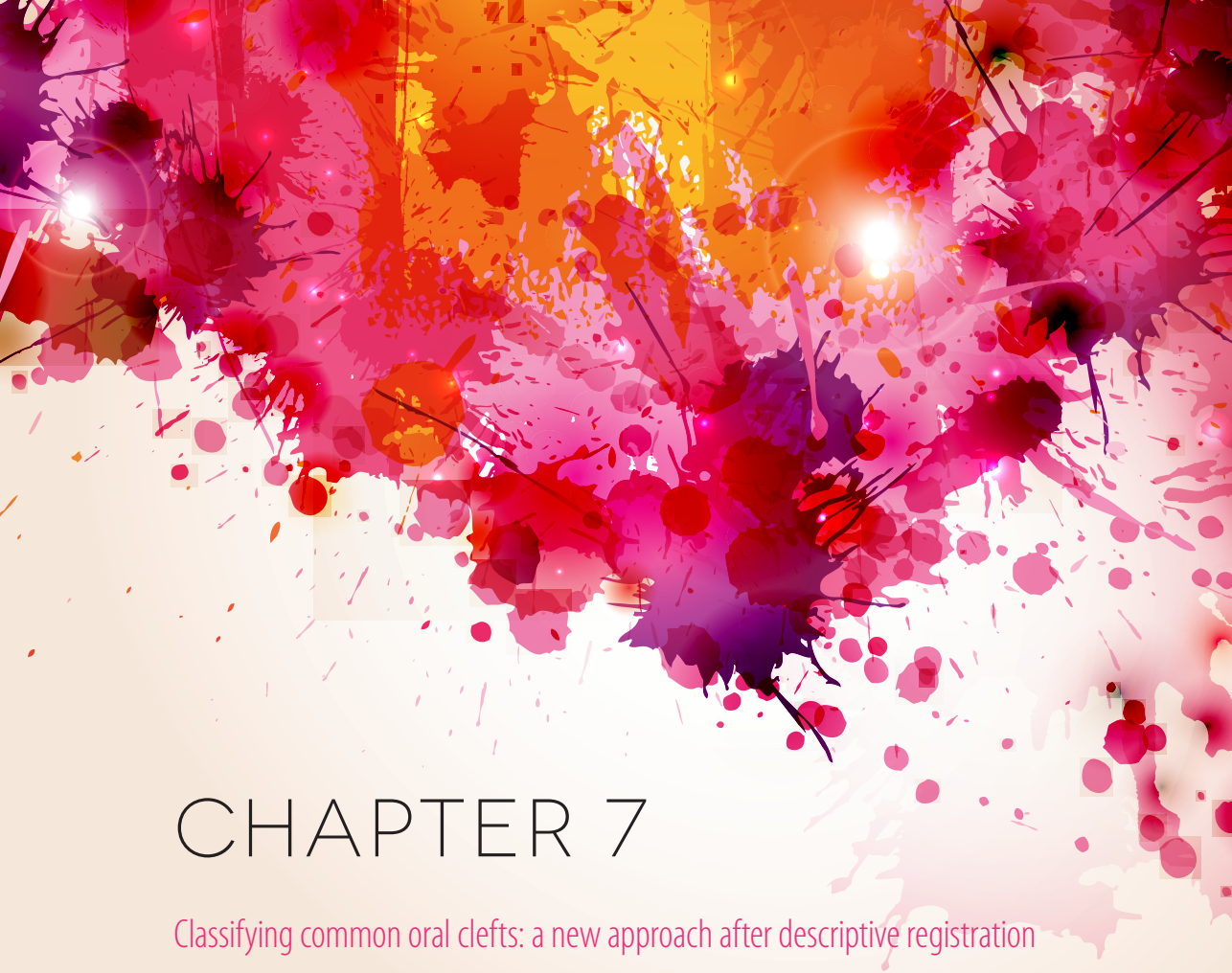
PART III

Postnatal classification



CHAPTER 7

Classifying common oral clefts: a new approach after descriptive registration

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ABSTRACT

Objective: Using the Dutch Oral Cleft Registration, which records the morphology and topography of common oral clefts, a new classification based on the (patho)embryology of the primary and secondary palates was tested.

Design: Prospective observational study.

Setting: The fifteen cleft palate teams in the Netherlands register patients to the national registry.

Patients: All unoperated patients with common oral clefts reported between 1997 and 2006 inclusive were included.

Main outcome measures: The classification is based on the pathoembryological events that ultimately result in various sub-phenotypes of common oral clefts. Patients within the three categories cleft lip/alveolus (CL/A), cleft lip/alveolus and palate (CL/AP) and cleft palate (CP) were divided into three subgroups: fusion defects, differentiation defects, and fusion and differentiation defects. A timetable was constructed to relate the type of clefting to the time of derailment during embryonic development.

Results: 3512 patients were included. Patients with CL/A showed 22% fusion defects, 75% differentiation defects, and 3% fusion and differentiation defects. CL/AP patients and CP patients mostly showed fusion defects (70% and 89%, respectively). We were able to relate almost all (over 90%) cleft sub-phenotypes to specific weeks in embryonic development.

Conclusions: This classification provides new cleft subgroups that may be used for clinical and fundamental research. The sub-phenotypes of these subgroups originate from different time frames during embryonic development and different cell biological mechanisms, thereby enabling more accurate data for, e.g., gene identification and/or environmental factors.

INTRODUCTION

Common oral clefts are one of the most frequent congenital anomalies worldwide.[1] Ethnic, socioeconomic, and geographic variations may partly account for the large multifactorial group of nonsyndromic common oral clefts.[2-4] A quest for identifying genes and environmental factors responsible for these anomalies has been done for years. However, only a small part of the nonsyndromic common oral clefts have been related to specific genes and/or environmental factors, such as MSX1 or smoking.[4-8] Within this multifactorial group, huge variations in cleft sub-phenotypes exist. These various cleft types originate from different developmental time periods (Vermeij-Keers, unpublished data),[9] and therefore have different exposures to genes and environmental factors.[10] If patients with different cleft sub-phenotypes are treated as a single group, linkage studies with genes and/or environmental factors may not be as fruitful as hoped.[8] Therefore, a new classification based on the human embryology of the primary and secondary palates was previously introduced (Vermeij-Keers, unpublished data).[11, 12] In this classification, different sub-phenotypes of common oral clefts are distinguished based on different cell biological mechanisms and related to different time periods in embryonic development.

Such a classification can be applied only if detailed phenotype descriptions of the common oral clefts are available. In 1997, a new descriptive recording system was developed on behalf of the Dutch Association for Cleft Palate and Craniofacial Anomalies (NVSCA).[13] This system, the NVSCA registry, consistently records all abnormalities of each anatomic structure that form the common oral cleft.

Recently, the feasibility of our new classification was shown for clefts of the primary palate using adult unoperated patients from Indonesia (Vermeij-Keers, unpublished data). In addition, we used this embryological approach to validate NVSCA registry data on the specific oral cleft features.[11] Previously, we divided broad categories into fusion and/or differentiation defects,[12] but it is unknown whether this classification is complete and feasible for all cleft sub-phenotypes of the primary and secondary palates among newborns.

In this study, we applied the classification to unoperated infants with common oral clefts using detailed data of the cleft sub-phenotypes from the NVSCA registry. After considering the normal and abnormal development of the primary and secondary palates, their clefts were first traditionally classified into three categories: cleft lip/alveolus (CL/A), cleft lip/alveolus and palate (CL/AP), and cleft palate (CP). Subsequently, we classified the various cleft sub-phenotypes within these categories into fusion and/or differentiation defects. Finally, we constructed a timetable, relating the various fusion and/or differentiation defects to weeks in embryonic development.

MATERIAL AND METHODS

Patients

In this study, we included all unoperated patients with a common oral cleft that had been reported by the 15 multidisciplinary cleft palate teams to the NVSCA registry between 1997 and 2006 inclusive. After careful examination, the consulting physicians (plastic surgeon, orthodontist or pediatrician) recorded these patients using the NVSCA recording form.[13] All forms were examined for incorrect, inconsistent, or insufficient data by the authors. If additional information was needed, it was provided by the cleft palate teams. In addition, the registry data were systematically validated.[11, 14, 15]

In this study, only common oral clefts were included. Median cleft lip and atypical facial clefts were excluded for their different pathogenesis.[9, 16]

Embryological basis of the classification

To place the different sub-phenotypes of oral clefts into the correct time periods and cell biological mechanisms during human embryonic development, the normal and abnormal development of the primary and secondary palates should be understood and is therefore briefly reviewed here.

Normal development of primary and secondary palates: fusion and differentiation

Normal embryonic development of the primary palate (the presumptive lip and alveolus) can be divided into early and late embryonic development (i.e., 4 to 7 weeks of development and 7 to 12 weeks of development [postconception], respectively).[9, 10, 17] In contrast, the development of the secondary palate (the presumptive hard and soft palates, including the uvula) takes place in the late embryonic period (7 to 12 weeks of development). During early development, the primary palate is formed in an occipito-frontal direction by fusion of three outgrowing facial swellings around each nasal placode (left and right). First, the maxillary process (occipitally) and subsequently the lateral nasal process (frontally) adhere and fuse with the medial nasal process.[9, 17, 18] As a consequence, the lateral and medial nasal processes always surround the nasal apertura. During the fusion process, the ectoderm covering the mesenchymal cores of the swellings on the fusion side is enclosed and an epithelial plate (the nasal fin) is formed. From the occipital part of this plate, the oronasal membrane (i.e., bucconasal membra ne) develops and subsequently ruptures by cell death (6 to 7 weeks of development). During the same weeks, the epithelial plate gradually disappears by programmed cell death followed by epitheliomesenchymal transformation (EMT) and/or migration (Vermeij-Keers, unpublished data).[17, 19-23] The last location for the epithelial plate to disappear is at the fusion of the pre-sumptive lip, beneath the nostril.

When late development starts, the mesenchymal cores of the facial swellings have fused completely. Subsequently, the primary palate differentiates by (1) outgrowth of the lip and

alveolar process in a caudal direction, thereby causing the labial groove, and (2) the development of a left and right bone center of the maxilla and two bone centers in each premaxilla.[9, 17] These bone centers approach each other and fuse without forming sutures, except between the two premaxillae (the intermaxillary suture). Bony differentiation is accompanied by the development of facial musculature.

During the development of the secondary palate, the palatine processes grow out, elevate, adhere, and fuse bilaterally with the primary palate and then in the median plane in a fronto-occipital direction.[23-28] They fuse with each other and with the nasal septum. Again, ectoderm of the various processes is enclosed during the fusion process, and a Y-shaped epithelial plate forms. Subsequently, this plate disappears gradually by programmed cell death followed by EMT, and/or migration of epithelial cells towards the nasal side of the plate.[9, 23, 24, 29-40] Although the cell fate underlying the disappearance of the epithelial plate has been controversial for many years, two recent review papers[41, 42] showed that none of the three possible cell biological mechanisms (programmed cell death, EMT, and migration) can be excluded.

While the palatine processes grow out, the bone centers of the palatine bones develop bilaterally. During the fusion process, they approach each other and the bone centers of the maxilla. The same holds for the maxilla and premaxillae. In this way, the median and transverse palatine sutures develop, as well as the bilateral incisive sutures. In addition, bony differentiation is accompanied by muscular differentiation. In conclusion, the primary and secondary palates develop in opposite directions: the facial swellings fuse in an occipito-frontal direction, while the palatine processes fuse in a fronto-occipital direction.

In view of the above, disturbances during the development of the primary and/or secondary palates give rise to fusion and/or differentiation defects. Examples of different cleft subphenotypes in relation to the various developmental periods and cell biological mechanisms are discussed below.

Abnormal development of primary and secondary palates: fusion and differentiation defects

Complete cleft lip and alveolus, early embryonic development

This type of clefting represents no fusion at all and is therefore considered a fusion defect, because of insufficient outgrowth of the facial swellings, lack of adherence of these swellings, or failure of programmed cell death/EMT/migration, that is, the epithelial plate does not develop or it remains intact. During the latter situation, further differentiation causes the ectoderm to separate again at the fusion site, resulting in a complete cleft lip and alveolus extending to the incisive foramen (Figures 1 and 2). As this process is completed before the secondary palate starts to fuse, these primary palatal defects are independent of the secondary palatal defects.[9] As a result, complete cleft lip and alveolus can be observed with a normal secondary palate (Figure 1b), or with an abnormal secondary palate, such as a complete cleft palate (Figure 2b). In the last case, it is readily possible that the palatal shelves could not have reached each other because of the width of defect of the primary palate.

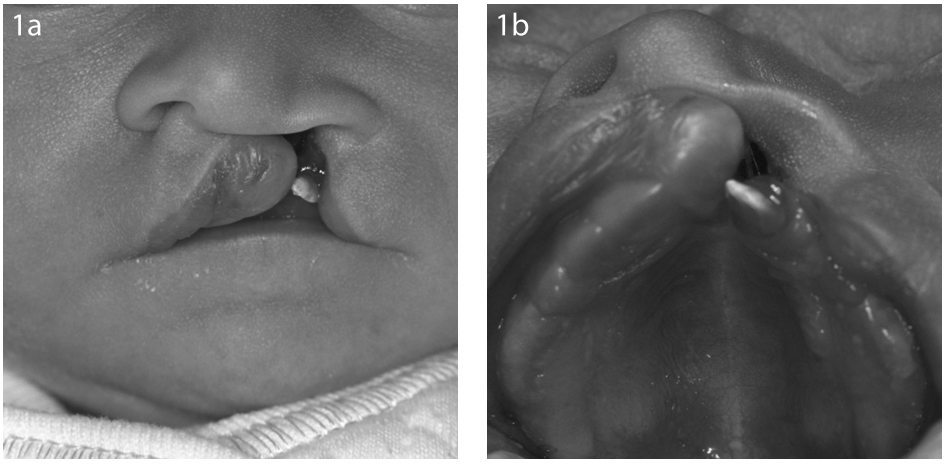

Figure 1. A complete left cleft of the lip and alveolus (a); the secondary palate is intact (b).

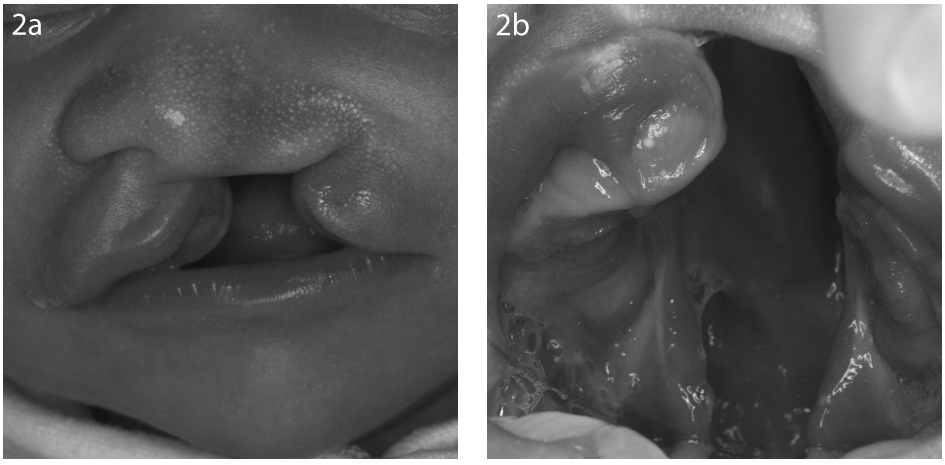

Figure 2. A complete left cleft of the lip/alveolus (a), hard and soft palate (b).

If fusion of the primary palate stops at a certain place along the fusion line, this always gives rise to a complete cleft lip combined with an intact alveolar process, or an incomplete cleft of the alveolar process.

Incomplete cleft lip with or without an incomplete cleft alveolus, late embryonic development

After fusion of the maxillary and lateral nasal processes with the medial nasal process, the primary palate differentiates by outgrowth of the lip and alveolus into a caudal direction. Since the fusion process has been completed at that stage, an incomplete cleft lip always displays a tissue bridge below the nostril (Vermeij-Keers, unpublished data).[10, 12] Consequently, the left

incomplete cleft lip and cleft alveolus of the patient in Figure 3a have their origin in incomplete caudal outgrowth and/or differentiation of the primary palate during late embryonic development (i.e., a differentiation defect). The right side of the same patient shows an incomplete cleft lip and a normal alveolus, demonstrating the about same starting point of disruption (incomplete outgrowth of the lip during late embryonic development). The presence of incomplete outgrowth of the alveolus at one side with normal outgrowth of the contralateral alveolus in the same individual might be explained by left/right asymmetry in the timing of the bony differentiation. The tissue bridge under de right nostril is larger than at the left side, suggesting that the outgrowth of the right lip started earlier than that of the left lip. Likewise, we presume that differentiation of the right alveolus preceded the differentiation of the left alveolus. When the event of disruption occurred, it is readily possible that differentiation of the right alveolus had already been completed, while that of the left alveolus was still differentiating, resulting in a normal right alveolus and an incomplete cleft of the left alveolus. A notch in the arch, hypoplasia, or a submucous cleft of the alveolar arch can also accompany the incomplete cleft lip. It is most likely that the abnormalities of the alveolar arch are the result from insufficient outgrowth of the premaxillary bone centers rather than the maxillary centers (Vermeij-Keers, unpublished data).

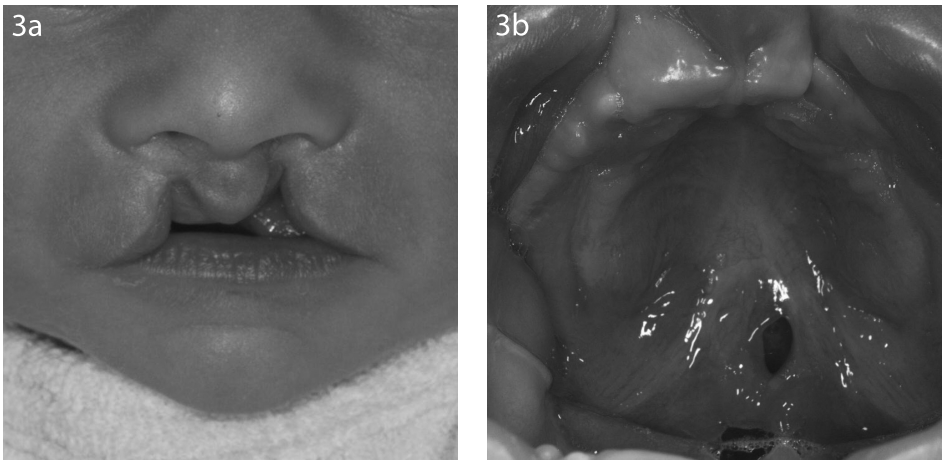

Figure 3. A bilateral asymmetric incomplete cleft of the lip with a normal right alveolus, and an incomplete cleft of the left alveolus (a) combined with a complete cleft of the soft palate (b).

Incomplete cleft lip and ipsilateral complete cleft alveolus, early and late embryonic development

In an incomplete cleft lip with an ipsilateral complete cleft alveolus (Figures 4a,b and 5a), the fusion process of the lip has been completed because a tissue bridge beneath the nostril has been formed. It is therefore a differentiation defect of the lip, which arises during late embryonic development. In the case of a small tissue bridge combined with an ipsilateral complete

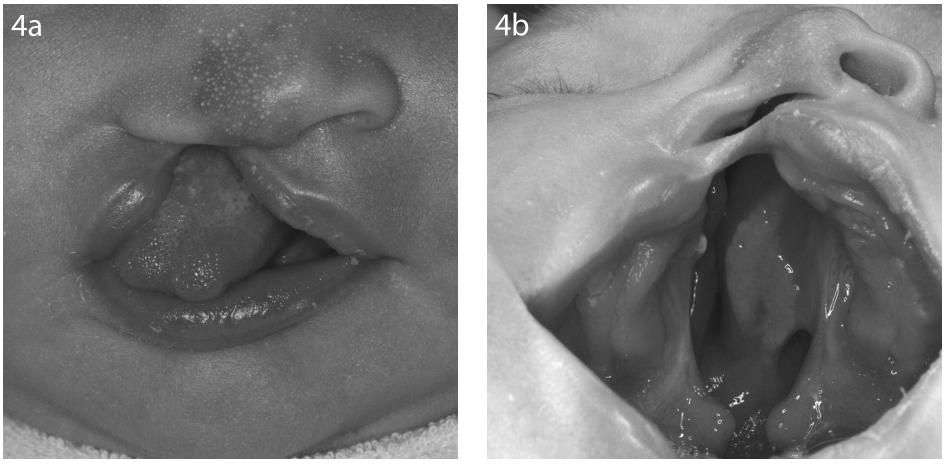

Figure 4. An incomplete right cleft of the lip, a complete alveolar cleft (a), combined with a complete cleft of the hard and soft palate (b).

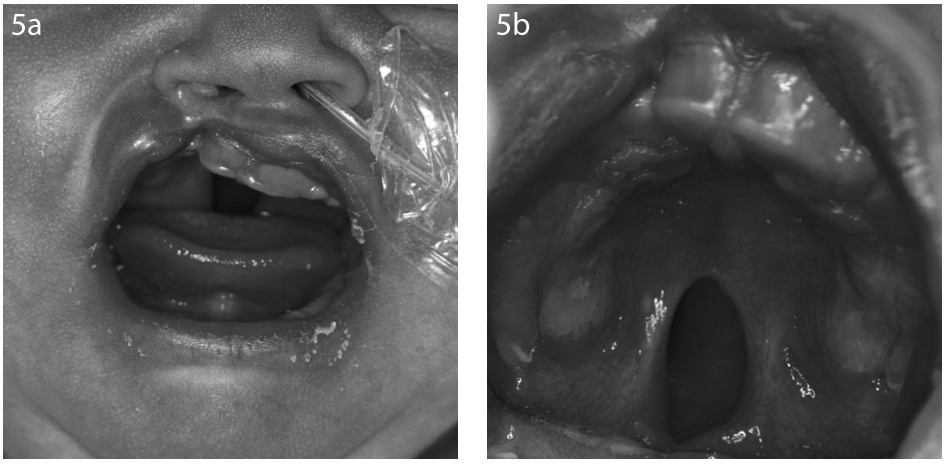

Figure 5. An incomplete cleft of the right lip and a complete alveolar cleft (a), combined with an incomplete cleft of the hard palate and a complete cleft of the soft palate (b).

cleft alveolus (Figure 4b), the term *Simonart's band* is used. The alveolar defect is a fusion defect that can be explained by a too wide oronasal membrane or a local persistence of the epithelial plate in front of the oronasal membrane. This part of the epithelial plate does not disappear by programmed cell death/EMT/migration during the early embryonic development (Vermeij-Keers, unpublished data). As is shown by these two patients, the appearance of the primary palate does not predict the appearance of the secondary palate (Figures 4b and 5b).

Complete cleft hard and soft palate, late embryonic development

If the palatine processes do not grow out or elevate insufficiently, a complete cleft of the hard and soft palate will result. This type of cleft can also occur when the palatine processes elevate, but do not adhere or fuse with the primary palate, with each other, and with the nasal septum (Figures 2b and 4b). These fusion defects develop early in secondary palatogenesis during the late embryonic period.

Incomplete cleft hard palate and complete cleft soft palate, late embryonic development

After elevation of the palatine processes, adhesion/fusion occurs in a fronto-occipital direction. If along this fusion line the fusion process is disrupted, various types of cleft palate can be observed (i.e., fusion defects). Relative early disruption of this fusion process may result in an incomplete cleft of the hard palate and complete cleft of the soft palate (including the uvula; Figure 5b). Somewhat later in development the hard palate is fused. If the fusion process stops after fusion of the hard palate, an intact hard palate will result, combined with a complete or incomplete cleft of the soft palate. Whether there will be a complete or incomplete cleft of the soft palate depends on the time of disruption. If disruption occurs later during the fusion process, more of the soft palate will be intact (Figure 3b). Therefore, an incomplete cleft of the hard palate combined with a complete cleft of the soft palate precedes complete and incomplete clefts of the soft palate.

Subclinical features of clefting regarding the primary and/or secondary palates

Milder expression of clefting can also be observed, such as a submucous cleft lip (also known as forme fruste, congenital scar, and microform, subsurface or subcutaneous cleft), submucous cleft palate, and bifid uvula. Except for the latter cleft type, which results from a fusion defect at the end of the fusion process of the secondary palate, these subclinical phenotypes can be considered as differentiation defects. Submucous clefts result from defective differentiation into bone and/or musculature, after completion of the fusion process. Other differentiation defects of the secondary palate include: (1) absence (agenesis) of the palatine bone, (2) a palatine bone and/or maxilla (palatine part) that is undersized (hypoplasia), or a submucous cleft, and/or (3) hypoplastic musculature.

Furthermore, with our concept of fusion/differentiation defects, special types of human cleft sub-phenotypes can be explained, such as an (in)complete cleft of the hard palate combined with an intact soft palate and uvula.[43, 44] This type may be the result of local insufficient programmed cell death/EMT/migration within the enclosed epithelial plates. Recently, it was reported that differential expression of proteins in the developing anterior and posterior secondary murine palate may cause too short anterior palatal shelves because of diminished cell proliferation and increased programmed cell death. The anterior palatal shelves do not reach each other, and a cleft of the hard palate remains. At that spot, the epithelium of the palatine processes persists, which causes a local fusion defect.[45] Another explanation of non-fusion

of the anterior palatal shelves was described based on thickened palatal epithelium in *Tbx1*$^{-/-}$ mice.[46]

Classification

In line with recent studies,[11, 13, 47, 48] we divided our study population into the three categories (CL/A, CL/AP, and CP). As we have shown previously, these categories manifest very heterogeneous cleft sub-phenotypes.[11, 13] To classify these types, the common oral clefts were divided into fusion and/or differentiation defects of the primary palate (lip and alveolus), the secondary palate (hard and soft palate, including the uvula), or both. The template for deciding which abnormality of the lip, alveolus, and hard and soft palates is a fusion defect or a differentiation defect is listed in Table 1. Theoretically, any combination of clefts of the lip, alveolus, hard and/or soft palates is possible, so each category was subdivided into three subgroups: fusion (F) defects, differentiation (D) defects, and fusion and differentiation (FD) defects.

Table 1. Classification of cleft sub-phenotypes of the primary and secondary palates: division into fusion and/or differentiation defects. Any combination of abnormalities of the lip, alveolus, hard and soft palate is allowed (adapted from Rozendaal et al.)[11]

Fusion defects	Primary palate	Complete cleft lip
		Complete cleft alveolus (extending to the foramen incisivum)
		Incomplete cleft alveolus **(only if the lip is normal or has a complete cleft)**
	Secondary palate	Complete cleft hard palate
		Incomplete cleft hard palate
		Complete cleft soft palate
		Incomplete cleft soft palate Complete uvular cleft Incomplete uvular cleft
Differentiation defects	Primary palate	Incomplete cleft lip
		Submucous cleft lip* Hypoplastic lip
		Incomplete cleft alveolus **(only if the lip has an incompleet or submucous cleft)**
		Submucous cleft alveolus
		Hypoplastic lip/alveolus
	Secondary palate	Submucous cleft hard palate
		Hypoplastic hard palate
		Submucous cleft soft palate (including uvula)
		Hypoplastic soft palate (including uvula)

* Synonyms: congenital scar, forme frust, subsurface cleft lip, subcutaneous cleft lip, and microform cleft lip.

RESULTS

The national registry recorded 3512 patients with a common oral cleft from 1997 to 2006. Twenty-eight percent of all patients showed a CL/A, 39% showed a CL/AP, and 33% exhibited a CP. The subdivision of the cleft sub-phenotypes—within these categories—into F defects, D defects, and FD defects is presented in Table 2. CL/A patients showed in 22% an F defect, in 75% a D defect, and in 3% an FD defect. CL/AP patients showed most frequently F defects (70%) and FD defects (29%). The vast majority of the CP patients displayed an F defect (85%).

As FD defects in CL/AP patients (n = 389) may involve F defects and D defects of the primary palate as well as of the secondary palate, we divided the study group into F, D, and FD defects concerning the primary and secondary palates (Table 3). The FD defects in CL/AP patients were mostly D defects (n = 159, 41%) or FD defects (n = 205, 52%) of the primary palate combined

Table 2. Classification of the sub-phenotypes within the three cleft categories: division into fusion and/or differentiation defects (n = 3512, Dutch Oral Cleft Registry 1997-2006)*

Type	Subgroups			Total
	F	*D*	*FD*	
CL/A	213	729	35	977
CL/AP	960	14	389	1363
CP	997	101	74	1172
Total	2170	844	498	3512

* CL/A = cleft lip and/or cleft alveolus, CL/AP = cleft lip and/or cleft alveolus and cleft palate, CP = cleft palate, F = fusion defects, D = differentiation defects, FD = fusion and differentiation defects.

Table 3. Classification of all patients with common oral clefts (n = 3512) into fusion and/or differentiation defects of the primary and/or secondary palates, based on data from the Dutch Oral Cleft Registry 1997-2006*

Primary palate	Secondary palate				Total
	No defect	*F*	*D*	*FD*	
F	213	960	6	3	1182
D	729	159	14	12	914
FD	35	205	2	2	244
No defect		997	101	74	1172
Total	977	2321	123	91	3512

* F = fusion defects, D = differentiation defects, FD = fusion and differentiation defects.

with F defects of the secondary palate. Of the 2340 patients with a defect of the primary palate, 1182 (51%) patients showed an F defect, 914 (39%) patients exhibited a D defect, and 244 (10%) patients showed an FD defect. A total of 2535 patients had a defect of the secondary palate and

an intact primary palate. In 92% of the patients (n = 2321), an F defect was observed, and in the remaining 8%, a D defect (n = 123) or FD defect (n = 91) was identified.

Fusion and/or differentiation defects of the primary palate

As shown in Table 4, F defects of the primary palate (n = 1183) mostly were of complete clefts of the lip/alveolus (62% in CL/A and 96% in CL/AP patients). Complete cleft lip combined with an incomplete cleft alveolus, as well as complete cleft lip, were less frequently observed. Together, these three types of clefting accounted for 99% of all F defects of the primary palate.

Ninety-two percent (n = 914) of D defects of the primary palate were incomplete clefts of the lip/alveolus (37% CL/A; 69% CL/AP), or incomplete clefts of the lip (51% CL/A; 20% CL/AP), or submucous clefts of the lip (5% CL/A; 4% CL/AP).

FD defects (n = 244) were mainly incomplete clefts of the lip combined with ipsilateral complete clefts of the alveolus (51% CL/A, 42% CL/AP). In 23% of the CL/A patients and in 40% of the CL/AP patients, a complete cleft lip/alveolus was observed with a contralateral incomplete cleft lip, an incomplete cleft lip/alveolus, or an incomplete cleft lip and complete cleft alveolus.

Table 4. Distribution of the sub-phenotypes of the primary palate: division of the cleft lip/alveolus patients (n = 977) and the cleft lip/alveolus and palate patients (n = 1363) into fusion and/or differentiation defects*

		CL/A	CL/AP
F	CCLA	134	932
	CCL+ICA	39	9
	CCL	37	18
	Miscellaneous	3	10
D	ICLA	267	128
	ICL	374	37
	SCL	36	7
	Miscellaneous	52	13
FD	ICL+CCA	18	88
	CCLA; ICL	3	33
	CCLA; ICLA	5	27
	CCLA; ICL+CCA	0	24
	Miscellaneous	9	37

* F = fusion defects, D = differentiation defects, FD = fusion and differentiation defects, CCLA = complete cleft lip + complete cleft alveolus, CCL+ICA = complete cleft lip + incomplete cleft alveolus, CCL = complete cleft lip, ICLA = incomplete cleft lip + incomplete cleft alveolus, ICL = incomplete cleft lip, SCL = submucous cleft lip, ICL+CCA = incomplete cleft lip + complete cleft alveolus (differentiation defect + fusion defect),
CCLA; ICL = complete cleft lip + complete cleft alveolus combined with a contralateral incomplete cleft lip; CCLA; ICLA = complete cleft lip + complete cleft alveolus combined with a contralateral incomplete cleft lip + incomplete cleft alveolus, CCLA; ICL+CCA = complete cleft lip + complete cleft alveolus combined with a contralateral incomplete cleft lip + complete cleft alveolus (differentiation defect + fusion defect**).**

Fusion and/or differentiation defects of the secondary palate

Table 5 presents that F defects of the secondary palate (n = 2321) were mostly complete cleft palates in CL/AP patients (86%). Complete cleft palate, incomplete cleft of the hard palate combined with a complete cleft of the soft palate, and complete cleft of the soft palate were observed in 91% of the CP patients.

D defects (n = 123) were mostly submucous clefts of the hard and/or soft palate (80% CL/AP, 68% CP). FD defects (n = 91) were predominantly submucous clefts of the hard and/or soft palate combined with an (in)complete uvular cleft (71% CL/AP, 69% CP), or submucous cleft of the hard palate combined with a complete cleft of the soft palate (12% CL/AP, 26% CP).

Timetable common oral clefts

As fusion and differentiation defects of the primary and secondary palates originate at different time periods, a timetable was constructed, relating the observed defects to weeks of development (Figure 6). For FD defects consisting of a fusion defect and a contralateral differentiation defect of the primary palate, both defects were considered to originate at different time points

Table 5. Distribution of the cleft sub-phenotypes of the secondary palate: division of the cleft lip/alveolus and palate patients (n = 1363) and cleft palate patients (n = 1172) into fusion and/or differentiation defects*

		CL/AP	CP
F	CCP	1142	274
	ICHP; CCSP	93	237
	CCSP	51	394
	ICSP	19	52
	I/CCU	17	37
	Miscellaneous	2	3
D	SCSP	12	55
	SCHP+SCSP	6	14
	HH/SP	1	29
	Miscellaneous	3	3
FD	SCH/SP+I/CCU	12	51
	SCHP+CCSP	2	19
	Miscellaneous	3	4

* F = fusion defects, D = differentiation defects, FD = fusion and differentiation defects, CCP = complete cleft palate, CCSP = complete cleft of the soft palate, ICHP = incomplete cleft of the hard palate, ICSP = incomplete cleft of the soft palate, I/CCU = (in)complete cleft of the uvula, SCSP = submucous cleft of the soft palate, SCHP = submucous cleft of the hard palate, HH/SP = hypoplastic hard and/or soft palate, SCH/SP = submucous cleft of the hard and/or soft palate.

(independently). For example, a patient with a complete cleft lip/alveolus combined with a contralateral incomplete cleft lip was considered to have sustained two disruptions during development. The first disruption was a fusion defect of the primary palate at one side (early embryonic development), and the second disruption concerned insufficient outgrowth/differentiation of the lip after fusion of the primary palate (late embryonic development). Both disruptions were counted in the timetable, once in the F group, and once in the D group (e.g., 3 CL/A patients and 33 CL/AP patients; Table 4).

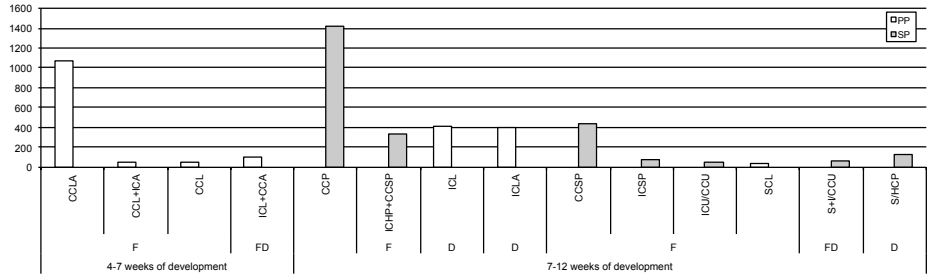

Figure 6. Timetable for the most frequent sub-phenotypes of the primary and secondary palates (n = 3512, Dutch Oral Cleft Registry 1997–2006).

DISCUSSION

This study demonstrates that our unique classification system can be applied successfully to unoperated newborns/infants having various sub-phenotypes of common oral clefts. Using detailed cleft data from the NVSCA registry, we were able to classify all clefts into fusion and/or differentiation defects. This was possible because we previously introduced the NVSCA registry, which describes the individual abnormalities of the common oral cleft.[11, 13] Furthermore, we were able to construct a timetable expressing fusion and/or differentiation defects in weeks of development, based on early and late embryonic development of the primary palate and on late embryonic development of the secondary palate.

The main strength of our study was the use of the national validated NVSCA database, which allowed us to analyze detailed data on a relatively large sample of patients affected with many different cleft sub-phenotypes. The NVSCA registry records all individual abnormalities that form the oral cleft, that is, the morphology and side of each anatomic structure (lip, alveolus, hard palate, and soft palate including the uvula). These data can be translated to any classification, new or old.[49] In contrast to this system, most available classification systems interpret the observed abnormalities that form the common oral cleft.[1] As a consequence, morphological details such as whether the cleft is complete, incomplete or submucous are lost. As

interpretations of these abnormalities will change by increasing knowledge about normal and abnormal development, adjustment of previously classified patients to new insights—such as a new classification—is often impossible.

Another strength of our study is that we used morphological sequelae that are more or less independent of progress in developmental biology. All parts of the primary and secondary palates grow out, adhere and fuse in a given time period, and somewhat later (primary palate) or during the same time period (secondary palate) they differentiate into bone and/or musculature. Therefore, it seems logical to divide the common oral clefts into fusion defects, differentiation defects, or a combination of fusion and differentiation defects (Tables 1 and 2). During the last decades, immense progress has been made concerning identification of candidate genes and environmental factors with respect to non-syndromic common oral clefts.[7, 10, 20, 50-55] However, elucidating pathways in their development is extremely difficult because of the multigenetic influences and their interaction with environmental factors.[8, 22, 56, 57] Furthermore, the classification systems that have been used for these studies are interpretations of the observed abnormalities. In other words, one does not reckon with the time periods at which various common oral clefts are originated. If one could relate groups of cleft types to specific time periods, identification of specific known and unknown genes that are expressed during these periods may follow. Also, submucous and microform clefts (including orbicularis oris muscle defects) are often not registered in other classifications. However, these subclinical forms may be just as important for further delineating the pathogenesis, clinical genetics, and understanding of the epidemiology.[7, 8]

As shown by our findings, the pathoembryological sequelae can be described in any individual case. Transfer of our data to this classification caused no problems, all patients fitted in a subgroup (Table 3). In addition, we constructed a timetable that can be used as a guideline for relating the type of clefting to the time period expressed in weeks of development. For instance, complete cleft lip/alveolus arises significantly earlier in development than incomplete cleft lip (Figure 6). In identifying genes and/or environmental factors, one should therefore distinguish these types and restrict the possible/candidate genes and environmental factors to the time period involved.

At the same time, this timetable also had some limitations. First, over 90% of the common oral clefts, but not all clefts, fitted in the timetable. Also, some fusion defects of the secondary palate were difficult to fit in the table. Theoretically, a complete cleft palate can originate from different mechanisms during two different time periods in late embryonic development. Complete cleft palate can originate relatively early during late development (7 to 9 weeks of development) because of insufficient outgrowth and elevation of the palatal shelves. However, lack of adhesion/ programmed cell death and/or EMT and/or migration later during late embryonic development (9 to11 weeks of development) may cause the same defect. Arbitrarily, all complete cleft palate cases were accumulated and placed in the early period of late embryonic development (Figure 6). Because of the possible different cell biological mechanisms and the

different originating timeframes, investigating complete cleft palate patients for common pathways may be hazardous. If one selects defects of the secondary palate in which a part of the hard palate or the whole hard palate has been fused, one can rule out insufficient outgrowth and elevation of the shelves, thereby limiting the number of mechanisms, and relating only to one time period.

In conclusion, our unique classification of common oral clefts provides subgroups reckoning with morphology and underlying cell biological mechanisms, and with the time period during which a given common oral cleft evolves. In this way, more accurate data may become available for further clinical and fundamental research. For international use of this new classification adjustment of the ICD-10 cleft coding system (Q35-Q37) is required with regard to sub-phenotypes, such as incomplete cleft lip/alveolus and submucous cleft palate.

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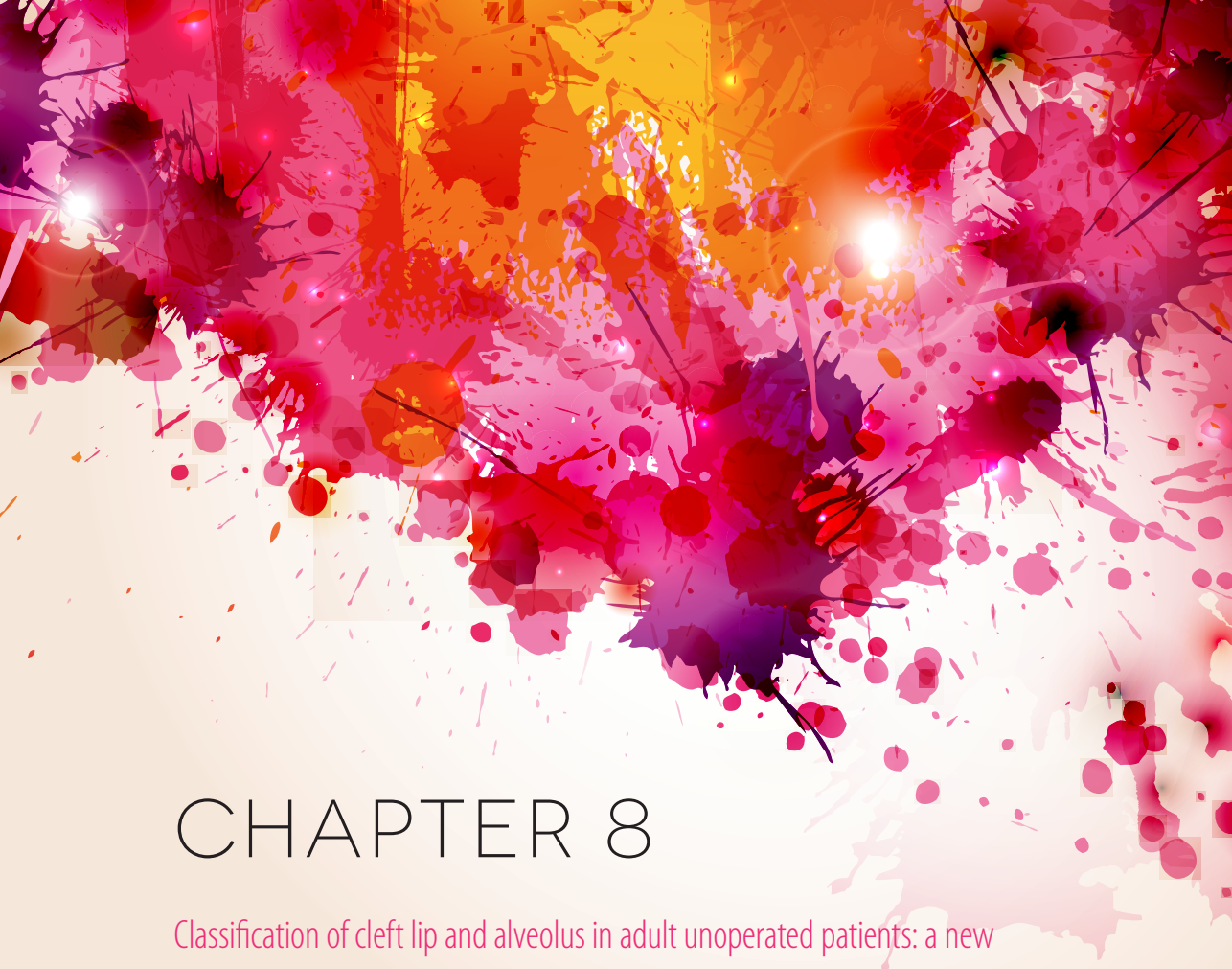

CHAPTER 8

Classification of cleft lip and alveolus in adult unoperated patients: a new embryological approach

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ABSTRACT

Background: To improve the outcome of oral clefts and further understand their etiopathogenesis, a new embryological cleft classification was previously introduced. We aimed to investigate whether this classification is complete and feasible for all cleft sub-phenotypes of the lip/alveolus, which have rather complex and underexposed patho-embryological mechanisms. Additionally, we investigated whether further morphological grading of incomplete cleft lips is clinically and embryologically relevant.

Methods: After local announcements, 108 adult unoperated patients from Indonesia with clefts of the lip/alveolus only were included. Using color photographs, X-rays, and dental casts, clefts were classified—according to their timing and mechanisms in embryogenesis—as fusion defects, differentiation defects, or combined defects. We further graded the morphology of incomplete cleft lips and analyzed whether these grades were related to the severity of alveolar clefts/hypoplasia. Permanent dentition was analyzed to investigate which alveolar part is deficient in fusion/differentiation defects.

Results: All sub-phenotypes—comprising 96 unilateral and 12 bilateral clefts—could be classified into *differentiation* (79%), *fusion* (17%), *fusion-differentiation* (2%), or *fusion & differentiation* (2%) defects. We found that the various morphological grades of cleft lip were not related to the associated alveolar clefts/hypoplasia. Additionally, all alveolar and dental deformities were located in the premaxillae.

Conclusions: This study demonstrates that this classification is complete and feasible for all clefts of the lip/alveolus, that further morphological grading of incomplete cleft lip is neither clinically nor embryologically relevant, and that the premaxilla forms the deficient part in alveolar deformities. This approach provides new subgroups for clinical/fundamental research considering timing and underlying mechanisms in embryogenesis.

INTRODUCTION

Oral clefts are very complex and heterogeneous birth defects affecting the lip/alveolus/hard palate/soft palate including the uvula. Development of these structures entails a complex series of embryonic processes, which are related to different time frames and regulated by different cell biological mechanisms and genes in embryogenesis.[1-4] In short, the primary palate (presumptive lip and alveolus) and secondary palate (presumptive hard and soft palates) are formed by subsequent outgrowth, fusion, and differentiation (into bone and musculature) of the facial swellings and palatine processes, respectively.[1, 4-7] Disruption of any of these tightly regulated processes by genetic or environmental factors during different developmental periods may result in various cleft sub-phenotypes.[4, 8-10] Therefore, accurate and detailed phenotyping and subsequent classification of clefts are vital to further understand their etiopathogenesis. In other words, the power to detect influencing factors may be weakened when heterogeneous cleft groups are treated as a single entity.[3] Moreover, it is crucial to help arrive at correct diagnosis, thereby improving clinical care and outcome.

Many systems have been developed to classify clefts. Classically, they are divided into two categories: cleft lip with or without cleft palate; and cleft palate only.[11-13] However, recent embryological and epidemiological data suggest that clefts affecting the lip only have unique genetic and etiologic features and should therefore be distinguished from those affecting both the lip and palate.[7, 9, 14-18] Besides this broad division, more detailed systems have been developed. They distinguish the different affected anatomical structures (lip/alveolus/hard palate/soft palate),[19-23] as well as various morphological features (complete/incomplete/submucous (subcutaneous) clefts).[24-30] However, none of these systems are fully based on the embryology of the primary and secondary palates, thereby lacking detailed information needed to gain more insight into the causes of clefts.

Therefore, we have developed a new classification that distinguishes the various cleft subphenotypes according to their timing and developmental mechanisms in embryogenesis.[4, 7, 10] More specifically, it allows classifying complete, incomplete, and submucous clefts, as well as hypoplasia, of the lip/alveolus and hard/soft palates (including the uvula) into groups of defects resulting from defective fusion, differentiation, or both, which are termed as fusion and/or differentiation defects. Previously, this classification was successfully applied to broad groups of clefts in Dutch newborns.[4, 7, 10] However, it is unknown whether this system is complete and feasible for all cleft sub-phenotypes, especially for those of the primary palate, which have more complex underlying mechanisms. While the embryogenesis of the secondary palate has extensively been investigated resulting in general consensus on this topic,[12] the developmental processes of the primary palate have insufficiently been discussed and several questions remain unanswered. For example, it is unknown whether further morphological grading of incomplete clefts of the lip/alveolus—as has been proposed by several studies[24-27, 29, 30]—is embryologically and clinically relevant. More specifically, it has not been described whether

the morphological severity of clefts of the lip is related to that of the associated alveolar clefts/hypoplasia. Additionally, it remains indefinite which part of the alveolar process—that formed by the premaxilla or maxilla—is deficient in these alveolar deformities.

The aim of this study was to investigate whether our new embryological classification is complete and feasible for all cleft sub-phenotypes of the primary palate. Using adult unoperated patients from Indonesia with clefts of the primary palate only, we were able to classify sub-phenotypes that had not been influenced by defective fusion/differentiation of the secondary palate. In addition, we analyzed whether the morphological severity of clefts of the lip is related to that of associated alveolar deformities, thereby investigating whether further morphological grading of incomplete cleft lip is embryologically and clinically relevant and should be added to our classification. Finally, we related permanent dentition to the location and morphological severity of alveolar deformities in order to investigate which part of the alveolar process is deficient in fusion/differentiation defects.

MATERIAL AND METHODS

Patients and data collection

After local announcement, 350 adult Indonesian patients with oral clefts presented themselves for operation. To be included, patients had to have clefts of the primary palate only without a syndrome diagnosis, previous cleft surgery, or extractions of teeth in the cleft area.[31] Median cleft lip/alveolus or atypical facial clefts were excluded because of their different pathogenesis.[6, 8, 32] For each patient, cephalograms, standard intraoral and extraoral color photographs, and dental casts were made prior to surgery. The principles outlined in the Declaration of Helsinki were followed, and details of the patient and data collection have been described elsewhere.[31]

Embryological classification

To relate the various cleft sub-phenotypes of the lip/alveolus—including further morphological grading—to their timing and underlying developmental processes, it is essential to understand the normal and abnormal embryological development of the primary and secondary palates, which therefore are described first.

Normal embryonic development of the primary and secondary palates

Fusion processes of the primary and secondary palates and their directions

It is generally accepted that the formation of the secondary palate is based on fusion of the palatal shelves (processes).[12] However, there is no consensus on the formation of the primary palate, which is thought to result from *fusion,* [2, 33-36] *merging,* [30, 37-40] or a combination of fusion and merging of the facial swellings (prominences or processes) that surround the nasal placode.[21, 41] The formation of both the primary and secondary palates can be subdivided into three

phases: 1) outgrowth of the facial swellings or palatal shelves, which are mesenchymal cores covered with epithelium; 2) opposition of the swellings/shelves followed by adhesion of their epithelium and subsequent epithelial plate (seam) formation; and 3) programmed cell death (apoptosis) followed by epitheliomesenchymal transformation and migration, resulting in disruption and subsequent disappearance of the epithelial plate and fusion of the mesenchymal cores of the swellings/shelves. Similar to the secondary palate, all three phases are present during formation of the primary palate,[5, 6, 33, 42, 43] underlining that the primary palate is formed by fusion and not by merging, as the last two phases do not occur in the process of merging.[6]

Although the fusion processes of the primary and secondary palates are roughly similar, there are also some differences. The first is that the facial swellings and palatal shelves fuse in opposite directions. The development of the primary palate starts with widely separated nasal placodes, which are located at the lateral sides of embryonic head. These placodes are transformed into nasal pits, grooves, and tubes, respectively, by the three outgrowing facial swellings (medial nasal process, maxillary process, and lateral nasal process) (Figure 1: 1-3). These outgrowing facial swellings, which are separated by grooves, adhere from occipital to frontal and form an epithelial plate (the nasal fin).[36, 43-45] Within this plate, cell death occurs before, during, and after formation, which results in a disruption halfway the epithelial plate.[6, 41, 46] Then cell death continues within the remnants of the epithelial plate, the three swellings fuse gradually, and the primary palate is formed (human embryos 11-17 mm crown-rump length (CRL)).[6, 46] The medial nasal process fuses first occipitally with the maxillary process and then frontally with the lateral nasal process.[6, 47] As a consequence, the external nasal aperture (nostril) is formed by the medial nasal and lateral nasal processes (Figure 1: 1-3).

In contrast to the primary palate, the secondary palate is formed from frontal to occipital, starting with adherence and fusion of the primary palate to the frontal borders of the palatal shelves (Figure 1: 3-6). Note that fusion of the secondary palate occurs after fusion of the primary palate; the facial swellings fuse during the early embryonic period, 4-7 weeks of development (≤17 mm CRL), while the palatal shelves fuse during the late embryonic period, 7-12 weeks of development (≥17 mm - ≤60 mm CRL).[4, 6]

Another difference between the primary and secondary palates is that the formation of the primary choanae is based on the development and subsequent rupture of the oronasal membranes (bucconasal membranes), while the secondary choanae are formed by the openings into the nasopharynx after formation of the secondary palate. During the development of the primary palate, the nasal pits/grooves/tubes have newly formed cavities, which belong in fact to the amniotic cavity and not to the stomodeum (oral cavity).[36, 44] The floors of these two blind ending cavities are formed by the fusing facial swellings, which enclose the epithelial plates. From the occipital part of these epithelial plates, the oronasal membranes develop and subsequently rupture by cell death, thereby forming the primary choanae (12-17 mm CRL; Figure 1: 3-4).[6, 36, 41] Occipitally, the nasal tubes now open into the stomodeum. Then the secondary palate develops and the primary choanae are frontally demarcated by the incisive canals. Finally,

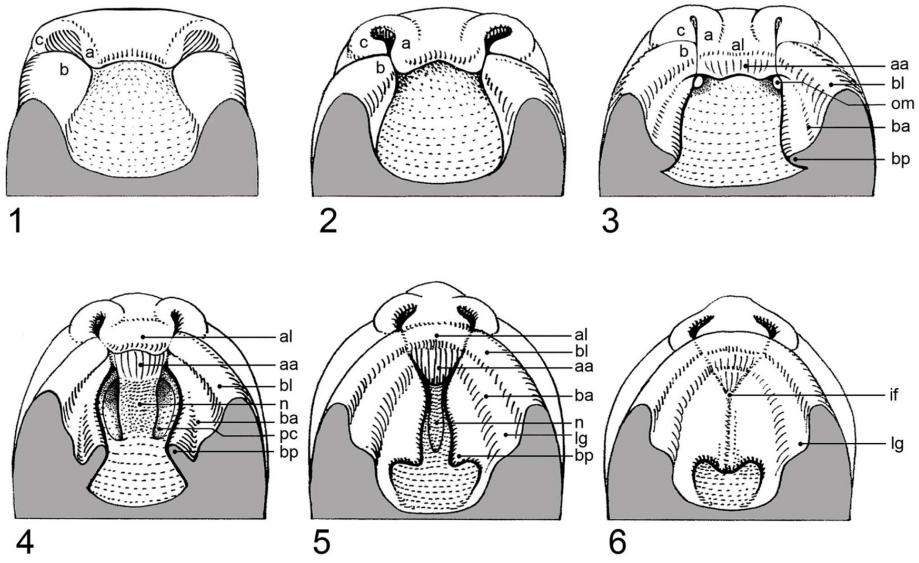

Figure 1. Embryonic development in successive stages viewed from the oral side: the fusion processes of the primary palate (1-3) and secondary palate (3-6), and differentiation of the lip and alveolus (3-6).

1. The nasal groove surrounded by the facial swellings (a-c) at five weeks;
2. Outgrowth and fusion of two (a-b) of the three facial swellings in occipito-frontal direction forming the nasal tubes at six weeks;
3. Further outgrowth and fusion of the three swellings (a-c), resulting in the formation of the primary palate and the external nasal aperture at about seven weeks (note that the swellings are separated by grooves), including the position of the oronasal membrane (om) and the beginning of development of the lip (al + bl), alveolus (aa +ba) and the shelves of the secondary palate (bp);
4. Formation of the primary choanae (pc), outgrowth of the nasal septum (n) and palatal shelves in vertical direction, and outgrowth of the lip and alveolus in caudal direction forming the presumptive labial groove at eight weeks;
5. Elevation and outgrowth of the palatal shelves in horizontal position, and start of the fusion of the shelves with the primary palate, frontally from the primary choanae (level presumptive incisive foramen) at eight to nine weeks;
6. Completed fusion of the shelves in fronto-occipital direction with the primary palate and nasal septum, as well as with each other, and completion of the lip, alveolus, incisive foramen (if), and labial groove (lg) at 10-12 weeks.

Abbreviations: a = medial nasal process; b = maxillary process; c = lateral nasal process; al = lip developed from a; bl = lip developed from b; aa = alveolus (premaxillae) developed from a; ba = alveolus (maxillae) developed from b; bp = palatal shelves developed from b; om = oronasal membrane; n = nasal septum; pc = primary choana; lg = labial groove; if = incisive foramen.

the definitive nasal cavities are formed from the nasal tubes and from a part of the stomodeum (captured through fusion of the palatal shelves); their openings into the pharynx are formed by the secondary choanae.[48]

Formation of the lip and alveolus & mesenchymal differentiation of the lip, alveolus, hard and soft palates

During the late embryonic period, the grooves between the swellings in the embryonic face are eliminated by merging.[6, 36, 46] The lip and alveolus develop from the primary palate, and the mesenchyme of the primary and secondary palates subsequently differentiates into bone centers and musculature. The presumptive lip and alveolus grow out in caudal direction, resulting in formation of the labial groove (Figure 1: 3-6). The first bone centers, which form the alveolus and secondary palate, arise in the mesenchyme of each maxillary process, i.e., the presumptive maxilla (17 mm CRL). Each maxilla and each palatal bone (anlage at 23 mm CRL) develops from a single bone center, while the premaxilla—bearing both incisors—develops from two centers in the medial nasal process (the frontal center at 23 mm CRL and occipital center at 50 mm CRL). All bone centers grow out and either fuse with each other (the frontal premaxillary center with the maxillary center and with the occipital premaxillary center, respectively) or form a suture (that is, the incisive suture between the occipital premaxillary center and maxillary center, and the median and transverse palatine sutures between both maxillary and palatine centers).[6]

Classification

As presented in Table 1, derailments in the fusion phases of the primary and/or secondary palates, including the formation of the primitive choanae, may cause various fusion defects along the respective fusion lines. For example, interrupted fusion of the primary palate during the early embryonic period (4-7 weeks of development) may result in a *complete cleft lip with complete/ incomplete cleft alveolus*, while defective fusion of the secondary palate during the late embryonic period (7-12 weeks of development) may give rise to *a complete/incomplete hard palate with complete soft palate*. Note that differences in anatomical extent and morphological severity are explained by the fusion direction of the facial swellings (occipito-frontal) and palatal shelves (fronto-occipital), and by the development of the primary choanae during fusion of the facial swellings.[4] If disruption occurs at a later stage during the fusion process, more of the primary and secondary palates will be intact.

Similarly, derailments in the formation of lip/alveolus—after the completed fusion process—and in the subsequent mesenchymal differentiation (into bone and musculature) of the primary and secondary palates may cause differentiation defects (Table 1). More specifically, insufficient merging, outgrowth, or differentiation of the lip/alveolus may result in *incomplete clefts of the lip and/or alveolus*. These sub-phenotypes always show a tissue bridge at the base of the nostril, as the fusion process of the lip/alveolus has been completed at this stage.[4] Additionally, derailments in the differentiation of musculature and bone anlage and in the outgrowth,

Table 1. Classification of the individual cleft anomalies of the primary and secondary palates according to timing and underlying fusion and differentiation mechanisms in embryogenesis (adapted from Rozendaal et al. and Luijsterburg et al.)[4, 10]

Early Embryonic Period (4 to 7 Weeks Postconception)	**Late Embryonic Period (7 to 12 Weeks Postconception)**	
Primary palate		
Fusion defects	*Differentiation defects*	
• Complete cleft lip	• Incomplete cleft lip	
• Complete cleft alveolus (extending to the incisive foramen)	• Submucous cleft lip*	
• Incomplete cleft alveolus (if the lip is normal or has a complete cleft)	• Hypoplastic lip	
	• Incomplete cleft alveolus (if the lip has an incomplete/submucous cleft)	
	• Submucous cleft alveolus	
	• Hypoplastic alveolus	
Secondary palate		
	Fusion defects	*Differentiation defects*
	• Complete cleft hard palate	• Submucous cleft hard palate
	• Incomplete cleft hard palate	• Hypoplastic hard palate
	• Complete cleft soft palate	• Submucous cleft soft palate (including uvula)
	• Incomplete cleft soft palate	• Hypoplastic soft palate (including uvula)
	• Complete cleft uvula	
	• Incomplete cleft uvula	

* Synonyms: congenital scar, forme fruste, and subsurface, subcutaneous, or microform cleft lip.

fusion, or suture formation of bone centers may cause bony defects, such as submucous clefts or hypoplastic bone/musculature in the primary or secondary palates.

Data analysis

Using patients' intraoral and extraoral color photographs, we analyzed the morphological severity (complete vs. incomplete/submucous (subcutaneous)) of lip defects. Subsequently, incomplete clefts of the lip were further graded into three groups: I) vermilion notch; II) cleft 1/3 to 2/3 lip height; and III) cleft 2/3 lip height to subtotal. Dental casts were used to analyze the location and morphological severity of alveolar clefts or hypoplasia; on these casts, we identified and counted teeth in the premaxilla and maxilla. Absent (agenetic), impacted, or supernumerary teeth in the cleft area were examined on the corresponding lateral cephalograms.

To classify the various cleft sub-phenotypes of the lip/alveolus, they were divided into fusion and/or differentiation defects of the primary palate (Table 1). Theoretically, more than one developmental process can be disturbed during embryogenesis, and therefore any combination of defects is possible. To be complete and feasible, the system must allow all combinations to be grouped into one of the following subgroups: *fusion defects*, *differentiation defects*, or combined defects (*fusion-differentiation defects*). An example of the latter group is an incomplete cleft lip (differentiation) with complete cleft alveolus (fusion) at the same side, termed as Simonart's band. As a bilateral cleft can consist of a fusion defect at one side and differentiation defect at the other side, we presented these defects as *fusion & differentiation defects.*

RESULTS

In total, 108 adult patients—comprising 63 males and 45 females, all over 12 years of age—were included. Table 2 shows the classification of their unilateral (n = 96) and bilateral (n = 12) clefts of the primary palate. All sub-phenotypes could be classified into *differentiation defects* (n = 85, 79%), *fusion defects* (n = 19, 17%), *fusion-differentiation defects* (n = 2, 2%), or *fusion & differentiation defects* (n = 2, 2%).

Table 2. Classification of the various sub-phenotypes in adult unoperated patients with clefts of the primary palate (n = 108) into fusion and/or differentiation defects

	Fusion Defect	Differentiation Defect	Fusion - Differentiation defect*	Fusion & Differentiation defect†	Total
Unilateral	17	77	2	–	96
Bilateral	2	8	–	2	12

* Combination of a fusion and differentiation defect at one side.

† Fusion defect at one side and differentiation defect at the other side.

Unilateral clefts

Complete clefts of the lip

Of the 96 patients with unilateral clefts, 18% (n = 17) showed a *complete cleft lip*. Table 3 presents the different alveolar deformities, including abnormal dentition, found in these patients. 59% (n = 10) showed a *complete cleft alveolus* (extending to the incisive foramen; Figure 2a,b), and 41% (n = 7) showed an *incomplete cleft alveolus* along the fusion line between the premaxilla and maxilla (Figure 3a,b). All 17 patients showed abnormal dentition at the cleft side and the alveolar deformity was located between the central and malformed/hypoplastic lateral incisors or at the level of the absent lateral incisor. As all observed clefts result from defective fusion of the facial swelling, they were classified as fusion defects (Table 2).

Table 3. Alveolar deformities in unilateral complete (n = 17) and incomplete (n = 79) cleft lip; those with abnormal dentition at the cleft sides are presented between parentheses*

	Normal Alveolus		Complete Cleft Alveolus		Incomplete Cleft Alveolus		Hypoplastic Alveolus		Total	
Complete cleft lip	–	–	10	(10)	7	(7)	–	–	17	(17)
Incomplete cleft lip	31	(23)	2	(2)	28	(28)	18	(18)	79	(71)
I. Vermilion notch	4	(3)	–	–	2	(2)	2	(2)	8	(7)
II. Cleft 1/3 to 2/3 lip height	17	(12)	–	–	8	(8)	7	(7)	32	(27)
III. Cleft 2/3 lip height to subtotal	10	(8)	2	(2)	18	(18)	9	(9)	39	(37)

* Including absence (agenesis), malformation, or hypoplasia of the lateral incisor, as well as supernumerary teeth or a persistent milk canine.

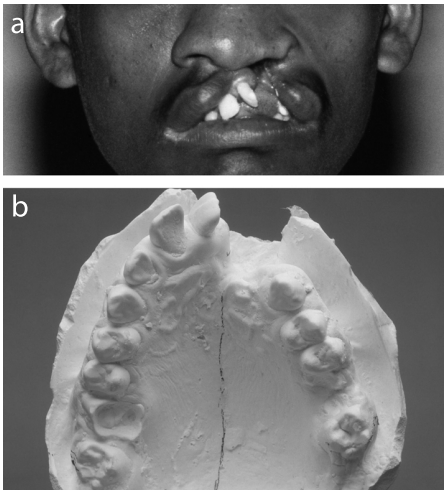

Figure 2. Unilateral complete cleft lip (a) with complete cleft alveolus (extending to the incisive foramen) at the level of the absent lateral incisor and with rotation of the premaxilla (b).

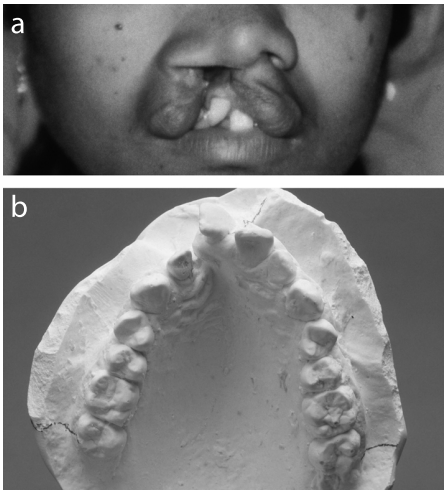

Figure 3. Unilateral complete cleft lip (a) with incomplete cleft alveolus along the fusion line between the premaxilla and maxilla located between the central and hypoplastic lateral incisors (b)

Incomplete clefts of the lip

Incomplete cleft lip was diagnosed in 82% (n = 79) of the unilateral cleft patients and further divided into three morphological groups (Table 3).

Group I comprised eight patients, with a normal alveolar process in 50% (n = 4; Figure 4a,b), *incomplete cleft alveolus* in 25% (n = 2), and horizontal or vertical alveolar hypoplasia in 25% (n = 2). For the last four patients and for one with a normal alveolar process, the photographs indicated that the vermilion notch was accompanied by a submucous (subcutaneous) cleft of the lip. Almost all patients (88%, n = 7) showed dental anomalies at the cleft side (Table 3).

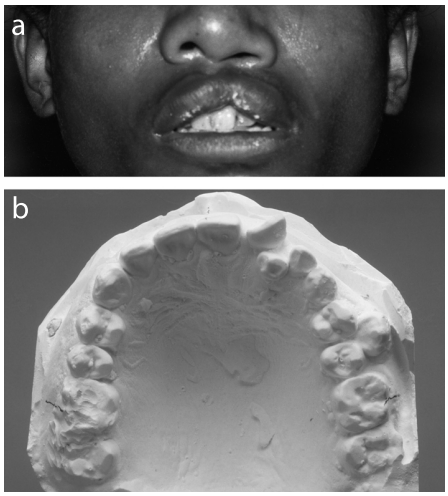

Figure 4. Notch of the vermilion (a) with a normal alveolus and rotated lateral incisor, persistent milk canine, and supernumerary teeth (b).

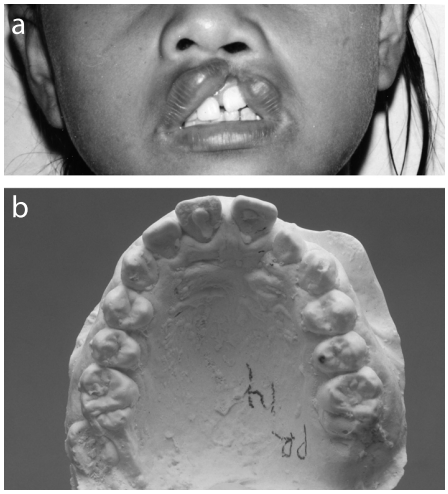

Figure 5. Incomplete cleft lip (a) with incomplete cleft alveolus between the central and malformed lateral incisors (b).

Group II comprised 32 patients, with a normal alveolar process in 53% (n = 17), *incomplete cleft alveolus* in 25% (n = 8; Figure 5a,b), and horizontal or vertical alveolar hypoplasia in 22% (n = 7). The majority (84%, n = 27) showed dental anomalies at the cleft side.

Group III comprised 39 patients, with a normal alveolar process in 26% (n = 10), *incomplete cleft alveolus* in 46% (n = 18), horizontal or vertical alveolar hypoplasia in 23% (n = 9), and *complete cleft alveolus* in 5% (n = 2; Figure 6a,b). Almost all (95%, n = 37) showed dental anomalies at the cleft side.

Finally, all *complete/incomplete clefts of the alveolus* were located between the central and malformed/hypoplastic lateral incisors or at the level of the absent lateral incisor, and alveolar hypoplasia was always located at the position of the lateral incisor. Patients with a normal

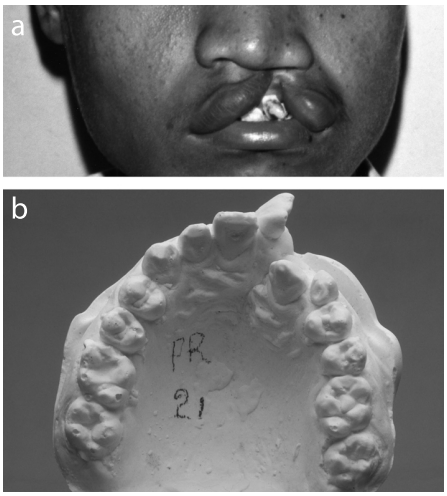

Figure 6. Simonart's band: incomplete (subtotal) cleft lip (a) with complete cleft alveolus (extending to the incisive foramen) at the level of the absent lateral incisor with a persistent milk canine and rotation of the premaxilla (b).

alveolus, *incomplete cleft alveolus*, or alveolar hypoplasia (98%, n = 77) were classified as having a differentiation defect, because their defects result from defective differentiation. Only two patients (3%) showed a *complete cleft alveolus*, which results from defective fusion, and they were therefore classified as fusion-differentiation defects (Table 2).

Bilateral clefts

Complete clefts of the lip

Of the 12 patients with bilateral clefts, two (17%) showed a bilateral *complete cleft lip* (Table 4). In one of them, this defect was accompanied by a bilateral *complete cleft alveolus* with absence of both lateral incisors. The other patient showed a left-sided *complete cleft alveolus* at the level of the absent lateral incisor, as well as a right-sided *incomplete cleft alveolus* in between two cone-shaped teeth without a normal lateral incisor. Both patients were classified as bilateral fusion defects (Table 2).

Complete and incomplete clefts of the lip

Two (17%) of the 12 bilateral cleft cases had a *complete cleft lip* at one side and *incomplete cleft lip* (1/3 to 2/3 lip height) at the other side (Table 4). One of them also showed a bilateral *incomplete cleft alveolus* between the right central and lateral incisors and at the level of the left absent lateral incisor. The other patient had a left-sided hypoplastic alveolus with a malformed lateral incisor, and a right-sided *complete cleft alveolus* at the level of the absent lateral incisor. Both patients were classified as having a fusion defect at one side and a differentiation defect at the other side, that is a fusion & differentiation defect (Table 2).

Table 4. Alveolar deformities in bilateral cleft lip (n = 12); those with abnormal dentition at the cleft sides are presented between parentheses*

	Normal Alveolus		Bilateral Complete Cleft Alveolus		Complete & Incomplete Cleft Alveolus		Bilateral Incomplete Cleft Alveolus		Complete Cleft & Hypoplastic Alveolus		Normal & Incomplete Cleft Alveolus		Total	
Bilateral complete cleft lip	–	–	1	(1)	1	(1)	–	–	–	–	–	–	2	(2)
Complete & incomplete cleft lip	–	–	–	–	–	–	1	(1)	1	(1)	–	–	2	(2)
Bilateral incomplete cleft lip	3	(3)†	–	–	–	–	4	(4)	–	–	1	(1)	8	(8)
Total	3	(3)	1	(1)	1	(1)	5	(5)	1	(1)	1	(1)	12	(12)

* Including absence (agenesis), malformation, or hypoplasia of the lateral incisor, as well as supernumerary teeth or a persistent milk canine.

† One patient had normal dentition at the left side.

Incomplete clefts of de lip

Eight (67%) of the 12 bilateral cleft cases showed a bilateral *incomplete cleft lip* extending to 2/3 lip height to subtotal (Table 4). In 38% (n = 3), a normal alveolar process was observed at both sides. In the other patients, an associated bilateral (50%, n = 4) or unilateral (13%, n = 1) *incomplete cleft alveolus* was observed. While two alveolar defects were located between the lateral incisor and a cone shaped extra tooth, the other seven were located between the central and malformed/hypoplastic lateral incisors or at the level of the absent lateral incisor. Finally, all patients showed abnormalities in dentition and were classified as bilateral differentiation defects (Table 2).

DISCUSSION

This study demonstrates that this new embryological approach is complete and feasible for clefts of the primary palate. We were able to classify all observed sub-phenotypes into fusion defects, differentiation defects, or combinations of these. By further grading incomplete clefts of the lip according to morphological severity, we found that these grades are not related to the severity of associated alveolar deformities and thus are neither clinically nor embryologically relevant. Finally, we found that all alveolar and dental deformities were located in the premaxillae.

Strengths and weaknesses

The main strength of our study is that we used unoperated adult patients with clefts of the primary palate only, allowing us to evaluate mature sub-phenotypes that had not been influenced by defective fusion/differentiation of the secondary palate. Furthermore, their permanent dentition was essential to accurately determine the location of the alveolar deformity and which part of the alveolar process is deficient in fusion/differentiation defects. However, this design also had its limitation. Because only patients who required cleft surgery had presented themselves after local announcements in Indonesia, some sub-clinical features—such as isolated hypoplasia or submucous (subcutaneous) clefts of the lip—were not included in our study. However, these anomalies were recently successfully classified in a study with unoperated newborns in the Netherlands.[4]

Explanations and implications

Classification with embryological basis

Our unique classification is based on normal palatogenesis, thereby fulfilling the criteria described by Sandham et al.: "a classification of clefting should be descriptively clear and have an embryological basis".[49] With regard to the search for causal factors, it is important to divide clefts into fusion and/or differentiation defects, because these defects develop in different

successive periods and are related to different genes and cell-biological mechanisms.[1, 4, 7] For example, a *complete cleft lip with complete/incomplete cleft alveolus* results from defective fusion of the facial swellings and consequently develops only during the early embryonic period (4-7 weeks postconception). By contrast, an *incomplete cleft lip* results from defective merging/outgrowth/differentiation, after the fusion process during the late embryonic period (7-12 weeks postconception; Table 1).[1, 4, 7] Whether an *incomplete cleft alveolus* is a fusion or differentiation defect depends on the morphology of the lip (complete vs. incomplete/submucous), and therefore the lip should always be evaluated first.

Furthermore, with our concept of fusion/differentiation defects, special sub-phenotypes—such as Simonart's bands (Figure 5)—can also be explained. Previous studies described two types of Simonart's bands: a soft tissue bridge covered with skin and located at the base of the nostril, or a mucous tissue bridge located between the segmented alveolar processes.[50, 51] Although the exact developmental mechanisms of these bands have not been identified yet, Semb and Shaw described two mechanisms.[50] The first is that these abnormalities result from incomplete fusion of the lateral and medial nasal processes, and the second is that the deformities are caused by postfusion rupture of tissues. However, based on the earlier-described embryology, we believe that the two types of Simonart's bands may be explained differently. Given that the skin covered band at the base of the nostril is formed by normal fusion of the lateral and medial nasal processes (i.e., the frontal side of the nasal fin), this Simonart's band can be considered as an incomplete cleft of the lip.[47] Therefore, we classified the tissue bridge in our two cases with *incomplete cleft lip and complete cleft alveolus* as a differentiation defect. The *complete cleft alveolus*, however, is a fusion defect, as it is caused by derailments within the occipital part of the nasal fin, which is formed by fusion of the medial nasal and maxillary processes (the region of the oronasal membrane).[6, 36] To explain the *complete cleft alveolus* in case of the Simonart's band, we have two hypotheses. The first is that if the oronasal membrane develops too wide, subsequent rupture of this membrane by cell death may cause a *complete cleft alveolus* in front of the primary choana.[4] Although it is well known that persistence of this membrane causes choanal atresia,[52] this membrane has, to our knowledge, not been related to *complete cleft alveolus* before. Another possible explanation is that the remnant of the nasal fin that connects with the oronasal membrane does not disappear by cell death and consequently opens again resulting in a *complete cleft alveolus*.[4]

Regarding the second type of Simonart's band, we postulate that the mucous tissue bridge between the segmented alveolar processes can be considered as a submucous cleft of the alveolus caused by insufficient outgrowth of the bone centers of the premaxilla and maxilla. This anomaly can be classified as a differentiation defect of the alveolus combined with fusion defect of the lip (*complete cleft lip)*.

Morphological grading

Our results do not support the suggestion of Jensen et al.[25] that the morphological severity of the cleft lip can predict the deformity of the alveolar process, and therefore, further morphological grading of incomplete clefts seems to be neither clinically nor embryologically relevant. More specifically, complete cleft lip (extent grade 4, Jensen et al.[25]) was associated with either complete or incomplete cleft alveolus (fusion defects in both combinations), as well as with abnormal dentition similar to those in incomplete cleft lip. Additionally, by further grading of incomplete cleft lip into three groups of different cleft heights (extent grades 1-3, Jensen et al.[25]), we found that all three groups included patients with normal alveolar processes and dentition, as well as patients with alveolar clefts or hypoplasia. Apart from the complete cleft alveolus in the Simonart's cases (group III, n = 2), all anomalies were classified as differentiation defects. Therefore, our results underline that deformities of the lip and alveolus should be evaluated as separate entities,[22, 24, 28, 29] because of their independent morphological characteristics.

Deficient part alveolar process

Analysis of permanent dentition revealed that all observed alveolar deformities were located between the central and lateral incisors, or at the level of the lateral incisor. The latter tooth was often absent, hypoplastic, or replaced by one or two malformed teeth. According to Stark and Kaplan [53] and Sperber [46], both incisors develop within the premaxilla. This implies that the developmental arrest involves the premaxilla, which develops from the medial nasal process during early embryonic stages. Our findings are in line with those of Lekkas et al.,[54] who did not found any missing permanent teeth in the canine-postcanine region of the maxilla in adult unoperated Indonesian patients.

Description and registration of clefts

Adequate characterization and description of the individual anomalies that form the various cleft sub-phenotypes is critical for both clinical and research purposes, as it helps to arrive at correct diagnosis, underlying mechanisms, and causal factors. It is important to also describe submucous and microform clefts (including orbicularis oris muscle defects), given that these sub-clinical forms have serious clinical implications and may be just as important for furthering our understanding of clefts.[2, 3] However, in most registration systems,[11, 55, 56] subclinical features are often not included, and clefs are not described but interpreted and directly coded according to clinical diagnosis, which may lead to important information being lost. Therefore, the cleft palate teams in the Netherlands have used a different approach, similar to that of the current classification. For over 15 years, they have registered oral clefts in the Dutch Oral Cleft Registry, using a unique system—based on craniofacial embryology—that records the morphology and side of each affected anatomical structure.[10, 57, 58] These descriptive data can be translated to any classification, new or old, and have enabled clinical, epidemiological, and fundamental research and improved clinical care and outcome.[4, 7, 9, 13, 16, 17] In line with other

recent studies emphasizing the need of accurate and detailed phenotyping,[1-3] we encourage future studies and registries to use a similar approach to further elucidate the etiopathogenesis of clefts.

CONCLUSIONS

This study demonstrates that this new embryological approach is complete and feasible for clefts of the primary palate, as all sub-phenotypes—including Simonart's bands—could be classified into fusion and/or differentiation defects. Whether a cleft lip/alveolus is a fusion or differentiation defect depends on the morphology of the lip (complete vs. incomplete/submucous), and therefore the lip should always be evaluated first. Additionally, our study showed that further morphological grading of incomplete cleft lip does not predict the severity of alveolar deformities and is thus neither clinically nor embryologically relevant. Therefore, the alveolar process should always be evaluated separately in order to have complete and accurate diagnoses and improve outcome and research. Finally, we found that the deficient part in alveolar deformities is formed by the premaxilla. This approach provides new subgroups considering timing and underlying developmental mechanisms in embryogenesis, thereby enabling more accurate clinical and fundamental research.

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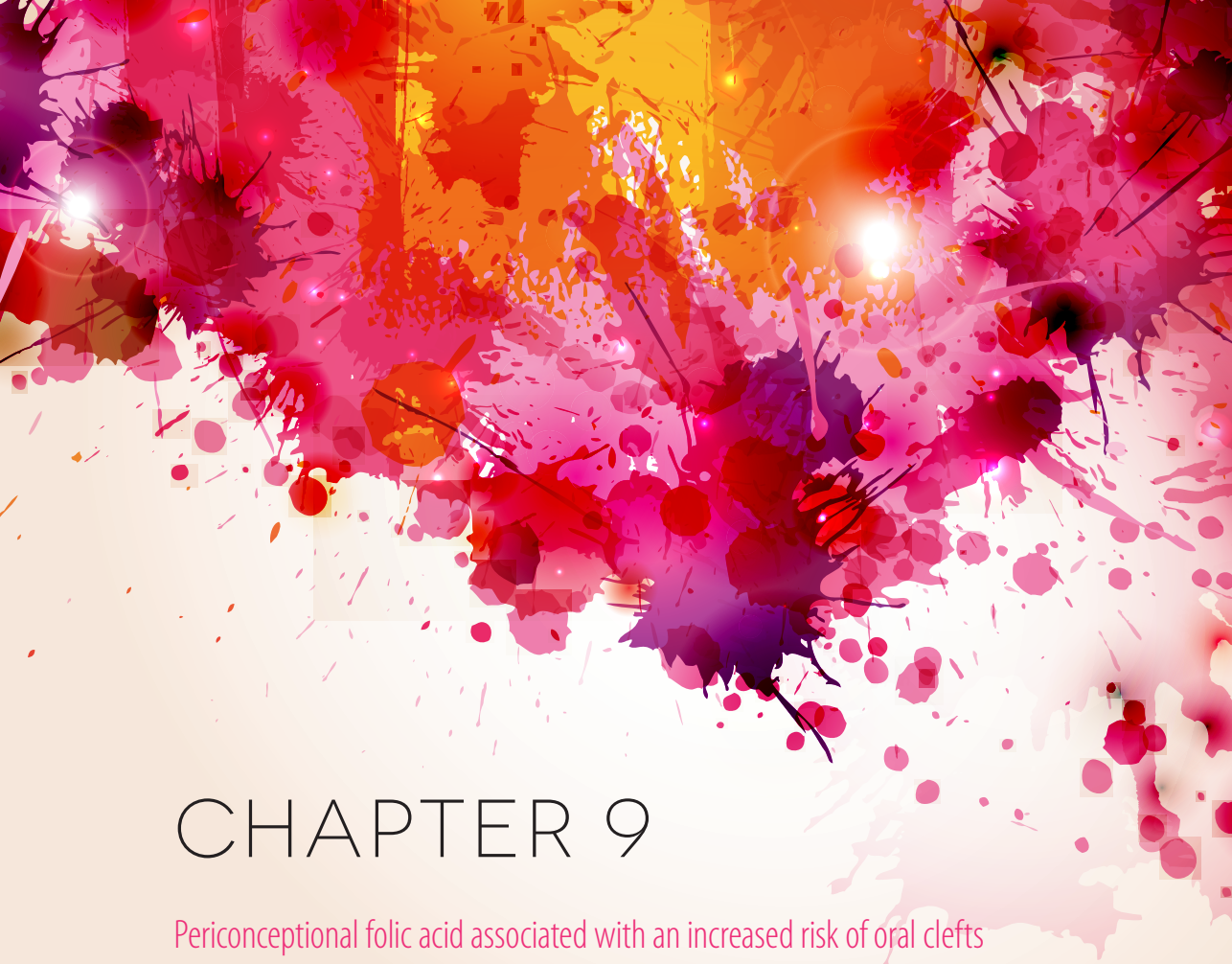
PART IV

Effects of periconceptional folic acid supplementation



CHAPTER 9

Periconceptional folic acid associated with an increased risk of oral clefts relative to non-folate related malformations in the Northern Netherlands—a population based case-control study

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ABSTRACT

Periconceptional folic acid has been associated with a reduced risk of neural tube defects, but findings on its effect in oral clefts are largely inconclusive. This case-control study assesses the effects of periconceptional folic acid on cleft risk, using complementary data from the Dutch Oral Cleft Registry and a population-based birth defects registry (Eurocat) of children and fetuses born in the Northern Netherlands between 1997-2009. Cases were live-born infants with non-syndromic clefts (n = 367) and controls were infants or fetuses with chromosomal/syndromal (n = 924) or non-folate related anomalies (n = 2021). We analyzed type/timing/duration of supplement use related to traditional cleft categories as well as to their timing (early/late embryonic periods) and underlying embryological processes (fusion/differentiation defects). Consistent supplement use during the etiologically relevant period (weeks 0-12 postconception) was associated with an increased risk of clefts (adjusted odds ratio 1.72, 95% confidence interval 1.19-2.49), especially of cleft lip/alveolus (3.16, 1.69-5.91). Further analysis systematically showed two- to three-fold increased risks for late differentiation defects—mainly clefts of the lip/alveolus—with no significant associations for early/late fusion defects. Effects were attributable to folic acid and not to other multivitamin components, and inclusion of partial use (not covering the complete etiologically relevant period) generally weakened associations. In conclusion, this study presents several lines of evidence indicating that periconceptional folic acid in the Northern Netherlands is associated with an increased risk of clefts, in particular of cleft lip/alveolus. This association is strengthened by the specificity, consistency, systematic pattern, and duration of exposure-response relationship of our findings, underlining the need to evaluate public health strategies regarding folic acid and to further investigate potential adverse effects.

INTRODUCTION

There is general consensus that periconceptional folic acid supplementation reduces the risk of neural tube defects. However, although the role of folic acid in oral clefts has been investigated for over 20 years, evidence for a preventive effect in clefts is still lacking and its role remains unresolved.[1-3]

Oral clefts—one of the most common congenital anomalies in humans—are complex and heterogeneous defects, ranging from mild types to complete clefts affecting the lip, alveolus, and palate. While they can occur as part of a broad range of Mendelian, chromosomal, or teratogenic syndromes,[4, 5] they most commonly occur in isolation. Despite extensive research, the etiopathogenesis of these non-syndromic (isolated) forms remains largely unknown. They are thought to result from a complex interplay of genetic and environmental factors.[4-7] Development of the lip, alveolus, and palate entails a complex series of embryological processes, which are related to different time frames in embryogenesis and regulated by many different genes and cell-biological mechanisms.[4, 6-8] Disruption of any of these tightly regulated processes may result in various cleft sub-phenotypes.[4, 6-10] Therefore, accurate and detailed phenotyping and subsequent subdivision according to timing and underlying processes is crucial for furthering our understanding of cleft etiology.

Folate deficiency has been causally related to several congenital anomalies, especially neural tube defects. The neural tube and craniofacial region have quite similar developmental mechanisms,[1, 3] leading to the hypothesis that folate deficiency might also contribute to non-syndromic clefts. Although multiple (non-randomized) observational studies have suggested a beneficial role of folic acid supplements in decreasing cleft risk, the evidence remains largely inconclusive, as many studies—including randomized and cohort controlled trials—identified no significant effects on clefts.[1-3, 11-13] Results are often mixed in terms of estimated effects, whether they affect certain or all cleft categories, and whether they are attributable to folic-acid containing multivitamins or folic acid alone. This is partly caused by differences in study populations and designs (including adjustment for confounders).[1, 2] Additionally, the composition of supplements as well as timing and duration of use varies greatly between studies. Often, supplementation is not subdivided by type (folic acid alone or combined with multivitamins) and does not completely cover the embryonic period of clefts (4-12 weeks postconception).[2, 6, 8] Another explanation for insufficient evidence might be that heterogeneous cleft groups are generally treated as a single entity to reach adequate statistical power. However, this crude approach may weaken the power to detect effects, given the etiologic and genetic heterogeneity underlying non-syndromic clefts.[4, 7]

We conducted a case-control study to assess the effects of periconceptional folic acid supplements on the risk of oral clefts relative to other non-folate related congenital anomalies. By combining unique complementary data from the Dutch Oral Cleft Registry (NVSCA) and a population-based birth defects registry (Eurocat Northern Netherlands), we were able to

analyze type, timing, and duration of supplement use in relation to timing and embryological mechanisms underlying cleft development.

METHODS

Study design and population

We used NVSCA and Eurocat data on children/fetuses born in the Northern Netherlands between 1997 and 2009 inclusive.

The NVSCA is a national register maintained by the Dutch Association for Cleft Palate and Craniofacial Anomalies. Since 1997, the 15 multidisciplinary cleft palate teams in the Netherlands have reported their anonymous live-born patients with clefts (no age limit), prior to cleft surgery. The teams treat virtually all surviving children with clefts who reside in the Netherlands, with a yearly average of 331 new patients, including 40 patients from the Northern Netherlands.[14] To optimize data quality, data are verified on a case-by-case basis, and the teams perform case-ascertainment activities. Additionally, the NVSCA database has been systematically validated.[9]

The population-based Eurocat registry has registered congenital anomalies in the Northern Netherlands since 1981 and now monitors about 18,500 births annually. While it has registry-specific methods for case ascertainment,[15] coding and classification are in concordance with EUROCAT Central Registry guidelines.[16] Children (up to ten years of age at notification) and fetuses with congenital anomalies are reported by midwifes, well-baby clinic doctors, and specialists. In addition, various sources—mainly hospital registries—are actively searched to find children or pregnancies eligible for registration, including spontaneous abortions and pregnancies terminated for congenital anomalies. Written parental informed consent is needed for registration, and the participation rate is approximately 80%.

Data collection

Live-born infants with clefts in the Northern Netherlands are reported to the NVSCA by a plastic surgeon during the first patient visit to the team. Using a unique recording system based on the embryology of the head and neck area,[8-10] the individual cleft anomalies are described in detail by recording the affected anatomical structure (lip/alveolus/hard palate/soft palate/uvula), the morphology (complete/incomplete/submucous), and the side (left/right/median). Additionally, infant and parental characteristics and diagnoses of associated congenital anomalies are recorded.

In Eurocat, congenital anomalies—including clefts—are coded according to the "International Classification of Diseases" (ninth/tenth revision).[16] Once an infant has been reported, further information is gathered from the mother. Parents are asked to complete a written questionnaire on medical and reproductive history, occupation, demographic characteristics,

maternal weight and height, smoking habits, alcohol consumption, and use of medications and supplements (with specific questions about folic acid and multivitamins) from three months before pregnancy through to delivery. In addition, data on any prescribed medication is retrieved from the pharmacy after written parental consent. Subsequently, specific information on whether medications have been actually taken, including the period of use, is verified with the mother in a telephone interview.

Definition of cases and controls

We defined cases as live-born infants with non-syndromic clefts, in other words, those not associated with other major non-folate related congenital anomalies. Median cleft lip/alveolus and atypical facial clefts were not considered to be oral clefts because of their different pathogenesis.[17] For inclusion, cases had to be registered in both databases, which allowed us to combine detailed information on cleft phenotype (NVSCA) with complementary data on maternal characteristics and folic acid supplement use (Eurocat). Data linkage was performed using key (e.g., date of birth and gender) and cleft information. Discrepancies between registries regarding this information were verified with the cleft palate teams and, if applicable, corrected in the study database. Non-matching cases from either registry were similarly verified, and if sufficient matching information was found they were added to the database. Using these steps, 91.6% (463/505) of the Eurocat and 76.0% (443/583) of the NVSCA cases were matched. The reasons why cases could not be matched are displayed in Figure 1. As shown here, the main reason that NVSCA cases had not been registered in Eurocat was the lack of permission from parents for registration in Eurocat (n = 34). Note that these cases showed a cleft distribution generally similar to that of the matched cases. Conversely, Eurocat cases were not registered in the NVSCA mainly due to postnatal death (n = 31). However, except for 2 cases, all died because of syndromal or chromosomal anomalies, and therefore these infants would have been excluded from the study anyway. As presented in Figure 1, a total of 474 cases were finally matched, and after exclusion of the syndromic (associated) matched cases (n = 48), a total of 426 potential cases remained in the study.

Eurocat does not register non-malformed children, and controls were therefore defined as infants or fetuses with chromosomal/syndromal defects or non-folate related congenital anomalies. The rationale for choosing chromosomal and syndromal defects is that the origin of these disorders is not related to folic acid. The use of malformed controls from birth years and geographical areas similar to those of the cases is widely accepted and beneficial with regard to internal validity, as it minimizes the potential for differential recall bias and other possible sources of differential exposure ascertainment compared to controls without congenital anomalies.[18-22] We excluded infants with anomalies previously associated with folic acid or having developmental mechanisms similar to clefts (neural tube defects, n = 213; congenital heart defects, n = 1692; hypospadias, n = 472; body wall defects, n = 91; limb reduction defects,

n = 157; iris colobomas, n = 16; and diaphragmatic hernias, n = 43),[1, 3, 22-24] resulting in a total of 3754 potential controls.

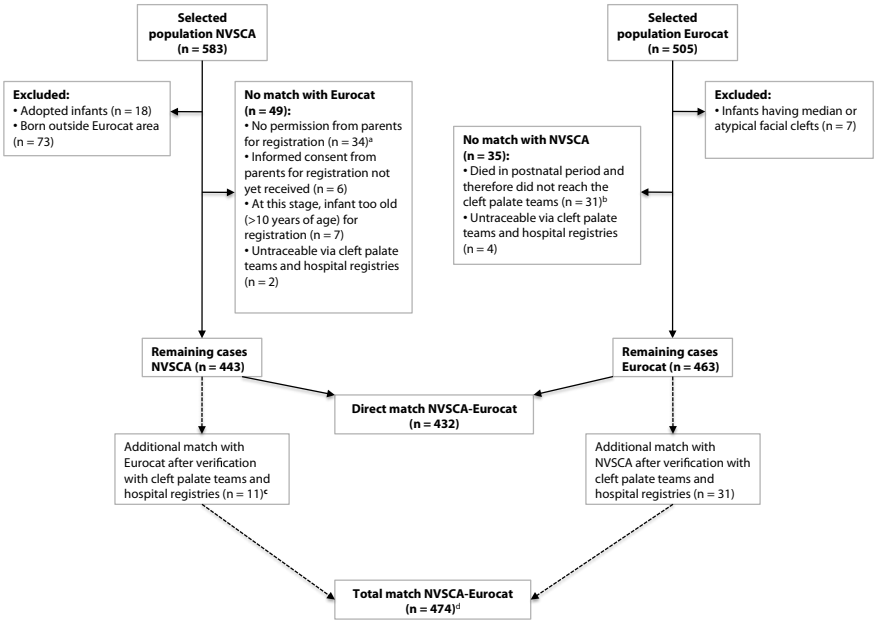

Figure 1. Flow diagram of selection of cases with oral clefts and matching these cases between the Dutch Oral Cleft Registry (NVSCA) and the population-based Eurocat Northern Netherlands.

[a] With regard to timing (early or late defects) and underlying embryological processes (fusion and/or differentiation defects), these infants showed a cleft distribution generally similar to that of the matched cases.

[b] Except 2 infants, all died because of syndromal or chromosomal anomalies, e.g., trisomy 13 or 18.

[c] At this stage, infants were ≤ 10 years of age and therefore eligible for registration in Eurocat.

[d] Including both syndromic and non-syndromic cases.

Oral cleft classification

To be consistent with literature,[4, 7, 10, 25-27] we first divided cases into three categories: cleft lip/alveolus only, cleft lip/alveolus and palate, and cleft palate only. Besides this generally accepted broad division, cases were also classified according to timing and underlying developmental mechanisms.

In short, embryogenesis of the primary palate (presumptive lip and alveolus) and secondary palate (presumptive hard and soft palates, including uvula) can be subdivided into an early (4-7 weeks postconception) and late (7-12 weeks postconception) developmental period. During the early embryonic period, the primary palate is formed by outgrowth and fusion of the facial swellings. Subsequently—during the late period—the definitive lip and alveolus are formed by

outgrowth and differentiation of mesenchyme into bone and musculature. By contrast, the secondary palate develops only during the latter period by outgrowth and fusion of the palatine processes and subsequent differentiation of its mesenchyme into bone and musculature.[6, 8, 9]

Derailments in fusion and/or differentiation processes may result in various cleft sub-phenotypes. For example, interrupted fusion of the primary palate may cause a *complete cleft lip/alveolus*, while defective fusion of the secondary palate may give rise to a *complete or incomplete cleft palate*. Additionally, disruptions in differentiation of the primary or secondary palates may result in an *incomplete or submucous cleft lip/alveolus* or *submucous cleft palate,* respectively. Note that more than one of these processes can be disturbed during development, resulting in different combinations of defects.[6, 8, 9]

Based on the early and late embryogenesis described above, cases were first classified into early and late defects and then into fusion and/or differentiation defects. Details of this classification have been described elsewhere.[8, 9]

Periconceptional folic acid supplements

To prevent neural tube defects, Dutch women planning a pregnancy are recommended to take 400µg folic acid/day from four weeks before until eight weeks after conception.[28, 29] However, this period covers only the early, but not the late, embryonic period for clefts. Therefore, we evaluated supplement use during the recommended period, as well as during the early and late embryonic periods mentioned above,[6, 8, 9] i.e., the etiologically relevant time periods for clefts. As for neural tube defects, we included four weeks prior to these embryonic periods to reach adequate folate status for prevention, resulting in the following etiologically relevant periods: all clefts (0-12 weeks postconception), early defects (0-7 weeks postconception), and late defects (3-12 weeks postconception).

Infants were initially excluded from the study if the mothers' use of folic acid supplements was unknown or the period of use was unknown (50 cases, 724 controls). They were also excluded if the mother had used folic acid antagonists, which interfere with folate metabolism,[18-20] including dihydrofolate reductase inhibitors (methotrexate, sulfasalazine, triamterene, pyrimethamine, and trimetroprim) or antiepileptic drugs (5 cases, 47 controls). Additionally, infants with reported maternal diabetes mellitus prior to or during pregnancy were excluded (4 cases, 38 controls), because clefts have been associated with maternal diabetes.[30]

Statistical analysis

The following potential confounders were explored using the chi-squared test: year of baby's birth, number of babies/fetuses delivered, number of previous live births, fertility problems, maternal age and BMI, mother's education level, and smoking and alcohol use. To estimate cleft risks, we calculated crude and adjusted odds ratios using univariate and multivariate logistic regression models, respectively. First, we evaluated the use of any supplement, and if possible, we subsequently stratified the analyses into folic acid alone and multivitamins (containing

folic acid). Two-tailed values of $p < 0.05$ and 95% confidence intervals (CI) excluding 1.0 were considered statistically significant. Analyses were performed using SPSS software version 19.0.

RESULTS

In total, 367 cases and 2945 controls (924 infants with chromosomal defects and 2021 with non-folate related anomalies) were included. Apart from the higher proportions of boys and previous live births among cases, no further significant differences in infant or maternal characteristics were found between cases and controls (Table 1). Generally, mothers who had

Table 1. Characteristics of cases and non-folate related controls in the Northern Netherlands, based on data from Eurocat Northern Netherlands (1997-2009)

Characteristic	Cases (n = 367)		Controls (n = 2945)		*p* Value[a]
	n	*(%)*	*n*	*(%)*	
Child sex					**0.002**
Boy	222	(60.5)	1535	(52.1)	
Girl	145	(39.5)	1410	(47.9)	
Year of birth					0.22
1997-2000	119	(32.4)	1082	(36.7)	
2001-2005	142	(38.7)	1105	(37.5)	
2006-2009	106	(28.9)	758	(25.7)	
Number of babies/fetuses delivered					0.87
1	348	(95.9)	2821	(96.1)	
≥2	15	(4.1)	116	(3.9)	
Unknown	4		8		
Number of previous live births[b]					**0.004**
0	146	(40.1)	1411	(48.1)	
1	139	(38.2)	1071	(36.5)	
2	61	(16.8)	334	(11.4)	
≥3	18	(4.9)	118	(4.0)	
Unknown	3		11		
Fertility problems					0.51
Yes	49	(13.7)	434	(15.0)	
No	308	(86.3)	2451	(85.0)	
Unknown	10		60		
Maternal age at delivery					0.89
15-19	2	(0.5)	26	(0.9)	
20-24	30	(8.2)	252	(8.6)	

Table 1. *(Continued)*

Characteristic	Cases (n = 367)		Controls (n = 2945)		*p* Value[a]
	n	*(%)*	*n*	*(%)*	
25-29	120	(33.0)	993	(33.8)	
30-34	149	(40.9)	1178	(40.0)	
35-39	57	(15.7)	423	(14.4)	
≥40	6	(1.6)	70	(2.4)	
Unknown	3		3		
Body mass index (kg/m^2)					0.22
10-18.5	14	(4.0)	95	(3.3)	
18.5-25	207	(58.6)	1831	(64.4)	
25-30	92	(26.1)	646	(22.7)	
>30	40	(11.3)	273	(9.6)	
Unknown	14		100		
Education level					0.24
Low	83	(23.3)	571	(19.7)	
Middle	175	(49.2)	1443	(49.9)	
High	98	(27.5)	878	(30.4)	
Unknown	11		53		
Smoking during pregnancy[c]					0.59
Yes	92	(25.6)	708	(24.2)	
No	268	(74.4)	2212	(75.8)	
Unknown	7		25		
Alcohol during pregnancy[c]					0.80
Yes	75	(20.8)	625	(21.4)	
No	285	(79.2)	2294	(78.6)	
Unknown	7		26		

[a] *p* value represents statistical significance level for differences between cases and controls (tested two-sided with χ^2 test). *p* value <0.05 is used to determine statistical significance and is presented in bold format.

[b] Proportions of previous stillbirths or terminated pregnancies did not differ between cases and controls.

[c] Maternal smoking and alcohol use were considered positive if mothers had reported any use during pregnancy, even if they had stopped when they became aware of their pregnancy.

used supplements had either taken folic acid alone or multivitamins (containing folic acid), at a daily dose of 400µg. As presented in Table 2, case mothers reported any periconceptional folic acid supplement use more frequently than control mothers. More specifically, consistent use of any supplement during the entire recommended period or etiologically relevant cleft periods was more frequently reported among cases, while partial use was more frequently reported among controls. Stratum analysis revealed similar figures for use of folic acid alone (not as a multivitamin). As shown in Table 3, consistent use of any supplement or folic acid alone during

Table 2. Distribution of timing and duration of periconceptional use of folic acid supplements by mothers of case and control infants in the Northern Netherlands, based on data from Eurocat Northern Netherlands (1997-2009)

Timing and Duration of Maternal Supplement Use	Case Mothers (n = 367)		Control Mothers (n = 2945)	
	n	*(%)*	*n*	*(%)*
Any folic acid supplement[a]				
4 weeks before until 8 weeks after conception[b]				
Consistent use[c]	173	(47.1)	1233	(41.9)
Partial use[d]	92	(25.1)	815	(27.7)
No use or use beyond the advised period	102	(27.8)	897	(30.4)
Period of supplement use unknown[e]	0	(0.0)	0	(0.0)
Weeks 0 to 12 postconception[f]				
Consistent use[c]	100	(27.3)	624	(21.2)
Partial use[d]	173	(47.1)	1484	(50.4)
No use or use beyond the advised period	93	(25.3)	828	(28.1)
Period of supplement use unknown[e]	1	(0.3)	9	(0.3)
Weeks 0 to 7 postconception[g]				
Consistent use[c]	181	(49.3)	1311	(44.5)
Partial use[d]	84	(22.9)	737	(25.0)
No use or use beyond the advised period	102	(27.8)	897	(30.5)
Period of supplement use unknown[e]	0	(0.0)	0	(0.0)
Weeks 3 to 12 postconception[h]				
Consistent use[c]	131	(35.7)	887	(30.1)
Partial use[d]	128	(34.9)	1073	(36.4)
No use or use beyond the advised period	97	(26.4)	865	(29.4)
Period of supplement use unknown[e]	11	(3.0)	120	(4.1)
Specific folic acid supplement				
4 weeks before until 8 weeks after conception[b]				
Folic acid alone (not as a multivitamin)				
Consistent use[c]	135	(36.8)	991	(33.7)
Partial use[d]	73	(19.9)	654	(22.2)
Multivitamins (containing folic acid)				
Consistent use[c]	21	(5.7)	160	(5.4)
Partial use[d]	23	(6.3)	187	(6.3)
No use or use beyond the advised period	102	(27.8)	897	(30.5)
Type of supplement use unknown[e]	13	(3.5)	56	(1.9)
Weeks 0 to 12 postconception[f]				
Folic acid alone (not as a multivitamin)				
Consistent use[c]	64	(17.4)	383	(13.0)
Partial use[d]	142	(38.7)	1262	(42.9)

Table 2. *(Continued)*

Timing and Duration of Maternal Supplement Use	Case Mothers (n = 367)		Control Mothers (n = 2945)	
	n	*(%)*	*n*	*(%)*
Multivitamins (containing folic acid)				
Consistent use[c]	19	(5.2)	157	(5.3)
Partial use[d]	35	(9.5)	250	(8.5)
No use or use beyond the advised period	93	(25.4)	828	(28.1)
Type or period of supplement use unknown[e]	14	(3.8)	65	(2.2)
Weeks 0 to 7 postconception[g]				
Folic acid alone (not as a multivitamin)				
Consistent use[c]	143	(39.0)	1051	(35.7)
Partial use[d]	65	(17.7)	594	(20.2)
Multivitamins (containing folic acid)				
Consistent use[c]	21	(5.7)	173	(5.9)
Partial use[d]	23	(6.3)	174	(5.9)
No use or use beyond the advised period	102	(27.8)	897	(30.4)
Type of supplement use unknown[e]	13	(3.5)	56	(1.9)
Weeks 3 to 12 postconception[h]				
Folic acid alone (not as a multivitamin)				
Consistent use[c]	83	(22.6)	576	(19.6)
Partial use[d]	113	(30.8)	944	(32.0)
Multivitamins (containing folic acid)				
Consistent use[c]	31	(8.5)	247	(8.4)
Partial use[d]	17	(4.6)	139	(4.7)
No use or use beyond the advised period	97	(26.4)	865	(29.4)
Type or period of supplement use unknown[e]	26	(7.1)	174	(5.9)

[a] Total group of folic acid supplements comprising folic acid alone and folic acid-containing multivitamins.

[b] Recommended period for periconceptional use of folic acid supplements in the Netherlands to support the prevention of neural tube defects.

[c] Daily use during the entire above-mentioned period.

[d] Daily/intermittent use during part of the above-mentioned period.

[e] If the period of mother's use of folic acid supplements was not known at all, infants were initially (before analysis) excluded from the study and therefore not shown in the current table.
If the specific type (folic acid alone or multivitamins) or period of use beyond the recommended period was unknown when stratified, infants were presented in this table as '*type or period of supplement use unknown*'. Note that these infants were excluded from further analysis of the specific stratum.

[f] Etiologically relevant period for all oral clefts, starting 4 weeks prior to the embryonic development of clefts to reach adequate folate status for prevention.

[g] Etiologically relevant period for early defects, starting 4 weeks prior to the embryonic development of early defects to reach adequate folate status for prevention.

[h] Etiologically relevant period for late defects, starting 4 weeks prior to the embryonic development of late defects to reach adequate folate status for prevention.

the entire etiologically relevant cleft period (0-12 weeks postconception) was associated with a significantly increased risk of all clefts, and in particular of cleft lip/alveolus only. Adjustment for potential confounders resulted in about one-and-a-half time higher risks for all clefts and three-fold increased risks for cleft lip/alveolus. After inclusion of partial use during the etiologically relevant period, the size, but not the direction, of the effects were reduced (except for multivitamins, Table 3). Overall, associations were also somewhat weakened by restricting analysis of use to the recommended period until 8 weeks, instead of the etiologically relevant period until 12 weeks postconception (Table 4). Exploration of a possible genetic background revealed that a positive family history for clefts was less frequently reported for cases with cleft lip/alveolus (2.5%, 3/120) than cases with other cleft categories (11.4%, 28/245; $p = 0.004$, data not further shown).

To gain more insight into the detected effects, clefts were also classified according to timing and underlying processes in embryogenesis. Table 4 shows that clefts of the lip/alveolus mainly consisted of late differentiation defects (70.2%, 85/121). The estimated risks for early and late defects—including fusion and/or differentiation defects—are shown in Table 5. Consistent supplement use during 0-7 weeks postconception was not significantly associated with higher risks of early defects (mainly fusion defects). Similarly, consistent use during 3-12 weeks postconception did not significantly increase the risk of late defects. Further subgroup analysis also showed no significant crude associations. However, after adjustment for potential confounders, we found two- to three-fold increased risks just for late differentiation defects, regardless of supplement type. Similar to the cleft category analysis, inclusion of partial use during the etiologically relevant periods reduced the size, but not the direction, of associations (Table 5).

Finally, if analyses were restricted to only chromosomal defects (n = 924) or only live births (n = 2895) as controls, no significant changes in our risk estimates were detected (data not further shown).

Table 3. Odds ratios for non-syndromic oral clefts, stratified by cleft category, associated with maternal periconceptional use of folic acid supplements relative to non-folate related anomalies in the Northern Netherlands, based on data from the NVSCA and Eurocat Northern Netherlands (1997-2009)

Timing and Duration of Supplement Use	Diagnosis	Users	Non-Users	Total	Crude Odds Ratio (95% CI)		Adjusted Odds Ratio[a] (95% CI)	
Recommended period in the Netherlands								
Consistent use from 4 weeks before until 8 weeks after conception[b]								
Any folic acid supplement[c]	Controls	1233	897	2130	Ref		Ref	
	Any oral cleft	173	102	275	1.23	(0.95-1.60)	**1.41**	**(1.05-1.91)**
	Cleft lip/alveolus only	57	31	88	1.34	(0.86-2.09)	**1.70**	**(1.01-2.87)**
	Cleft lip/alveolus and palate	66	38	104	1.26	(0.84-1.90)	1.49	(0.93-2.40)
	Cleft palate only	50	33	83	1.10	(0.70-1.73)	1.10	(0.66-1.85)
Folic acid alone (not as a multivitamin)[d]	Controls	991	897	1888	Ref		Ref	
	Any oral cleft	135	102	237	1.20	(0.91-1.57)	1.31	(0.96-1.79)
	Cleft lip/alveolus only	43	31	74	1.26	(0.78-2.01)	1.58	(0.91-2.72)
	Cleft lip/alveolus and palate	52	38	90	1.24	(0.81-1.90)	1.44	(0.88-2.34)
	Cleft palate only	40	33	73	1.10	(0.69-1.76)	1.00	(0.58-1.70)
Multivitamins (containing folic acid)[e]	Controls	160	897	1057	Ref		Ref	
	Any oral cleft	21	102	123	1.15	(0.70-1.90)	1.24	(0.69-2.22)
	Cleft lip/alveolus only	8	31	39	1.45	(0.65-3.21)	2.59	(0.97-6.91)
	Cleft lip/alveolus and palate	6	38	44	0.89	(0.37-2.13)	0.68	(0.24-1.94)
	Cleft palate only	7	33	40	1.19	(0.52-2.74)	1.15	(0.44-2.97)
Consistent and partial use from 4 weeks before until 8 weeks after conception[f]								
Any folic acid supplement[c]	Controls	2048	897	2945	Ref		Ref	
	Any oral cleft	265	102	367	1.14	(0.89-1.45)	1.23	(0.94-1.61)
	Cleft lip/alveolus only	90	31	121	1.27	(0.84-1.93)	1.42	(0.89-2.27)

Table 3. *(Continued)*

Timing and Duration of Supplement Use	Diagnosis	Users	Non-Users	Total	Crude Odds Ratio (95% CI)		Adjusted Odds Ratio[a] (95% CI)	
	Cleft lip/alveolus and palate	104	38	142	1.20	(0.82-1.75)	1.36	(0.89-2.08)
	Cleft palate only	71	33	104	0.94	(0.62-1.44)	0.91	(0.57-1.46)
Folic acid alone (not as a multivitamin)[d]	Controls	1645	897	2542	Ref		Ref	
	Any oral cleft	208	102	310	1.11	(0.87-1.43)	1.16	(0.87-1.53)
	Cleft lip/alveolus only	73	31	104	1.28	(0.84-1.97)	1.42	(0.88-2.29)
	Cleft lip/alveolus and palate	79	38	117	1.13	(0.76-1.68)	1.27	(0.82-1.97)
	Cleft palate only	56	33	89	0.93	(0.60-1.43)	0.81	(0.49-1.33)
Multivitamins (containing folic acid) [e]	Controls	347	897	1244	Ref		Ref	
	Any oral cleft	44	102	146	1.12	(0.77-1.62)	1.28	(0.82-2.01)
	Cleft lip/alveolus only	15	31	46	1.25	(0.67-2.35)	**2.18**	**(1.01-4.73)**
	Cleft lip/alveolus and palate	17	38	55	1.16	(0.64-2.08)	1.02	(0.50-2.07)
	Cleft palate only	12	33	45	0.94	(0.48-1.84)	1.00	(0.45-2.19)
Etiologically relevant period oral clefts								
Consistent use during weeks 0 to 12 postconception[g]								
Any folic acid supplement[c]	Controls	624	828	1452	Ref		Ref	
	Any oral cleft	100	93	193	**1.43**	**(1.06 - 1.93)**	**1.72**	**(1.19 - 2.49)**
	Cleft lip/alveolus only	38	28	66	**1.80**	**(1.09 - 2.97)**	**3.16**	**(1.69 - 5.91)**
	Cleft lip/alveolus and palate	34	34	68	1.33	(0.82 - 2.16)	1.45	(0.80 - 2.65)
	Cleft palate only	28	31	59	1.20	(0.71 - 2.08)	1.12	(0.59 - 2.11)

Table 3. *(Continued)*

Timing and Duration of Supplement Use	Diagnosis	Users	Non-Users	Total	Crude Odds Ratio (95% CI)		Adjusted Odds Ratio[a] (95% CI)	
Folic acid alone (not as a multivitamin)[d]	Controls	383	828	1211	Ref		Ref	
	Any oral cleft	64	93	157	1.49	**(1.06 - 2.09)**	**1.65**	**(1.10 - 2.47)**
	Cleft lip/alveolus only	24	28	52	1.85	**(1.06 - 3.24)**	**3.11**	**(1.57 - 6.14)**
	Cleft lip/alveolus and palate	21	34	55	1.34	(0.77 - 2.33)	1.50	(0.78 - 2.88)
	Cleft palate only	19	31	50	1.33	(0.74 - 2.38)	1.03	(0.52 - 2.04)
Multivitamins (containing folic acid)[e]	Controls	157	828	985	Ref		Ref	
	Any oral cleft	19	93	112	1.08	(0.64 - 1.82)	1.13	(0.61 - 2.08)
	Cleft lip/alveolus only	8	28	36	1.51	(0.67 - 3.37)	2.61	(0.97 - 7.07)
	Cleft lip/alveolus and palate	4	34	38	0.62	(0.22 - 1.77)	0.44	(0.12 - 1.59)
	Cleft palate only	7	31	38	1.19	(0.52 - 2.75)	1.07	(0.40 - 2.81)
Consistent and partial use during weeks 0 to 12 postconception[h]								
Any folic acid supplement[c]	Controls	2108	828	2936	Ref		Ref	
	Any oral cleft	273	93	366	1.15	(0.90 - 1.48)	1.29	(0.97 - 1.71)
	Cleft lip/alveolus only	93	28	121	1.31	(0.85 - 2.01)	1.49	(0.91 - 2.41)
	Cleft lip/alveolus and palate	108	34	142	1.25	(0.84 - 1.85)	1.45	(0.93 - 2.26)
	Cleft palate only	72	31	103	0.91	(0.59 - 1.40)	0.89	(0.55 - 1.44)

Table 3. *(Continued)*

Timing and Duration of Supplement Use	Diagnosis	Users	Non-Users	Total	Crude Odds Ratio (95% CI)		Adjusted Odds Ratio[a] (95% CI)	
Folic acid alone (not as a multivitamin)[d]	Controls	1645	828	2473	Ref		Ref	
	Any oral cleft	206	93	299	1.12	(0.86 - 1.44)	1.21	(0.90 - 1.62)
	Cleft lip/alveolus only	73	28	101	1.31	(0.84 - 2.05)	1.50	(0.91 - 2.48)
	Cleft lip/alveolus and palate	80	34	114	1.18	(0.77 - 1.78)	1.35	(0.85 - 2.14)
	Cleft palate only	53	31	84	0.86	(0.55 - 1.35)	0.76	(0.46 - 1.27)
Multivitamins (containing folic acid)[e]	Controls	407	828	1235	Ref		Ref	
	Any oral cleft	54	93	147	1.18	(0.83 - 1.69)	1.35	(0.87 - 2.09)
	Cleft lip/alveolus only	18	28	46	1.31	(0.72 - 2.39)	**2.15**	**(1.00- 4.63)**
	Cleft lip/alveolus and palate	20	34	54	1.20	(0.68 - 2.11)	1.14	(0.56 - 2.29)
	Cleft palate only	16	31	47	1.05	(0.57 - 1.94)	1.00	(0.47 - 2.14)

[a] Odds ratio adjusted for year of baby's birth, number of babies/fetuses delivered, number of previous live births, fertility problems, maternal age (continue variable) and BMI, mother's education level, and smoking and alcohol use.

[b] Daily use during the entire recommended period for periconceptional use of folic acid supplements in the Netherlands to support the prevention of neural tube defects.

[c] Total group of folic acid supplements comprising folic acid alone and folic acid-containing multivitamins.

[d] Infants whose mothers had used multivitamins (containing folic acid) were excluded from this part of the analysis.

[e] Infants whose mothers had used folic acid alone were excluded from this part of the analysis.

[f] Daily use during the entire recommended period for folic acid use in the Netherlands or daily/intermittent use during part of this recommended period.

[g] Daily use during the entire etiologically relevant period for oral clefts, starting 4 weeks prior to the embryonic development of clefts to reach adequate folate status for prevention.

[h] Daily use during the entire etiologically relevant period for oral clefts or daily/intermittent use during part of this period.

Table 4. Classification of cases with various cleft sub-phenotypes in the Northern Netherlands according to timing and underlying processes in embryogenesis, based on data from the NVSCA (1997-2009)

Cleft Type	Cases	
	n	*(%)*
Any oral cleft	367	
Early defects[a]	162	(44.1)
Fusion defects	140	(38.1)
Complete cleft lip/alveolus[b]	30	
Complete cleft lip/alveolus & complete or incomplete cleft palate[b]	110	
Fusion and differentiation defects	22	(6.0)
Incomplete cleft lip & complete cleft alveolus[b,c]	5	
Incomplete cleft lip & complete cleft alveolus & cleft palate[b,c]	8	
Complete cleft lip/alveolus & incomplete or submucous cleft lip/alveolus[d,e]	1	
Complete cleft lip/alveolus & incomplete or submucous cleft lip/alveolus & cleft palate[d,e]	8	
Late defects[f]	205	(55.9)
Fusion defects	81	(22.1)
Complete or incomplete cleft palate	81	
Differentiation defects	104	(28.3)
Incomplete or submucous cleft lip/alveolus[b]	85	
Incomplete or submucous cleft lip/alveolus & submucous cleft palate[g]	2	
Submucous cleft palate	17	
Fusion and differentiation defects	20	(5.4)
Incomplete or submucous cleft lip/alveolus & complete or incomplete cleft palate[b]	14	
Incomplete & submucous cleft palate	6	

[a] Embryonic development during 4-7 weeks postconception.
[b] This category comprised unilateral as well as bilateral clefts.
[c] Synonym for this phenotype: Simonart's band.
[d] This category comprised bilateral clefts only.
[e] Synonym for submucous cleft lip: microform, subsurface or subcutaneous cleft, forme fruste, and congenital scar.
[f] Embryonic development during 7-12 weeks postconception.
[g] This category comprised unilateral clefts only.

Table 5. Odds ratios for non-syndromic oral clefts, subdivided according to timing and embryological processes, associated with maternal periconceptional use of folic acid supplements relative to non-folate related anomalies in the Northern Netherlands, based on data from the NVSCA and Eurocat Northern Netherlands (1997-2009)

Timing and Duration of Supplement Use	Diagnosis	Users	Non-Users	Total	Crude Odds Ratio (95% CI)		Adjusted Odds Ratio[a] (95% CI)	
Etiologically relevant period early defects								
Consistent use during weeks 0 to 7 postconception[b]								
Any folic acid supplement[c]	Controls	1311	897	2208	Ref		Ref	
	Early defects	76	46	122	1.13	(0.78 - 1.65)	1.38	(0.89 - 2.14)
	Fusion defects	63	41	104	1.05	(0.70 - 1.57)	1.33	(0.84 - 2.11)
	Fusion & differentiation defects	13	5	18	1.78	(0.63 - 5.01)	2.05	(0.49 - 8.63)
Folic acid alone (not as a multivitamin)[d]	Controls	1051	897	1948	Ref		Ref	
	Early defects	60	46	106	1.11	(0.75 - 1.65)	1.33	(0.85 - 2.08)
	Fusion defect	51	41	92	1.06	(0.70 - 1.62)	1.29	(0.81 - 2.07)
	Fusion & differentiation defects	9	5	14	1.54	(0.51 - 4.60)	1.82	(0.41 - 8.16)
Multivitamins (containing folic acid)[e]	Controls	173	897	1070	Ref		Ref	
	Early defects	8	46	54	0.90	(0.42 - 1.94)	0.89	(0.36 - 2.22)
	Fusion defects	6	41	47	0.76	(0.32 - 1.82)	0.75	(0.27 - 2.12)
	Fusion & differentiation defects	2	5	7	2.07	(0.40 - 10.78)	1.91	(0.19 - 19.37)
Consistent and partial use during weeks 0 to 7 postconception[f]								
Any folic acid supplement[c]	Controls	2048	897	2945	Ref		Ref	
	Early defects	116	46	162	1.10	(0.78 - 1.57)	1.30	(0.88 - 1.93)
	Fusion defects	99	41	140	1.06	(0.73 - 1.54)	1.24	(0.82 - 1.88)
	Fusion & differentiation defects	17	5	22	1.49	(0.55 - 4.05)	2.06	(0.55 - 7.71)

Table 5. *(Continued)*

Timing and Duration of Supplement Use	Diagnosis	Users	Non-Users	Total	Crude Odds Ratio (95% CI)		Adjusted Odds Ratio[a] (95% CI)	
Folic acid alone (not as a multivitamin)[d]	Controls	1645	897	2542	Ref		Ref	
	Early defects	90	46	136	1.07	(0.74 - 1.54)	1.23	(0.82 - 1.84)
	Fusion defects	77	41	118	1.03	(0.70 - 1.51)	1.16	(0.76 - 1.78)
	Fusion & differentiation defects	13	5	18	1.42	(0.50 - 3.99)	2.07	(0.54 - 8.00)
Multivitamins (containing folic acid)[e]	Controls	347	897	1244	Ref		Ref	
	Early defects	20	46	66	1.12	(0.66 - 1.93)	1.25	(0.65 - 2.42)
	Fusion defects	17	41	58	1.07	(0.61 - 1.91)	1.23	(0.61 - 2.48)
	Fusion & differentiation defects	3	5	8	1.55	(0.37 - 6.53)	1.55	(0.20 - 12.03)
Etiologically relevant period late defects								
Consistent use during weeks 3 to 12 postconception[g]								
Any folic acid supplement[c]	Controls	887	865	1752	Ref		Ref	
	Late defects	71	53	124	1.31	(0.90 - 1.89)	1.40	(0.90 - 2.18)
	Fusion defects	29	25	54	1.13	(0.66 - 1.95)	0.90	(0.47 - 1.74)
	Differentiation defects	36	23	59	1.53	(0.90 - 2.60)	**2.34**	**(1.24 - 4.42)**
	Fusion & differentiation defects	6	5	11	1.17	(0.36 - 3.85)	1.06	(0.25 - 4.52)
Folic acid alone (not as a multivitamin)[d]	Controls	576	865	1441	Ref		Ref	
	Late defects	46	53	99	1.30	(0.87 - 1.96)	1.30	(0.80 - 2.09)
	Fusion defects	20	25	45	1.20	(0.66 - 2.18)	0.87	(0.44 - 1.75)
	Differentiation defects	22	23	45	1.44	(0.79 - 2.60)	**2.12**	**(1.05 - 4.28)**
	Fusion & differentiation defects	4	5	9	1.20	(0.32 - 4.49)	1.26	(0.27 - 5.79)

Table 5. *(Continued)*

Timing and Duration of Supplement Use	Diagnosis	Users	Non-Users	Total	Crude Odds Ratio (95% CI)		Adjusted Odds Ratio[a] (95% CI)	
Multivitamins (containing folic acid)[e]	Controls	247	865	1112	Ref		Ref	
	Late defects	17	53	70	1.12	(0.64 - 1.98)	1.28	(0.65 - 2.51)
	Fusion defects	6	25	31	0.84	(0.34 - 2.07)	0.68	(0.24 - 1.95)
	Differentiation defects	10	23	33	1.52	(0.72 - 3.24)	**2.90**	**(1.12 - 7.47)**
	Fusion & differentiation defects	1	5	6	0.70	(0.08 - 6.02)	0.56	(0.04 - 7.08)
Consistent and partial use during weeks 3 to 12 postconception[h]								
Any folic acid supplement[c]	Controls	1960	865	2825	Ref		Ref	
	Late defects	145	53	198	1.21	(0.87 - 1.67	1.24	(0.86 - 1.78)
	Fusion defects	53	25	78	0.94	(0.58 - 1.52)	0.80	(0.47 - 1.39)
	Differentiation defects	78	23	101	1.50	(0.93 - 2.40)	1.68	(0.99 - 2.85)
	Fusion & differentiation defects	14	5	19	1.24	(0.44 - 3.44)	1.79	(0.53 - 6.01)
Folic acid alone (not as a multivitamin)[d]	Controls	1520	865	2385	Ref		Ref	
	Late defects	110	53	163	1.18	(0.84 - 1.66)	1.18	(0.81 - 1.73)
	Fusion defects	41	25	66	0.93	(0.56 - 1.55)	0.78	(0.44 - 1.37)
	Differentiation defects	60	23	83	1.49	(0.91 - 2.42)	1.62	(0.94 - 2.81)
	Fusion & differentiation defects	9	5	14	1.02	(0.34 - 3.07)	1.64	(0.45 - 5.97)
Multivitamins (containing folic acid)[e]	Controls	386	865	1251	Ref		Ref	
	Late defects	27	53	80	1.14	(0.71 - 1.84)	1.13	(0.63 - 2.02)
	Fusion defects	10	25	35	0.90	(0.43 - 1.89)	0.64	(0.26 - 1.58)
	Differentiation defects	14	23	37	1.36	(0.69 - 2.68)	2.03	(0.87 - 4.73)
	Fusion & differentiation defects	3	5	8	1.35	(0.32 - 5.65)	0.89	(0.12 - 6.48)

[a] Odds ratio adjusted for year of baby's birth, number of babies/fetuses delivered, number of previous live births, fertility problems, maternal age (continue variable) and BMI, mother's education level, and smoking and alcohol use.
[b] Daily use during the entire etiologically relevant period for early defects, starting 4 weeks prior to the embryonic development of early defects to reach adequate folate status for prevention.
[c] Total group of folic acid supplements comprising folic acid alone and folic acid-containing multivitamins.
[d] Infants whose mothers had used multivitamins (containing folic acid) were excluded from this part of the analysis.
[e] Infants whose mothers had used folic acid alone were excluded from this part of the analysis.
[f] Daily use during the entire etiologically relevant period for early defects or daily/intermittent use during part of this period.
[g] Daily use during the entire etiologically relevant period for late defects, starting 4 weeks prior to the embryonic development of late defects to reach adequate folate status for prevention.
[h] Daily use during the entire etiologically relevant period for late defects or daily/intermittent use during part of this period.

DISCUSSION

This population-based case-control study provides the first evidence that periconceptional folic acid supplementation might be associated with elevated risks for certain types of oral clefts. Defects of the lip/alveolus—mainly resulting from defective differentiation in development—appeared to account for the largest proportion of the risk increase, being associated with more than three-fold higher risks after consistent maternal use during the entire etiologically relevant period. Further analysis systematically showed two- to three-fold increased risks for late differentiation defects, with no significant associations for fusion defects. Given that stratum analysis revealed similar figures for folic acid alone, effects were attributable to folic acid and not to other multivitamin components. Furthermore, a duration of exposure-response relationship was shown, as inclusion of partial use generally reduced the size, but not the direction, of observed associations.

Strengths and weaknesses

By combining complementary NVSCA and Eurocat data, we were able to investigate type, timing, and duration of periconceptional folic acid supplement use in relation to traditional cleft categories as well as to their timing and underlying embryological processes—an approach not used in earlier studies.[1, 2] The rationale of this approach was based on the early and late embryogenesis, in line with the theoretical basis of the NVSCA system.[6, 8-10] The unique data combination, allowing us to use a relatively large sample drawn from a well-defined and homogenous population, gave our study its main strength. However, there were also some limitations, mostly inherent to the observational nature of our study. As information on folic acid supplement use and potential confounders was mainly obtained from retrospective questionnaires, recall bias might be a concern. However, although misclassification and measurement errors inevitably occur, the use of malformed controls minimized differential recall between cases and controls. This is reflected in the equal distribution of other exposures among cases and controls for which socially desirable answers could be expected, like maternal smoking and alcohol use. Concerns about recall bias were further reduced by the specificity, consistency, and systematic character of the observed effects, including the duration of exposure-response relationship.

Another limitation is that we were not able to investigate dose effects because a fixed dose of 400µg is recommended for the periconceptional period in the Netherlands, and high dose (5mg) supplements are only prescribed for certain medical indications (e.g., previous pregnancy affected with a neural tube defect). Additionally, exact intake could not be measured because we had to rely on information about supplement use from retrospective questionnaires, and data on folate concentrations in serum and red blood cells were not available. While verification of the products that had been used by cases and controls showed dosages similar to the recommended periconceptional dose (400µg), dietary intake was not known, which limited our knowledge on folate status.

In addition, since parents have to give informed consent for registration in Eurocat, there is a possibility of selection bias due to this informed consent. However, we assume that not giving the consent is equally distributed among cases and controls, given that both had congenital anomalies. Furthermore, the cases with parental refusal showed a cleft distribution generally similar to that of the included cases, which suggests that refusal occurred not just in selective groups. Conversely, the NVSCA registers only patients who survive long enough to reach a cleft palate team. Theoretically, this might also affect results, but in our study, almost all postnatal deaths had syndromal or chromosomal anomalies and would therefore have been excluded anyway. Another source of selection bias might be the inclusion of pregnancy terminations and stillbirths among controls in our study. However, we believe this to be minimal, because restricting analyses to live births only did not significantly alter our risk estimates.

Another weakness might be the presence of unidentified confounding factors. Even though we used malformed controls and were well informed about maternal health and lifestyle factors as well as use of medications (folic acid antagonists), there may still have been confounding by other closely related factors. However, such confounders would have to be strongly related to specific cleft defects and folic acid use to produce the observed results.

Although the use of malformed controls is beneficial with regard to internal validity, it might also have its restrictions, as it could lead to risk over- or underestimations if anomalies in the control group were also associated with folic acid. However, we assume this to be minimal because suspected folate-related anomalies were excluded by design, and further restrictions to purely chromosomal anomalies (i.e., excluding the largest subgroup having non-chromosomal anomalies) did not substantially change our risk estimates. Additionally, Van Beynum and colleagues recently used similar methods and comparable controls drawn from the same Eurocat population to demonstrate significant reduced risks for congenital heart defects associated with periconceptional folic acid use in the Northern Netherlands.[22] As stated by these authors, their findings are in line with earlier findings of a Hungarian randomized controlled trial[12] and other observational studies,[22] thereby supporting the validity of malformed controls. Moreover, they demonstrated that such controls are representative for the general population, as they found overall similar risk estimates using reference groups from the general population. Because we investigated supplement use in great detail and considered many potential confounders, these reference groups—for which just minimal information on absence or presence of folic acid use was available—could not be used in our study.

Possible explanations

The specificity and systematic pattern in our findings is consistent with recent embryological and epidemiological data, suggesting that clefts of the lip/alveolus have unique genetic and etiologic features.[4, 6, 7, 10, 25-27] However, our results are in contrast with previous studies on the effects of periconceptional folic acid in clefts, which showed either preventive or no effects.[1-3, 31] More specifically, one of the first positive effects were reported by Tolarova, who found an

84% reduction in recurrence of cleft lip/alveolus with or without cleft palate after supplementation of multivitamins and high dose (10 mg) folic acid during 3 months before and after pregnancy.[32] However, this study was limited by the small number of cases as well as by its non-randomized nature. In contrast, the only two intervention trials that considered oral clefts and were reported—comprising a randomized controlled trial and cohort-controlled trial from Hungary in 1992 and 2004, respectively—showed no reduction or increase in the prevalence of cleft lip/alveolus with or without palate or cleft palate alone after supplementation of multivitamins with 800µg folic acid.[11, 13] This might partly be explained by the low statistical power due to limited number of subjects. Among observational studies, the Hungarian case-control study of Czeizel and colleagues (1999) indicated a dose-dependent preventive effect of folic acid on the risk of clefts.[33] Additionally, Li and colleagues recently performed a Chinese prospective cohort study, showing a reduced risk for cleft lip with or without palate among women who had used periconceptional folic acid in a northern rural region of China with a proven high prevalence of folate deficiency.[34] However, this reduced risk was mainly attributable to cleft lip and palate and not to cleft lip only, the category for which our study showed the most elevated risk. Furthermore, no significant effects were found in a more southern region with an overall higher socioeconomic status and generally greater availability of fresh vegetables, resulting in a better folate status. An important limitation of this study was that folic acid use was not randomized, and that women who had taken folic acid may thus have differed systematically in other factors that could have influenced the prevalence of clefts. As the authors did not have information on risk factors found in our and other studies to be important, such as smoking and alcohol,[1] they were not able to adjust for these factors.

The mechanisms by which folic acid might prevent certain congenital anomalies remains unexplained, but we do know that other aspects surrounding folate metabolism have also been shown to deviate for clefts. For example, in some studies, the association with the CT/TT genotype of the MTHFR gene appeared to be a protective factor instead of a risk factor for clefts,[35-38] or it appeared to be an even greater risk factor if the mother had used folic acid supplements.[38] Moreover, inhibiting folic acid binding to folate receptor has been shown to reduce the cleft risk.[39] Finally, some studies have found that increased plasma or erythrocyte folate (a parameter for long-term folate status) is associated with elevated, rather than decreased, cleft risks.[35, 40, 41]

Given that the Northern Netherlands has a rather homogenous population with relatively high cleft rates,[14] a higher genetic predisposition might have contributed to our findings. This could specifically have affected differentiation defects, as differentiation and fusion processes are regulated by different genes and cell-biological processes.[4, 6] However, this explanation was not supported by our exploratory analysis of relatives with oral clefts, which showed relatively low proportions of cleft relatives among cases with these specific defects.

To our knowledge, our study is the first to report a negative effect of folic acid supplementation on the risk of an isolated congenital anomaly. However, previous reports have shown comparable associations between periconceptional folic acid supplements/multivitamins/cereals

and the occurrence of multiple congenital anomalies.[42, 43] Furthermore, several animal studies have also demonstrated adverse effects linking high folate intake to embryonic delay, growth retardation, congenital heart defects.[44-46] In humans, folate intake and blood cell concentrations increased significantly due to folate fortification and additional supplementation,[47, 48] but the consequences of long-term high folate intake are not known yet. Recently, it has been hypothesized that folic acid intake might lead to changes in epigenetic patterns, thereby altering gene expression.[49-51] This might help to explain different health outcomes (e.g., congenital anomalies) among those with similar genetic backgrounds.

Possible implications

Our findings may have implications for healthcare and policy makers. First, oral clefts require extensive multidisciplinary treatment and account for substantial morbidity among infants. Therefore, higher cleft risks will increase the public health burden in terms of medical costs and emotional stress to patients and their families.[4, 5, 7] Second, if our findings are correct, it is vital to restrict the use of folic acid to the official recommended period of 4 weeks before to 8 weeks after conception, that is the etiologically relevant period for neural tube defects.[29] Minimizing pregnant women's exposure to folic acid in this way may then reduce cleft prevalence. More generally, our study underlines the importance of evaluating public health strategies regarding folic acid supplementation, including its timing, duration, and dose, which should be done in the light of potential dietary improvements. Together with other emerging studies on the potential adverse effects of increased folic acid intake,[44-46, 49-51] our findings also underscore the need for additional studies on the consequences of increased folic acid intake. Large population-based studies using other datasets, but the same approach and methodology as in the current study, are needed to confirm or refute our findings. To gain more insight into the role of folic acid in the etiology of clefts and other congenital anomalies, future studies should evaluate effects according to timing and embryological mechanisms underlying their development.

CONCLUSIONS

This study presents several lines of evidence indicating that periconceptional folic acid in the Northern Netherlands is associated with an increased risk of clefts, especially of the lip/alveolus, relative to non-folate related malformations. Although detected by an observational study, this association is strengthened by the specificity, consistency, systematic pattern, and duration of exposure-response relationship of our findings. Ideally, a randomized controlled trial should be conducted to confirm or refute our findings, but this would be unethical with the knowledge that folic acid can prevent neural tube defects. Therefore, it is advisable to restrict folic acid supplementation to the period recommended for neural tube defects until more information is available.

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PART V

Prenatal diagnosis and classification

CHAPTER 10

A systematic review of associated structural and chromosomal defects in oral clefts: when is prenatal genetic analysis indicated?

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ABSTRACT

Background: Oral clefts—comprising cleft lip (CL), cleft lip with cleft palate (CLP), and cleft palate (CP)—are being diagnosed prenatally more frequently. Consequently, the need for accurate information on the risk of associated anomalies and chromosomal defects to aid in prenatal counseling is rising. This systematic review was conducted to investigate the prenatal and postnatal prevalence of associated anomalies and chromosomal defects related to cleft category, thereby providing a basis for prenatal counseling and prenatal invasive diagnostics.

*Methods***:** Online databases were searched for prenatal and postnatal studies on associated anomalies and chromosomal defects in clefts. Data from literature were complemented with national validated data from the Dutch Oral Cleft Registry.

Results: Twenty studies were included: three providing prenatal data, 13 providing postnatal data, and four providing both. Data from prenatal and postnatal studies showed that the prevalence of associated anomalies was lowest in CL (0%-20.0% and 7.6%-41.4%, respectively). For CLP, higher frequencies were found both prenatally (39.1%-66.0%) and postnatally (21.1%-61.2%). Although CP was hardly detected by ultrasound, it was the category most frequently associated with accompanying defects in postnatal studies (22.2%-78.3%). Chromosomal abnormalities were most frequently seen in association with additional anomalies. In absence of associated anomalies, chromosomal defects were found prenatally in CLP (3.9%) and postnatally in CL (1.8%, 22q11.2 deletions only), CLP (1.0%) and CP (1.6%).

Conclusion: Prenatal counseling regarding prognosis and risk of chromosomal defects should be tailored to cleft category, and more importantly, to the presence/absence of associated anomalies. Irrespective of cleft category, clinicians should advise invasive genetic testing if associated anomalies are seen prenatally. In absence of associated anomalies, prenatal conventional karyotyping is not recommended in CL, although array-comparative genomic hybridization should be considered. In presumed isolated CLP or CP, prenatal invasive testing, preferably by array-based methods, is recommended.

INTRODUCTION

Oral clefts—one of the most common congenital malformations in humans—arise in approximately 1 of 700 live births.[1] It is has been well established that, although clefts can be isolated anomalies, they are frequently associated with other congenital anomalies, often as part of a syndrome or chromosomal defect. Oral clefts are traditionally subdivided into two categories: cleft lip with or without cleft palate (CL±P), and cleft palate only (CP).[1] However, recent studies have emphasized subdivision into three categories: cleft lip only (CL), cleft lip with cleft palate (CLP), and cleft palate only (CP), because of differences concerning embryologic development, prevalence, risk factors, and associations with other congenital anomalies.[2-5] Although many studies have also included median cleft lip or atypical facial clefts as oral clefts,[6-8] these anomalies should be considered as separate craniofacial anomalies because of their different pathogenesis.[9, 10]

As a result of advances in transabdominal 2D ultrasound technology and its routine use in obstetric practice, oral clefts with or without associated anomalies are being diagnosed prenatally more frequently.[11] Detection rates—predominantly on CL±P—increased from approximately 5% in the early 1980s to over 26% in the late 1990s,[12] and they are as high as 65% today.[13] Consequently, there is an increasing need for accurate information to aid in prenatal counseling. When informing parents on outcome and prognosis, the category of cleft as well as the presence of other congenital anomalies is crucial. Especially the identification of an underlying chromosomal defect will influence prenatal counseling and management of the pregnancy significantly. However, in clinical practice there is often discussion on whether further invasive tests should be performed prenatally to identify chromosomal defects.[14] It is unknown whether invasive diagnostics should be offered in all identified cleft cases or should be limited to specific cleft categories or the presence of associated anomalies.

To allow informed decisions on invasive prenatal diagnostics, clinicians and parents need to be informed about the prevalence of associated anomalies and underlying chromosomal defects in clefts. However, the reported rates in prenatal cleft populations vary greatly between studies.[6-8, 13-16] Furthermore, these findings may reflect selection bias,[17] as cases that are more likely to be diagnosed prenatally tend to be the more severe cases with associated anomalies and chromosomal defects.[11] Nowadays, increasing numbers of isolated clefts—not accompanied by growth retardation or other prenatal complications—are identified in utero.[11] Therefore, both prenatal and postnatal studies have to be interpreted in order to provide accurate information on frequencies of associated anomalies and underlying chromosomal defects for future prenatal cleft populations.

This systematic review presents a comprehensive summary of literature and complementary Dutch registry data on prenatal and postnatal findings of associated anomalies and chromosomal defects related to cleft category. The aim of this study was to provide a basis

for prenatal counseling of future parents and to advise on invasive genetic diagnostics in prenatally detected oral clefts.

METHODS

Literature search

In August 2011, the PubMed database was systematically searched using the search string "(cleft) AND (abnormalities OR anomalies) AND (chromosomal OR syndrome)". The search was limited to articles published in English after 31 December 1994. This restriction was applied because technologies to identify specific syndrome diagnoses and chromosomal abnormalities have been developed relatively recent. For example, fluorescence *in situ* hybridization (FISH) was introduced in clinical practice in the early 1990s enabling the detection of specific microdeletions.[18, 19] Consequently, studies published before 1995 may have reported relatively underestimated rates of associated anomalies and chromosomal defects.

The titles and abstracts of the citations were screened independently by two reviewers (MJB and WM) to identify potentially relevant papers for which full-text publications were retrieved. Additional studies were found by crosschecking references. Studies were included if they presented data on oral clefts that were analyzed prenatally and/or postnatally for associated anomalies and chromosomal defects, the latter preferably verified by karyotype. To ensure the quality and prevent our prenatal analysis from significant underreporting, we excluded prenatal studies in which several obvious structural defects (for example anencephaly or holoprosencephaly) had been missed by ultrasound. Because of the ethnic variation in prevalence of clefts and their associated anomalies,[1, 17] studies evaluating non-Caucasian populations (e.g., Asian populations) were excluded to keep a homogeneous study population.

Complementary data

Comparison of the existing literature on congenital anomalies and chromosomal defects associated with oral clefts is restricted, particularly due to differences in methodology. For example, there is a considerable variation in definitions and classifications of clefts and their accompanying defects, as well as in sample sizes, data sources, methods of data collection, and follow-up periods between studies.[17] For this reason, we complemented our review of postnatal studies with national data from the Dutch Oral Cleft Registry (NVSCA). Since 1997, the 15 Dutch cleft palate teams have registered oral clefts and their associated anomalies, using a unique detailed recording system based on the embryology of the head and neck area. Because major as well as minor anomalies (including dysmorphic features) are recorded in detail, the NVSCA data can be fit into any existing classification and are highly applicable for comparison with other studies. Moreover, a selection of registry data has recently been validated and completed by review of medical data, after a median follow-up period of 5 years.[20-22] This selection of validated data

was used to complement our analysis on associated anomalies and chromosomal defects in postnatally detected clefts.[22] In addition, the annual NVSCA reports 1997-2010 were used to inventory the different syndromes and chromosomal defects that had been identified postnatally.[23, 24] The methods of registration and validation have been described in detail elsewhere.[5, 20-22]

Data analysis

Data on associated anomalies and chromosomal defects were extracted from the selected articles and subdivided according to the three cleft categories: CL, CLP and CP. Also, the validated and completed NVSCA data were further analyzed according to these three categories.[22] For studies not distinguishing CL and CLP, the category of CL±P was used. Median cleft lip and atypical facial clefts were excluded because of their different pathogenesis.[9, 10]

For all cleft categories, frequencies of associated congenital anomalies and chromosomal defects were deducted from the reported data and presented in numbers and percentages. For studies providing karyotype information for isolated and/or associated cases, we calculated separate rates of chromosomal defects among isolated (if available) and associated clefts.[6-8, 13-15, 22, 25-27] If studies did not provide numbers of karyotyped cases, but reported routine karyotyping of associated clefts (as in daily practice), we assumed that the majority of associated clefts were karyotyped.[13, 22] Likewise, if chromosomal defects were reported from retrospective registry data without information about the presence or absence of associated anomalies, we assumed that chromosomal analysis had been performed in associated cases only.[16, 26, 28-31] If no specific data on chromosomal anomalies was available for the three cleft categories,[32-36] total rates of associated anomalies were calculated. Theoretically, these numbers might also include chromosomal anomalies detected in isolated clefts, without other congenital anomalies. Where possible, prevalence data were also subdivided according to unilateral and bilateral clefts.[6-8, 13, 14, 25]

To specify the detected chromosomal anomalies, we made an inventory of the different syndromes and chromosomal defects that had been identified in clefts prenatally and/or postnatally. Due to great differences in methodology,[17] we were not able to perform a meta-analysis with these data. To give more information about the reviewed studies and to illustrate the differences, we summarized the various study characteristics and designs, including the inclusion criteria and definitions of clefts and accompanying defects, as well as the sample sizes, data sources, and methods of data collection.

RESULTS

The literature search yielded 9,540 citations. Initial screening by title identified 88 potentially relevant abstracts, including 20 studies meeting the inclusion criteria. Subsequently, one of these

studies was excluded because obvious structural anomalies (e.g., lobar holoprosencephaly and severe congenital heart anomalies) had been missed prenatally, which raised doubts about the quality of the performed prenatal ultrasounds.[37]

Including the NVSCA study,[22] the remaining studies comprised three studies providing relevant prenatal data,[6-8] 13 studies providing relevant postnatal data,[22, 25-36] and four studies providing both.[13-16] All studies with postnatal data had a follow-up period of at least one year. Although the studies of Stoll et al.,[26] Vallino-Napoli et al.,[29] and Walker et al.[25] presented both prenatal and postnatal data, they were included only in the postnatal analysis for divergent reasons. First, the retrospective data of Stoll et al. did not allow extraction of frequencies of associated anomalies and chromosomal defects among prenatally detected clefts. Second, Vallino-Napoli et al. reported data on pregnancy outcome, but the prenatally detected cleft cases could not be identified from their data. Finally, Walker et al. evaluated anomalies that could theoretically have been detected by ultrasound instead of those that had actually been detected. The latter were not separately discussed in their paper. The various study characteristics and designs of the reviewed studies are presented in Table 1.

Prenatally detected associated anomalies and chromosomal defects

In the seven prenatal studies, a total of 407 fetuses with oral clefts were analysed.[6-8, 13-16] The prevalence of associated anomalies and chromosomal defects in prenatally detected clefts is summarized according to cleft category in Table 2. In the CL category, three out of 23 fetuses had associated anomalies, comprising a cardiac defect with a situs inversus,[6] an umbilical hernia, and a clubfoot.[13] One of these three CL cases had a chromosomal defect (trisomy 18).[13] CLP showed the highest prevalence of associated anomalies (54.0%, range 39.1% to 66.0%). For studies that grouped CL and CLP together as CL±P, the prevalence was somewhat lower (29.9%, range 17.2% to 57.1%). Only one study evaluated prenatally detected CP cases (n = 2); both cases had associated anomalies as well as an underlying chromosomal defect.[7] In addition to the three cleft categories, studies distinguishing unilateral and bilateral clefts generally found a higher prevalence of associated anomalies and chromosomal defects in bilateral than in unilateral CLP or CL±P (Table 3).

Analysis of chromosomal defects in isolated and associated clefts revealed that almost all chromosomal defects were associated with other congenital anomalies or ultrasound markers, such as intrauterine growth retardation (97.4%, 74/76; one case with a chromosomal defect not included, as information on associated anomalies was not available, Table 2).[6] For only two cases with chromosomal defects, no accompanying defects were found by ultrasound; one case showed a mosaic trisomy 22[6] and the other had a trisomy 18.[13] Consequently, the prevalence of chromosomal defects in cases with associated clefts was 50.7% (74/146), while it was 0.9 % (2/212) in cases with formerly presumed isolated clefts. In studies specifying the detected chromosomal abnormalities, trisomy 13 (56.3%, 36/64) and trisomy 18 (29.7%, 19/64)

Table 1. Summary of the various study characteristics and designs of the prenatal and postnatal studies included in the systematic review

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
Prenatal studies						
Nyberg 1995	Not given	High-risk obstetric cases referred to tertiary referral centers 1988-1993*	Live births, stillbirths, and TOP with oral clefts *(n = 40)*†	Prospective and retrospective analysis of sonograms & postnatal information retrieved by inspection at autopsy or after birth	Major and minor congenital anomalies; IUGR and other ultrasound markers‡	Karyotype given for most associated clefts (94%, 17/18); number of isolated clefts karyotyped not given
Berge 2001	Region of Bonn, Germany	High-risk obstetric cases referred to a single tertiary referral centre 1991-2000*	Live births, stillbirths, and TOP with oral clefts *(n = 59)*†	Prospective and retrospective analysis of sonograms & postnatal follow-up data from neonatologist and surgeons, and pathological and cytogenetic records	Major and minor congenital anomalies; IUGR and other ultrasound markers‡	Karyotype given for most associated clefts (94%, 33/35) and isolated clefts (76%, 16/21)
Perrotin 2001	Region of Tours, France	High-risk obstetric cases referred to a single tertiary referral centre 1991-1999*	Live births, stillbirths, and TOP with oral clefts *(n = 56)*†	Retrospective evaluation of ultrasonographic and clinical records, and ascertainment via autopsy reports or hospital records with a postnatal follow-up of 3-12 months	Major and minor congenital anomalies; IUGR and other ultrasound markers	Karyotype given for all associated clefts (n = 20), and some isolated clefts (39%, 14/36)

Table 1. *(Continued)*

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
Prenatal and Postnatal studies						
Offerdal 2008	Region of Trondheim, Norway	Pregnant women in a non-selected population who underwent routine ultrasound examination 1987-2004 *(n = 49,314)*	Live births, stillbirths, and TOP (≥16 weeks gestation) with oral clefts *(n = 101)*	Prospective collection of ultrasound examinations & prenatal and postnatal follow-up data from autopsy reports, photographs, physical examinations by pediatricians and review of medical records	Structural congenital anomalies, sequences, and non-chromosomal syndromes according to the ICD; Additional anomalies further subdivided into: - chromosome aberrations - syndromes/ sequences with normal chromosomes - structural anomalies without chromosomal aberrations, syndromes or sequences	Some clefts (31%, 31/101 were karyotyped; presence/ absence of abnormal karyotype given for most associated clefts 62%, 29/47); no specific data given
Russell 2008	Novia Scotia, Canada	Births to residents of Novia Scotia registered in the Perinatal Database, Fetal Anomaly Database, or Cleft Palate Database 1992-2002 *(n = 108,220)*	Live births, stillbirths, and TOP with oral clefts *(n = 225)*	Data from the population-based Novia Scotia Atlee Perinatal Database, the Fetal Anomaly Database, and Cleft Palate Database	Structural congenital anomalies; Additional anomalies further subdivided into: - with abnormal karyotype - structural/syndromic with normal karyotype	Data suggest that all associated clefts (n = 99) were karyotyped; no specific data given

Table 1. *(Continued)*

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
Gillham 2009	North-West England	Obstetric cases prenatally suspected of an oral cleft and referred to the Regional Fetal Management Unit & infants referred to the regional cleft team 2000-2006 *(n = 570)*	Live births, stillbirths, and TOP with oral clefts *(n = 490)*†	Retrospective review of prenatal diagnoses in the North-West Regional Fetal Management Unit Database & postnatal findings in the North-West Cleft Lip and Palate Database	Structural congenital anomalies	Almost all associated clefts (97%, 29/30) were karyotyped; all isolated clefts were karyotyped
Maarse 2011	Region of Utrecht, Netherlands	Pregnant women in a non-selected population who underwent routine ultrasound examination 2007-2008, including low-risk (n = 35,924) and high-risk cases *(n = 2,836)*	Live births, stillbirths, and TOP with oral clefts *(n = 60)*†	Retrospective evaluation of ultrasound examinations from prenatal screening centers and clinical records of the cleft palate team	Major and minor congenital anomalies; IUGR and other ultrasound markers (20 weeks gestation); abnormal karyotype defined as associated anomaly	Some clefts were karyotyped; total number of associated and isolated clefts karyotyped not given
Postnatal studies						
Druschel 1996	New York State, USA	Children born to New York residents, registered with an oral cleft before the age of 2 years in the Congenital Malformation Registry, and matched to their birth certificate 1983-1990 *(n = 2,786)*	Live births with oral clefts *(n = 2,786)*	Retrospective review of data from the population-based database of New York State Congenital Malformations Registry and from additional registry sources, including birth certificates	Major congenital anomalies, and minor congenital anomalies (only if they were associated with major anomalies)	No information given

Table 1. *(Continued)*

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
Kallen 1996	Central-East of France/ Sweden/ California, USA	Births registered to 3 registries of the International Clearinghouse for Birth Monitoring Systems: France, 1976-1992 *(n = 1.46 million)*; Sweden, 1973-1992 *(n = 2.07 million)*; California, 1983-1990 *(n = 1.62 million)*	Live births and stillbirths (≥28 weeks gestation) with oral clefts (*n = 8,315*)	Data from the population-based databases of Central-East France, National Sweden's Registry, and the California Birth Defects Monitoring Program; malformations were retrospectively ascertained up to an age of one year	Major non-facial congenital structural anomalies	Identified karyotypes presented; number of associated and isolated clefts karyotyped not given
Milerad 1997	Region of Stockholm, Sweden	Children with oral clefts referred to the regional cleft palate team of Stockholm or reported to the National Malformation Registry 1975-1992 *(n = 616)*	Live births with oral clefts (*n = 616*; submucous CP excluded)	Retrospective review of cleft palate team files, and birth and hospital records	Congenital anomalies that require follow-up or intervention; Abnormal karyotype defined as associated anomaly	Most identified karyotypes (94%, 16/17) presented; not classified according cleft category
Walker 2001	Utah, USA	Births to residents of the statewide non-selected non-referred population of Utah 1995-1999 *(n = 217,429)*	Live births, stillbirths, and TOP with CL or CLP (*n = 263*; CP only excluded)	Data from the Utah Department of Health Birth Defect Network population-based surveillance system	Major anatomic congenital anomalies (that is, those anomalies that would alter pregnancy management or result in functional impairment of the child)	Most associated clefts (75%, 38/51), and few isolated clefts (0.6%, 12/212) were karyotyped

Table 1. *(Continued)*

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
DeRoo 2003	Washington State, USA	Live-born infants to residents of the statewide population of Washington 1987-1990 *(n = 298,138)*	Live births with oral clefts *(n = 608)*	Data from the Washington State Birth Defects Registry population-based surveillance system on congenital anomalies diagnosed within the first year of life & Washington State birth certificates	Major congenital anomalies, confirmed genetic anomalies and recognized syndromes	No information given
Shaw 2004	California, USA	Deliveries (≥20 weeks gestation) to California women in non-military hospitals 1983-1997 *(n = 3,572,230)*	Live births, stillbirths, and TOP (≥20 weeks gestation) with oral clefts *(n = 6,415)*	Data from the California Birth Defects Monitoring Program, population-based active surveillance system on congenital anomalies diagnosed within 1 year of delivery	Structural congenital anomalies according to the BPA; Abnormal karyotype defined as associated anomaly	No information given
Vallino-Napoli 2006	Victoria, Australia	Pregnancies in Victoria 1983-2000 *(n = 1,140,668)*	Live births, stillbirths, and TOP (<20 weeks gestation) with oral clefts *(n = 2,022)*	Data from the population-based Victorian Birth Defects Registry	Major congenital anomalies, PRS, chromosomal anomalies, and non-chromosomal syndromes	Some clefts karyotyped; number of associated and isolated clefts karyotyped not given

Table 1. *(Continued)*

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
Calzolari 2007	Europe	Births registered to 23 registers in 14 European countries 1980-2000 *(n = 5,989,834)*	Live births, stillbirths, and TOP with CL or CLP *(n = 5,449)*	Data from the European network (EUROCAT) of 23 registers in 14 European countries, having various periods of follow-up	Two or more unrelated congenital anomalies according to the BPA; Additional anomalies further subdivided into: - recognized conditions (including chromosomal) - MCA	Some clefts were karyotyped; number of associated and isolated clefts karyotyped not given
Stoll 2007	Region of Strasbourg, France	Newborns and fetuses delivered in 11 maternity hospitals in Strasbourg and surrounding rural areas 1979-2003 (*n = 334,262*; no home deliveries in this area)	Live births, stillbirths, and TOP with oral clefts (n = 651; submucous CP excluded)	Data from the regional registry of congenital malformation on anomalies diagnosed within 1 year of age	One or more non-cleft major congenital anomalies§; Additional anomalies further subdivided into: - chromosomal - non-chromosomal	All associated clefts were karyotyped
Beriaghi 2009	Omaha, Nebraska, USA	Children with oral clefts referred to the cleft palate/craniofacial clinic 1980-2000 *(n = 1,127)*	Live births with oral clefts *(n = 1,127)*	Data from the craniofacial centre database obtained by the multidisciplinary team	Congenital anomalies (slight variations of normal & neurological and behavioral abnormalities excluded); Abnormal karyotype defined as associated anomaly	No information given

Table 1. *(Continued)*

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
Tan 2009	Victoria, Australia	Children born in Victoria and registered with an oral cleft in the Birth Defects Register 2000-2002 (n = 312)	Live births with oral clefts (n = 279)‖	Data from the Victorian Birth Defects Register on congenital anomalies diagnosed within 15 years of age	Structural, functional, genetic, chromosomal, and biochemical abnormalities after birth	Some clefts were karyotyped; number of associated and isolated clefts karyotyped not given
Rittler 2011	South America	Children with congenital anomalies ascertained at birth in 48 maternity hospitals from 7 countries of the ECLAMC network, within the framework of a special intervention study 2003-2005 (n = 10,371)¶	Live births with oral clefts (*n = 710*; those with a bifid uvula, congenitally 'healed' CL, submucous CP, or birth weight < 500 g excluded)	Information reported by pediatricians and retrieved by further evaluation by dysmorphologists and geneticists during a follow-up period of 1 year	Major unrelated defects (that is, those requiring medical or surgical intervention, or of substantial cosmetic importance, and clinically recognizable or suspected syndromes) detected and reported between birth and hospital discharge; PRS was classified as isolated CP; Additional anomalies further subdivided into: - chromosomal anomaly - syndromes without chromosomal anomalies - malformation complexes/sequences - MCA	All clefts were karyotyped; information available for most associated (58%, 108/185) and isolated clefts (54%, 281/525); FISH 22q11 was not regularly performed; array CGH was performed in some clefts

Table 1. *(Continued)*

Study Type	Study Area	Study Population and Setting	Inclusion Criteria	Data Sources and Collection	Definition & Classification Associated Congenital Anomalies	Karyotype Information
Rozendaal 2011	Netherlands	Children with oral clefts referred to the 15 Dutch cleft palate teams in the Netherlands	Live births with oral clefts	Registry data from the National Oral Cleft Database and review of medical records (including color photographs, X-rays, and dental casts) after a median follow-up period of 5 years	Major and minor congenital anomalies, including dysmorphic features	Some associated and isolated clefts karyotyped; number not given

TOP = termination of pregnancy; CL = cleft lip only; CLP = cleft lip with cleft palate; CP = cleft palate only; IUCR = intrauterine growth retardation; MCA = multiple congenital anomalies; PRS = Pierre Robin Sequence; EUROCAT = European Registry of Congenital Anomalies and Twins; ECLAM = Latin American Collaborative Study of Congenital Malformations; ICD = International Classification of Diseases (9th and 10th revision); BPA = malformation codes of the British Pediatric Association.

* Number of cases not given.

† Median cleft lip and/or atypical facial clefts analyzed, but excluded from this review.

‡ Other ultrasound markers: e.g., oligohydramnios, polyhdyramnios, single umbilical artery, or nuchal edema.

§ Stoll et al. excluded mental retardation and classified Pierre Robin sequence as isolated CP when it was present without congenital anomalies beyond micrognathia and glossoptosis.

|| Cases with PRS (n = 33) analyzed, but excluded from this review because of the inconsistent definition applied in clinical practice and consequently its over- or underreporting.

¶ Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, and Venezuela (total live births in this area: n = 422,240).

Table 2. Summary of published prevalence data on associated anomalies and chromosomal defects in prenatally and postnatally detected oral clefts

Study Type	Associated Anomalies	Chromosomal Defects		
		*Isolated clefts**	*Associated clefts**	*Total clefts**
	% (n)	% (n)	% (n)	% (n)
Prenatal studies				
CL				
Nyberg 1995†	20.0 (1/5)		0.0 (0/1)‡	0.0 (0/5)
Berge 2001†	0.0 (0/3)	0.0 (0/3)	0.0 (0/0)	0.0 (0/3)
Maarse 2011†	13.3 (2/15)		50.0 (1/2)	6.7 (1/15)
Total	13.0 (3/23)	0.0 (0/3)	33.3 (1/3)	4.3 (1/23)
CLP				
Nyberg 1995†	45.7 (16/35)	5.3 (1/19)	50.0 (8/16)	25.7 (9/35)
Berge 2001†	66.0 (35/53)	0.0 (0/18)	68.6 (24/35)	46.3 (25/54)§
Maarse 2011†	39.1 (9/23)	7.1 (1/14)	66.7 (6/9)	30.4 (7/23)
Total	54.0 (60/111)	3.9 (2/51)	63.3 (38/60)	36.7 (41/112)§
CL±P				
Perrotin 2001†	35.7 (20/56)	0.0 (0/36)	55.0 (11/20)‡	19.6 (11/56)
Offerdal 2008	57.1 (20/35)		40.0 (8/20)	22.9 (8/35)
Russell 2008	51.7 (15/29)		33.3 (5/15)‡‖	17.2 (5/29)
Gillham 2009†	17.2 (26/151)	0.0 (0/122)	34.6 (9/26)	6.0 (9/151)
Total	29.9 (81/271)	0.0 (0/158)	40.7 (33/81)	12.2 (33/271)
CP				
Berge 2001†	100.0 (2/2)		100.0 (2/2)‡	100.0 (2/2)‡
Postnatal studies				
CL				
Kallen 1996	10.4 (212 /2029)		10.4 (22/212)‖	1.1 (22/2029)
Milerad 1997	8.0 (13/163)¶			
Walker 2001**	8.3 (7/84)		14.3 (1/7)	1.2 (1/84)
Calzolari 2007	13.6 (245/1806)		13.1 (32/245)‖	1.8 (32/1806)
Tan 2009	11.9 (8/67)		12.5 (1/8)‖	1.5 (1/67)
Maarse 2011	11.8 (2/17)		0.0 (0/2)	0.0 (0/17)
Rittler 2011	7.6 (9/119)	1.8 (2/110)††	22.2 (2/9)	3.4 (4/119)
Rozendaal 2012	41.4 (29/70)		0.0 (0/29)	0.0 (0/70)
Total	12.1 (525/4355)	1.8 (2/110)	11.3 (58/512)	1.4 (60/4192)
CLP				
Kallen 1996	25.3 (819/3232)		24.5 (201/819)‖	6.2 (201/3232)
Milerad 1997	28.0 (60/214)¶			
Walker 2001**	24.6 (44/179)		31.8 (14/44)	7.8 (14/179)
Calzolari 2007	23.8 (693/2913)		22.1 (153/693)‖	5.3 (153/2913)
Tan 2009	23.2 (22/95)		13.6 (3/22)‖	3.2 (3/95)

Table 2. *(Continued)*

Study Type	Associated Anomalies		Chromosomal Defects					
			*Isolated clefts**		*Associated clefts**		*Total clefts**	
	%	(n)	%	(n)	%	(n)	%	(n)
Maarse 2011	21.1	(4/19)			25.0	(1/4)	5.3	(1/19)
Rittler 2011	23.5	(93/395)	1.0	(3/302)††	28.0	(26/93)	7.3	(29/395)
Rozendaal 2012	61.2	(60/98)			5.0	(3/60)	3.1	(3/98)
Total	25.1	(1795/7145)	1.0	(3/302)	23.1	(401/1735)	5.8	(404/6931)
CL±P								
Drushel 1996	29.2	(467/1599)¶						
DeRoo 2003	22.9	(64/280)¶						
Shaw 2004	60.2	(2453/4072)¶					10.3	(419/4072)
Vallino-Napoli 2006	25.1	(299/1189)			33.8	(101/299)‖	8.5	(101/1189)
Stoll 2007	27.9	(109/390)			33.0	(36/109)‖	9.2	(36/390)
Russell 2008	37.0	(47/127)			34.0	(16/47)‖	12.6	(16/127)
Offerdal 2008	33.3	(22/66)			4.5	(1/22)	1.5	(1/66)
Beriaghi 2009	26.4	(157/595)¶						
Gillham 2009	7.2	(16/222)	0.0	(0/206)	6.3	(1/16)	0.5	(1/222)
Total	42.6	(3634/8540)	0.0	(0/206)	31.4	(155/493)	9.5	(574/6066)
CP								
Drushel 1996	43.6	(517/1187)¶						
Kallen 1996	29.0	(732/2527)			18.3	(134/732)‖	5.3	(134/2527)
Milerad 1997	22.2	(53/239)¶						
DeRoo 2003	64.9	(144/222)¶						
Shaw 2004	71.1	(1665/2343)¶					10.6	(249/2343)
Vallino-Napoli 2006	41.7	(347/833)			21.0	(73/347)‖	8.8	(73/833)
Stoll 2007	47.9	(125/261)			14.4	(18/125)‖	6.9	(18/261)
Russell 2008	53.1	(52/98)			11.5	(6/52)‖	6.1	(6/98)
Offerdal 2008	50.0	(10/20)			30.0	(3/10)	15.0	(3/20)
Tan 2009‡‡	23.1	(27/117)			29.6	(8/27)‖	6.8	(8/117)
Beriaghi 2009	38.7	(206/532)¶						
Gillham 2009	26.6	(67/252)						
Maarse 2011	52.9	(9/17)			0.0	(0/9)	0.0	(0/17)
Rittler 2011	42.3	(83/196)	0.0	(0/113)	12.0	(10/83)	5.1	(10/196)
Rozendaal 2012	78.3	(54/69)	13.3	(2/15)	16.7	(9/54)	15.9	(11/69)
Total	45.9	(4091/ 8913)	1.6	(2/128)	18.1	(261/1439)	7.9	(512/6481)

CL = cleft lip only; CLP = cleft lip with cleft palate; CL±P = cleft lip with or without cleft palate; CP = cleft palate only. Blanc entry: data were not available or could not be deducted.

* Information on karyotype not available for all clefts, unless stated differently (see Table 1). Therefore, inclusion of undetected chromosomal defects cannot be ruled out. Null values were given only if information about chromosomal analysis was reported.

† Median cleft lip and atypical facial clefts were excluded because of their different pathogenesis.

‡ Karyotype available for all clefts.

§ For one case with a chromosomal defect, data on associated anomalies were not available.

|| Retrospective analysis of data from birth or birth defect registries. Although not specifically mentioned whether chromosomal defects were accompanied by additional anomalies, we assumed that karyotype analysis had been performed only in associated clefts (as is generally done in clinical practice).

¶ No specific data given about type of associated anomalies, including chromosomal defects. Therefore, inclusion of chromosomal defects in isolated clefts cannot be ruled out.

** Because of limited data, chromosomal defects among isolated clefts not given.

†† Including deletions 22q11.2 identified by array CGH.

‡‡ Pierre Robin sequence excluded.

were the most commonly observed defects.[6-8, 13, 14] Offerdal et al.[15] and Russell et al.[16] did not specify prenatally identified chromosomal defects in their study (n = 8 and n = 5, respectively).

Postnatally detected associated anomalies and chromosomal defects

Seventeen studies analyzed a total of 28,953 infants with oral clefts.[13-16, 22, 25-36] Table 2 shows the prevalence of associated anomalies and chromosomal defects in postnatally detected clefts. Similar to the prenatal analysis, postnatal studies showed that CL was less frequently associated with accompanying defects than the other two cleft categories. The prevalence of associated anomalies in CL was approximately 10%, except for the study of Rozendaal et al. (41.4%).[22] For CLP and CL±P, most studies showed a prevalence of approximately 25%. However, the studies of Shaw et al.[35] and Rozendaal et al.[22] found a prevalence of about 60%. All studies reported that CP was the category most frequently associated with additional anomalies (45.9%; range 22.2% to 78.3%). When analyzing the underlying chromosomal defects, the prevalence was highest in CL±P (9.5%, range 0.5% to 12.6%). The lowest prevalence of chromosomal defects was found in CL (1.4%, range 0% to 3.4%). Studies distinguishing unilateral and bilateral clefts showed a higher prevalence among bilateral than unilateral CLP (Table 3).

Analysis of chromosomal defects in isolated and associated clefts revealed that almost all chromosomal abnormalities were found in association with additional anomalies. Only two studies found chromosomal defects in isolated clefts. In the study of Rittler et al.,[27] information was available for 58% (108/185) of the isolated cleft cases (Table 1). They found diagnostic evidence for chromosomal defects in 1.8% (2/110) of the CL cases (both having a deletion 22q11.2), and for 1.0% (3/302) of the CLP cases. The latter three cases showed a deletion 22q11.2, a 46,X,del(X)(q1.3), and a 46,XY,add(15)(p11). As the 22q11.2 deletions were identified with array-comparative genomic hybridization (array CGH) during follow-up, the rate of chromosomal defects detected by standard karyotyping was 0% (0/110) and 0.7% (2/302) for CL and CLP, respectively. Although the rate of karyotyped cases was not known in the study of Rozendaal et al.,[22] they found that two out of 15 isolated CP cases had chromosomal defects (trisomy 21 and 46,XY,add(14)(p), respectively). In both cases, the identification of the chromosomal abnormality was delayed due to absence of additional congenital anomalies. An inventory of the reported chromosomal defects, non-chromosomal syndromes, and other diagnoses associated with prenatally and/or postnatally detected clefts is provided in Table 4.

Table 3. Summary of published prevalence data on associated anomalies and chromosomal defects in prenatally and postnatally detected unilateral and bilateral oral clefts

Study Type	Associated Anomalies		Chromosomal Defects					
			*Isolated clefts**		*Associated clefts**		*Total clefts**	
	Unilateral	Bilateral	Unilateral	Bilateral	Unilateral	Bilateral	Unilateral	Bilateral
	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Prenatal studies								
CLP								
Nyberg 1995†	40.0 (6/15)	55.0 (10/20)	11.1 (1/9)	0.0 (0/10)	33.3 (2/6)	54.4 (6/10)	20.0 (3/15)	30.0 (6/20)
Berge 2001†	52.0 (13/25)	78.6 (22/28)	0.0 (0/12)	0.0 (0/6)	61.5 (8/13)	72.7 (16/22)	32.0 (8/25)	58.6 (17/29)§
Maarse 2011†	35.3 (6/17)	50.0 (3/6)	0.0 (0/11)	33.3 (1/3)	66.7 (4/6)	66.7 (2/3)	23.5 (4/17)	50.0 (3/6)
Total	45.6 (26/57)	64.8 (35/54)	4.8 (1/32)	5.3 (1/19)	57.7 (15/25)	68.6 (24/35)	26.3 (15/57)	47.3 (26/55)§
CL±P								
Perrotin 2001†	24.1 (7/29)	48.1 (13/27)	0.0 (0/22)	0.0 (0/14)	57.1 (4/7)‡	53.8 (7/13)‡	13.8 (4/29)	25.9 (7/27)
Gillham 2009†	15.5 (18/116)	22.9 (8/35)	0.0 (0/98)	0.0 (0/27)	33.3 (6/18)	37.5 (3/8)	5.2 (6/116)	8.6 (3/35)
Total	17.2 (25/145)	33.9 (21/62)	0.0 (0/110)	0.0 (0/41)	40.0 (10/25)	47.6 (10/21)	6.9 (10/145)	16.1 (10/62)
Postnatal studies								
CL								
Walker 2001‖	8.3 (6/72)	8.3 (1/12)			16.7 (1/6)	0.0 (0/1)	1.4 (1/72)	0.0 (0/12)
CLP								
Walker 2001‖	20.5 (23/112)	31.3 (21/67)			21.7 (5/23)	42.9 (9/21)	4.5 (5/112)	13.4 (9/67)

CL = cleft lip only; CLP = cleft lip with cleft palate; CL±P = cleft lip with or without cleft palate; CP = cleft palate only. Blanc entry: data were not available or could not be deducted.

* Information on karyotype not available for all clefts, unless stated differently (see Table 1). Therefore, inclusion of undetected chromosomal defects cannot be ruled out. Null values were given only if information about chromosomal analysis was reported.

† Median cleft lip and atypical facial clefts were excluded because of their different pathogenesis.

‡ Karyotype available for all clefts.

§ For one case with a chromosomal defect, data on associated anomalies were not available.

‖ Because of limited data, chromosomal defects among isolated clefts not given.

Table 4. Observed chromosomal defects and non-chromosomal syndromes associated with oral clefts in prenatal and/or postnatal populations

	Studies												
	Prenatal			*Prenatal-Postnatal*			*Postnatal*						
	Nyberg 1995	Berge 2001	Perrotin 2001	Maarse 2010	Russell 2008	Gillham 2009	Kallen 1996	Milerad 1997	Stoll 2007	Vallino 2006	Walker 2001	Rittler 2011	NVSCA 1997-2010*
Chromosomal defect													
Trisomy 6											X		
Trisomy 9p				X									
Trisomy 13	X	X	X	X	X	X		X	X	X	X	X	X
Trisomy 16p													X
Trisomy 18	X	X	X	X	X	X		X	X	X	X	X	X
Trisomy 21				X				X	X	X		X	X
Trisomy 22								X					
Triploidy 69		X											
Monosomy 21								X					
Mosaic trisomy 22	X												
Mosaic tetrasomy 12p												X	
Partial autosomal trisomy	X								X				
Translocation			X		X								
Deletion 2q								X					
Deletion 4p			X									X	X
Deletion 4q								X					
Deletion 5p14.3p14.1													X
Deletion 5q21.1q23.3													X
Deletion 13q											X		X
Deletion 22q11.2									X			X	X
46,XY,der(3)del(p26)inv dup(3)(p24p25)													X
46,XX, der(6)t(2;6)(q37;q27) pat													X
dup(11)(p11.1p15.5)pat													X
46,XY,der(14)t(14;16) (p11;p12.3)													X
46,XY,add(15)(p11)												X	
46,XX,del(16)(q22.3q22.3)													X

Table 4. *(Continued)*

	Studies												
	Prenatal			*Prenatal-Postnatal*			*Postnatal*						
	Nyberg 1995	Berge 2001	Perrotin 2001	Maarse 2010	Russell 2008	Gillham 2009	Kallen 1996	Milerad 1997	Stoll 2007	Vallino 2006	Walker 2001	Rittler 2011	NVSCA 1997-2010*
46,XY,der(18)t(16;18)(q24;q23)pat													X
46,XY,del(18)(q21.3)													X
47,XX,+inv dup (22)(q11q11)													X
dup(22)(q11q11)													X
Partial autosomal deletion	X								X				X
Sex chromosomal abnormalities	X							X	X			X	X
Other chromosomal abnormalities		X								X			
Non-chromosomal syndrome													
Adams-Oliver syndrome									X				
Amniotic band association	X									X			
Anti-epileptic drugs							X						
Apert syndrome						X	X	X					X
Beckwith-Wiedeman syndrome				X				X					X
Branchio-oculo-facial syndrome													X
Bohring-Opitz syndrome													X
Caudal Regression syndrome					X								
CHARGE syndrome						X						X	X
Chondrodystrophy							X						
Cornelia de Lange syndrome							X		X			X	X
Crouzon syndrome								X					X
DiGeorge syndrome					X					X			
Duane retraction syndrome													X
Ectrodactyly-ectodermal dysplasia-clefting syndrome							X		X				X
Encephalocele-clefting syndrome								X					

Table 4. *(Continued)*

	Studies												
	Prenatal			*Prenatal-Postnatal*			*Postnatal*						
	Nyberg 1995	Berge 2001	Perrotin 2001	Maarse 2010	Russell 2008	Gillham 2009	Kallen 1996	Milerad 1997	Stoll 2007	Vallino 2006	Walker 2001	Rittler 2011	NVSCA 1997-2010*
Fetal alcohol syndrome							X						
Fraser syndrome													X
Fryns syndrome			X										
Goldenhar syndrome				X			X		X	X			X
Gordon syndrome													X
Gorlin syndrome													X
Greig syndrome													X
Hay-Wells (AEC) syndrome													X
Holoprosencephaly										X			
Ivemark syndrome									X				
Jeune syndrome													X
Kabuki syndrome													
Kartagener syndrome†								X					
Klippel-Feil syndrome							X		X				X
Larsen syndrome								X				X	
Loeys-Dietz syndrome													X
Meckel-Gruber syndrome					X								
Meckel syndrome							X		X				
Moebius syndrome							X		X				X
Mohr syndrome												X	
Multiple epifysial dysplasia†										X			
Multiple pterygium syndrome									X				
Nager syndrome							X						X
Noonan syndrome													X
Omenn reticuloendotheliosis†									X				
Opitz-Frias syndrome			X										
Opitz G/BBB												X	X
Oro-facio-digital syndrome							X		X				X

Table 4. *(Continued)*

	Studies												
	Prenatal			*Prenatal-Postnatal*			*Postnatal*						
	Nyberg 1995	Berge 2001	Perrotin 2001	Maarse 2010	Russell 2008	Gillham 2009	Kallen 1996	Milerad 1997	Stoll 2007	Vallino 2006	Walker 2001	Rittler 2011	NVSCA 1997-2010*
Osler-Weber syndrome†								X					
Osteogenesis imperfecta†							X						
Osteopathia striata with cranial sclerosis													X
Oto-palato-digital syndrome	X								X				X
Pentalogy of Cantrell (Thoraco-abdominal syndrome)													X
Poland syndrome†								X					
Popliteal pterygium syndrome			X				X						
Rieger syndrome													X
Roberts syndrome									X				
Robinow syndrome								X					
Rubinstein-Taybi syndrome†											X	X	X
Smith-Lemli-Opitz syndrome					X		X	X					X
Stickler syndrome				X		X	X	X	X			X	X
Treacher-Collins syndrome							X	X	X	X			X
VACTERL													X
Van der Woude syndrome				X	X			X			X	X	X
VATER association									X				
VCF syndrome§									X				
X-linked hydrocephalus†										X			
Other diagnosis													
Neonatal Abstinence syndrome													X
Pierre Robin sequence				X	X	X			X	X			X
Sebaceus Nevus syndrome													X

* Annual reports 1997-2010 of the Dutch Association for Cleft Palate and Craniofacial anomalies, comprising data without follow-up.

† Diagnosis uncertain.

§ Clinical diagnosis, not confirmed by karyotype.

DISCUSSION

This systematic review assessed the association of prenatally and postnatally detected oral clefts with other congenital anomalies and underlying chromosomal defects, thereby providing a basis for prenatal counseling and well-informed decisions on invasive prenatal diagnostics in clefts. We demonstrated that the prevalence of associated structural and chromosomal defects is evidently related to cleft category. Although varying in study characteristics and designs, both prenatal and postnatal studies showed a higher frequency of associated anomalies and chromosomal defects in CLP and CP than in CL. For all cleft categories, chromosomal defects were almost always seen in association with additional congenital anomalies. Therefore, the presence of additional anomalies on ultrasound is the most important predictor of underlying chromosomal defects in fetuses with oral clefts.

Methodological issues

The use of both prenatal and postnatal studies—including detailed Dutch registry data—gave our study its main strength. It allowed us to provide a more reliable and representative basis for prenatal counseling and genetic testing than when only prenatal studies were evaluated. As the proportion of detected isolated clefts in prenatal populations is rising, previous prenatal studies may not have provided representative samples of current/future prenatal cleft populations. Overall, prenatal rates of associated anomalies and chromosomal defects may have been too high, because associated clefts are more likely to be detected by ultrasound than isolated clefts,[11] and some prenatal cases never reach term due to lethal anomalies or termination of pregnancy (TOP).[16, 25, 26, 29] Another advantage of our evaluation of postnatal studies is that congenital anomalies not detected by ultrasound were also included. Especially studies with a longer follow-up allowed us to consider minor anomalies and features that become more evident later in life.[22] For example, individuals with the velo-cardio-facial (VCF) syndrome (22q11.2 deletion) are often diagnosed at school age when speech and learning difficulties become evident, unless a cardiac defect manifests earlier.[38] Our study was also strengthened by its focus on clinical genetic aspects. If provided, karyotype information was evaluated and separate rates of chromosomal defects among isolated (if available) and associated clefts were calculated. Besides these prevalence rates, we also composed an inventory of the different syndromes and chromosomal defects in prenatally and/or postnatally detected clefts reported by the reviewed studies and complemented with Dutch registry data, thereby specifying the detected anomalies (Table 4).

However, combining results from different studies also had its limitations, mainly due to methodological issues. As summarized in Table 1, we found many differences in study characteristics and designs between the reviewed studies, which are in line with those reported by Wyszynski et al.[17] The most important issue was non-uniform subdivision of oral clefts. Some studies distinguished CL and CLP,[6, 7, 13, 25, 27, 28, 30, 31, 33] while others grouped them as CL±P.[8, 14-16,

[26, 29, 32, 34-36] Together with previous studies,[2-5] our results stress the need of accurate prenatal subdivision into three categories (CL, CLP and CP). Obviously, analyzing CL and CLP as one group will result in different frequencies of associated anomalies and chromosomal defects than when they are analyzed separately. Unfortunately, prenatal distinction between CL and CLP can be limited because prenatal identification of involvement of the palate is still challenging.[11, 14] For this reason, data on prenatally detected CP were limited in the current study. However, there is evidence of improvements in imaging, as well as in experience in detection and interpretation of subtle signs on ultrasound,[39, 40] which will progressively reduce the lower limits for detection.

Another important factor was that associated anomalies were differently defined and classified in the evaluated studies, which partly explains the wide variation in the reported rates of associated anomalies.[17] The definitions in the reviewed studies ranged from only major (structural) non-facial congenital anomalies to all anomalies, including minor congenital anomalies and ultrasound markers, such as intrauterine growth retardation (Table 1). This might explain, at least partially, the relatively high rates of associated anomalies reported by Rozendaal et al., [22] who also included minor and dysmorphic features in their analysis. Although these minimal defects are hardly detected prenatally, they may be recognizable components of specific syndromes or chromosomal defects in postnatally detected clefts.[17] Similarly, the high prevalence of Shaw et al.[35] could also partly be due to the inclusion of minor defects, as they used diagnostic codes with low specificity, including the malformation groups "ear, face, neck" and "upper alimentary tract". Another source of variation is the inconsistent definition of Pierre Robin sequence applied in clinical practice and consequently its over or underreporting.[41] Some of the reviewed studies classified this condition—being CP combined with micrognathia, glossoptosis and airway compromise—as isolated CP,[26, 27, 33] while other studies considered it as a separate category[28, 31] or as associated CP.[11, 14, 16, 22, 29]

The reviewed studies also varied considerably in their reporting of karyotypic information (Table 1). While some studies provided explicit information about the number of karyotyped cases and their detected associated and chromosomal defects,[6-8, 13-15, 22, 25, 27] others reported only abnormal karyotypes, but not their associated anomalies,[16, 26, 28-31] or they did not give any specific data at all.[32-36] As a consequence, separate and complete rates of chromosomal defects could not always be obtained. Furthermore, in studies providing explicit information, chromosomal analysis was mostly performed in associated clefts only, which explains why almost all reported chromosomal defects were accompanied by additional anomalies. It is important to realize that most of these studies obtained chromosome results for just a part—and not all—of the clefts, and that the inclusion of cases with undetected chromosomal defects in their rates therefore cannot be ruled out. Besides karyotype analysis, most studies did also not report whether FISH analysis had been performed and whether microdeletions were included in the presented data. Only the studies of Tan et al.[31], Rittler et al.[27], and Rozendaal et al.[22] reported the inclusion of microdeletions or duplications, while Stoll et al.[26] included

the results of FISH22q11 screening as from 1994. In contrast, Kallen et al.[28] reported not to have included microdeletions, which might have led to an underestimation of the frequency of underlying chromosomal defects. On the other hand, some studies may have overrepresented chromosomal defects in association with oral clefts due to the inclusion of sex chromosome abnormalities. For example, Stoll et al.[26] showed that 12 out of 54 abnormal karyotypes concerned abnormalities of sex chromosomes, which may be coincidental findings and not related to clefts. From literature, no convincing evidence is provided that the most frequently detected sex chromosomal anomalies (e.g., 47,XXX; and 47,XXY) are actually related to clefts.

Differences in study settings and data sources between studies (Table 1) may also have accounted for the variation in the prevalence of associated anomalies and are possible sources of selection bias. For example, some studies were performed with data from prenatal centres,[6-8, 13, 15] while others were retrospectively conducted via the so-called 'cleft palate teams'.[14, 16] Consequently, the retrospective cleft-team studies did not include the fetuses that were not born alive and were thus not referred to the cleft palate teams, thereby inducing selection bias. Additionally, according to Wyszynski et al.,[17] information obtained from vital records (e.g., birth certificates) is neither complete nor accurate in detail due to passive ascertainment methods (i.e., data submitted by data sources and not actively collected by registry staff searching data sources for eligible cases) and lack of follow-up. Conversely, studies having active ascertainment methods or long follow-up periods, such as that of Rozendaal et al. (median follow-up 5 years),[22] may result in relatively high rates of associated anomalies. Also, the value of information depends on the interest and skills of the person who records the anomalies. This is in line with the study of Tan et al.,[31] who reported higher frequencies of associated anomalies in patients recruited for a clinical study than in cases derived from a birth defect register. They suggested this was explained by a combination of ascertainment bias and more complete diagnosis by detailed clinical assessment in the clinical study.

Nevertheless, despite the above-mentioned issues, we found unambiguous evidence that the three cleft categories are differently associated with structural and chromosomal defects. Due to the inclusion of large numbers of cases from both prenatal and postnatal populations, we were able to provide a rather reliable basis for clinicians and future parents, thereby allowing accurate counseling and informed decisions on whether to have invasive diagnostics if an oral cleft is detected prenatally.

Prenatal counseling and genetic testing

When counseling future parents regarding prognosis and risk of associated chromosomal defects, it is vital to tailor the discussion according to cleft category. As our results showed, CLP and CP are more frequently associated with additional anomalies and chromosomal defects than CL. Moreover, these frequencies are higher in bilateral than in unilateral CLP or CL±P. This emphasizes the need for accurate prenatal subdivision of clefts. However, accurate detection of additional anomalies appears to be even more significant to outcome. As we found, the

presence of other congenital anomalies is a strong predictor for chromosomal defects. For all cleft categories, both prenatal and postnatal studies showed that chromosomal abnormalities are almost always seen in association with other congenital anomalies. Therefore, invasive prenatal testing to identify chromosomal abnormalities in combination with genetic counseling should be offered in all cases with associated anomalies, irrespective of cleft category.

It should be realized, however, that the absence of associated anomalies does not exclude the possibility of the presence of an underlying chromosomal defect. As mentioned above, chromosomal analysis was often not performed in isolated cases, and therefore undetected chromosomal defects might have been included in our rates of isolated clefts. The few studies that reported chromosomal defects in isolated clefts showed that the prevalence differed by category. As standard karyotyping did not reveal any chromosomal defect, cases with isolated CL have the most favorable prognosis when it comes to chromosomal anomalies with a poor outcome. Therefore, if confident in ultrasound findings, conventional karyotyping is not recommended in isolated CL. However, based on the findings of Rittler et al.[27], array CGH to detect deletion 22q11.2 should be considered.

For CLP, prenatal studies together showed chromosomal defects in 3.9% of the presumed isolated cases, while just one postnatal study addressed this issue showing defects in 1.0%. In the latter study,[27] standard karyotyping revealed chromosomal defects in 0.7% of the isolated CLP cases, while array CGH during follow-up revealed a deletion 22q11.2 for one more case. Based on these data, it is recommended to inform future parents about the possible association of a chromosomal defect and to consider invasive prenatal testing in these cases, preferably by array-based methods. However, if not confident in ultrasound findings regarding cleft category, it should be noted that the overall prevalence in presumed isolated clefts (CP excluded) was 0.8% (7/830). Furthermore, when considering invasive testing, the baseline risk of complications (1%) should be weighed against the potential benefits.[42] Another concern might be the detection of unexpected or unclassified variants with array-based methods, which should be discussed with future parents.

Regarding CP, especially isolated CP, prenatal identification is still challenging, which has resulted in limited prenatal information on their underlying chromosomal defects. However, postnatal karyotyping of isolated CP cases revealed a chromosomal defect in 1.6%. Especially in this category, specific syndromes, such as VCF (22q11.2 deletion), Treacher-Collins, and Stickler, have to be considered. As presented in Table 4, these syndrome diagnoses were frequently reported in the evaluated literature. Therefore, until more information on chromosomal defects in prenatally presumed isolated CP is available, we advise to consider invasive genetic testing and consultation by a clinical geneticist if an isolated CP is detected prenatally. A prenatal diagnostic algorithm according to cleft category is presented in Figure 1.

Based on the above findings, more accurate prenatal ultrasound screening will improve counseling, especially regarding palatal involvement. Therefore, we advise to refer pregnant women with a fetus suspected of an oral cleft to a tertiary care centre where more specific

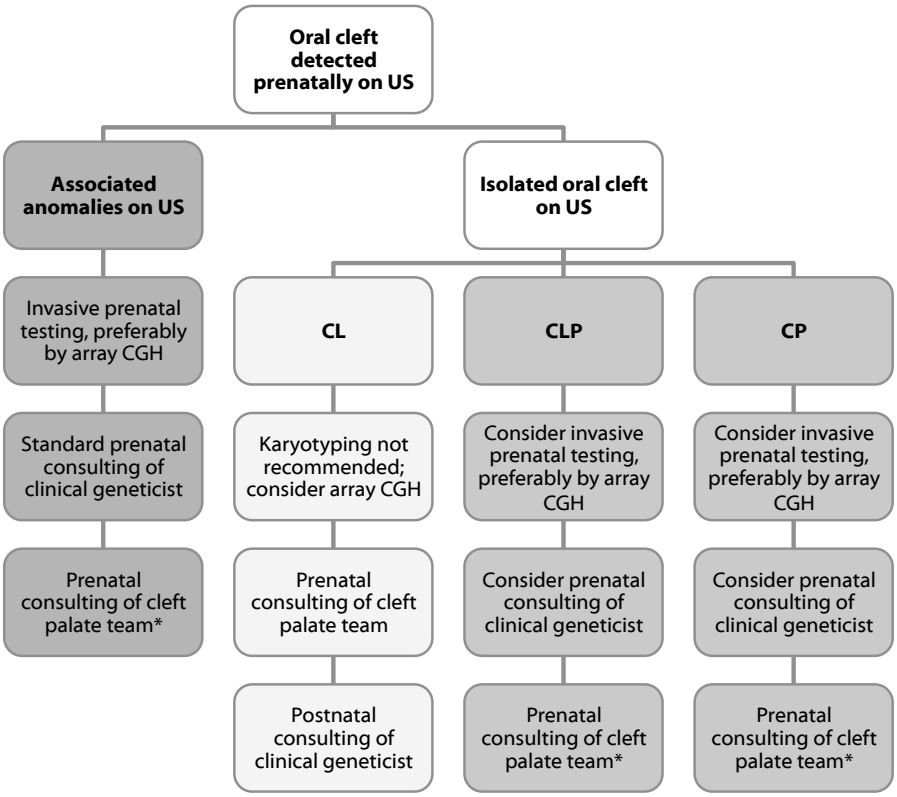

Figure 1. Algorithm for invasive genetic testing according to oral cleft category. CL = cleft lip only; CLP = cleft lip with cleft palate; CP = cleft palate only; US = ultrasound; array CGH = array-comparative genomic hybridization. * If a normal karyotype is confirmed or invasive genetic testing is declined.

ultrasound screening can be performed. In addition, if a normal karyotype is confirmed or invasive testing is declined, future parents should be counseled by a multidisciplinary cleft palate team that focuses on psychosocial support, education on management of clefts, and parents' options, TOP being one of them.[43-46] Finally, it is crucial to distinguish median clefts and atypical facial clefts from oral clefts. These different craniofacial anomalies are associated with other congenital anomalies and have a different prognosis, and should therefore be referred to and treated by specialized multidisciplinary craniofacial teams.

Future studies

The use of array CGH in clinical practice is rising, and it is expected that it will be implemented as standard prenatal diagnostics in the near future. Compared to conventional karyotyping,

array CGH can detect smaller chromosome deletions and duplications. To gain more insight in the yield of array CGH in cases with clefts, it would be interesting to perform array CGH in a large cohort of cases with prenatally and postnatally detected clefts. This would also give us more information about the proportion and types of chromosomal defects that are missed in cases that have not been karyotyped or studied by array-based methods. Especially with regard to prenatally presumed isolated clefts, this is essential to reach consensus on the role of invasive genetic testing in these cases. As was demonstrated by the NVSCA data,[23, 24] clefts can be associated with various microdeletions and duplications. This implies that array CGH should be the standard technique to identify chromosomal defects in children with oral clefts.

Finally, follow-up studies are needed to gain more insight into additional abnormalities and chromosomal anomalies identified after birth. This can aid in more optimal counseling of future parents, especially with regard to unexpected anomalies in presumed isolated clefts, and timely treatment of children with clefts.

CONCLUSIONS

This systematic review presents unambiguous evidence that the different cleft categories are variously associated with additional congenital anomalies and underlying chromosomal defects. This emphasizes the need of accurate subdivision of CL, CLP and CP for both ultrasound screening and postnatal follow-up. However, the most important predictor of chromosomal abnormalities is the presence of associated anomalies, and we urge clinicians to advise invasive testing in these cases. In absence of associated anomalies, cases with CL have the most favorable prognosis and do not require conventional karyotyping. In presumed isolated CLP and CP, professionals should explain the possible association of a chromosomal defect and consider invasive genetic testing, preferably by array-based methods. In all cleft categories, an association with deletion 22q11.2 should be considered.

Accurate prenatal diagnosis by ultrasound is essential in the quality of counseling, especially with regard to palatal involvement and associated anomalies. Therefore, pregnant woman—with a fetus suspected of an oral cleft—should be referred to a tertiary care centre where more specific ultrasound screening can be performed. Finally, follow-up studies, including array CGH, are needed to gain more insight in additional abnormalities and chromosomal defects missed in associated and presumed isolated clefts. This would aid in more optimal counseling and timely treatment of children with oral clefts.

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CHAPTER 11

Clinical implications of a new ultrasound classification of oral and craniofacial clefts based on embryology

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ABSTRACT

Orofacial clefts are being diagnosed prenatally more frequently. They comprise various subphenotypes that have different underlying embryological processes and vary in accompanying structural and chromosomal defects, and thus have a different prognosis. Therefore, accurate and detailed phenotyping and subsequent classification of clefts is essential to further understand their etiopathogenesis. Furthermore, it is crucial to help arrive at correct diagnosis, thereby improving clinical care and outcome. This is especially important in the prenatal setting, as it will influence counseling and management of the pregnancy significantly. While many systems have been developed to record or classify clefts, most are intended for the postnatal setting only, and those designed for fetal clefts do not incorporate the latest scientific insights and are not designed for modern ultrasound technologies. Therefore, we propose a new prenatal ultrasound classification for orofacial clefts based on their patho-embryology, recent epidemiological insights, and advances in ultrasound technology to aid in prenatal counseling, care, and research. In short, this paper discusses why oral clefts (i.e., unilateral or bilateral clefts of the lip/alveolus or palate) should be distinguished from midline and atypical facial clefts, and why the latter should be considered as craniofacial clefts based on their patho-embryogenesis and accompanying defects. Subsequently, both groups are further divided according to their specific underlying embryological processes as well as their accompanying defects, prognosis, and outcome. Finally, our system is discussed in relation to the only previously published—purely prenatal—cleft classification, the Nyberg classification.

INTRODUCTION

Orofacial clefts are complex and heterogeneous birth defects affecting various facial structures, including the lip, alveolus, and palate. Normal development of these structures entails a complex series of embryonic processes, which are related to different time frames and regulated by different cell biological mechanisms and genes.[1-4] Disruption of any of these tightly regulated processes may result in various cleft sub-phenotypes.[2-8] Therefore, accurate and detailed phenotyping and subsequent classification of clefts is crucial to further understand their etiopathogenesis. Furthermore, it is essential to help arrive at correct diagnosis and improve clinical care and outcome. Different cleft types are variously associated with accompanying structural and chromosomal defects and thus have a different prognosis.[9] Especially in the prenatal setting, early detection of these anomalies is of paramount importance, as it will influence counseling and management of the pregnancy significantly.

Clinically, it is crucial to distinguish oral clefts (often termed orofacial clefts and comprising unilateral or bilateral clefts of the lip/alveolus/palate) from midline and atypical facial clefts. While the latter anomalies should be considered as craniofacial clefts given their pathoembryogenesis, accompanying defects, and outcome,[5, 6, 10, 11] they are often interpreted as oral clefts.[12-20] Consequently, great discrepancies with regard to cleft definition and classification exist. Although many systems have been developed to record or classify clefts, most are intended for the postnatal setting only,[7, 8, 12, 14, 18, 21-28] and just a few have been specifically designed for fetal clefts. The first prenatal diagnosis of oral clefts was reported in the early 80s,[29] and Nyberg et al.[30] presented an ultrasound classification based on the embryology of the face in 1995. Later on, Sommerlad et al.[31] demonstrated the use of Kernahan's postnatal 'striped Y' model to record oral clefts detected with both two- and three-dimensional (2D and 3D) ultrasound. While these systems have brought structure in the prenatal diagnosis of clefts, they do not incorporate the latest insights into the embryonic and genetic mechanisms underlying clefts,[2-4, 32] and they have not been designed for modern ultrasound technologies. Because of the technical advances in prenatal imaging, as well as in the experience in detection and interpretation of subtle signs, fetal anomalies of the head and neck area are being diagnosed in detail more frequently.[20, 31, 33-38] Although prenatal detection rates for oral clefts by 2D ultrasound have historically been poor, recent studies have reported rates of over 75% for clefts of the lip/alveolus (with or without cleft palate).[39] Because prenatal identification of involvement of the palate by 2D is still challenging, detection rates for clefts of the palate only have been considerably lower (0%-22%).[39] However, there is evidence of improvements in imaging and detecting subtle signs that will progressively increase the prenatal detection of cleft palate.[37, 40, 41] Besides oral clefts, craniofacial clefts and their syndromes, such as frontonasal dysplasia, are also being diagnosed prenatally more frequently.[34, 42] As a consequence, there is an increasing demand for accurate and updated information and classification of clefts to aid in prenatal counseling and further research.

In this article, we propose a new prenatal classification of oral and craniofacial clefts based on their underlying embryological processes as well as on recent insights in their epidemiology (including accompanying defects and prognosis) and advances in ultrasound technology. To explain the rationale behind this classification, the epidemiology, registration and embryology of both oral and craniofacial clefts are outlined before introducing this new system. Finally, our system is compared with Nyberg's ultrasound classification and the differences are discussed.

EPIDEMIOLOGY AND REGISTRATION OF CLEFTS

Oral clefts

Oral clefts are among the most common congenital anomalies in humans, ranging from minor (subcutaneous or submucous) types to complete clefts of the lip, alveolus, and hard and soft palates (including the uvula).[4, 8] Worldwide, the prevalence varies from 4.8 to 28.6 per 10,000 births,[43] with considerable variations in ethnicity and geographic regions. Oral clefts may either be isolated or be associated with other congenital anomalies, often as part of a syndrome or chromosomal defect.[2, 9, 44, 45] Many genetic and environmental factors—such as maternal smoking, alcohol use, and nutrition—have been suggested to contribute to their development.[1, 44]

To gain more insight into the epidemiology and causes of congenital anomalies—including oral clefts—and to optimize their outcome and prevention, various birth defect registries have been established using different postnatal recording and classification systems. Classically, oral clefts are divided into two categories: cleft lip with or without cleft palate, and cleft palate only.[44, 46, 47] However, more recent epidemiological studies have also distinguished cleft lip only from those that affect both the lip and palate, because these categories may have unique embryological and etiological features.[7, 9, 28, 48, 49] To further classify oral clefts, many registries use the International Classification of Diseases (ICD) or its extensions.[46, 50, 51] Using these classifications, oral clefts are not described, but interpreted and directly coded according to clinical diagnosis. Because this approach may lead to important anatomical and morphological details being lost, more specific systems have been developed. These comprehensive systems incorporate the anatomical extent by distinguishing the different affected structures (lip, alveolus, hard palate, and soft palate including the uvula),[13, 21, 23, 25, 52] as well as the morphological severity (complete, incomplete, and submucous clefts).[12, 18, 24, 27] Although these systems seem to be clinically sufficient, none of them has been fully based on craniofacial embryology, thereby lacking detailed information needed to gain more insight into the causes of clefts. Therefore, the Dutch Oral Cleft Registry (NVSCA) has developed a unique descriptive system—based on the embryology of the head and neck area—to record craniofacial congenital anomalies (including oral clefts) in the postnatal setting. This system records all individual anomalies that form the craniofacial defect by describing the morphology and side of each affected anatomi-

cal structure, thereby expressing the various embryonic mechanisms underlying the various sub-phenotypes.[4, 7, 8, 28, 45]

Craniofacial clefts

In contrast to oral clefts, craniofacial clefts are rare congenital anomalies affecting the face and cranium in a great variety of sub-phenotypes, including midline cleft lip and palate or atypical facial clefts, such as hemifacial microsomia (Tessier 7) and Treacher-Collins (Tessier 6, 7, 8).[53] Different facial parts and tissue layers can be involved with various degrees of severity on clinical presentation. Rare craniofacial clefts can present themselves unilaterally or bilaterally, in the midline of the face, or in paramedian or oblique directions.[5, 6] Their low frequency has made their study and classification difficult. Consequently, little is known about their epidemiology, as most reports are based on small groups from various ethnic and geographic regions. In a review, Kawamoto reported a prevalence ranging from 1.4 to 4.9 per 100,000 live births.[54] Craniofacial clefts are almost always associated with other congenital anomalies and frequently part of a syndrome or chromosomal defect.[55]

Because craniofacial clefts are rare, just a few recording systems have specifically been designed for craniofacial clefts. In 1976, Tessier introduced a new comprehensive classification of craniofacial clefts and their syndromes. Using an anatomical cleft numbering system, he described the various sub-phenotypes of craniofacial clefts, but oral clefts were also included.[53] In contrast to this system, Van der Meulen et al.[5, 6] developed an embryological system classifying both oral and craniofacial clefts as well as other craniofacial malformations, such as craniosynostosis. This system is based on the underlying developmental processes of the head and neck area, similar to the above-described NVSCA system.

EMBRYOLOGY OF THE HEAD AND NECK AREA

From a clinical point of view, it is essential to prenatally distinguish between embryologically different cleft anomalies, because they have specific accompanying defects, outcome, and prognosis. Additionally, with regard to the search for causal factors, it is even more crucial to group clefts according to their timing and underlying mechanisms in embryogenesis, as the power to detect effects may be weakened when heterogenous cleft groups are treated as a single entity or divided in a too simplistic way.[3] To classify according to abnormal embryonic processes that result in various cleft deformities, normal craniofacial development need to be understand first. The embryogenesis of the various head and neck structures has been described elsewhere (Vermeij-Keers et al., submitted)[1, 4, 10, 11] and is summarized here.

The craniofacial region develops during two main successive periods. The first is the early embryonic period from 4 to 9 weeks gestation (1-17 mm Crown-Rump Length (CRL), starting with the development of the forebrain (prosencephalon) and early face, followed by formation

of the nose and primary palate. During the second period, which is the late embryonic period from 9 to 14 weeks gestation (17- 60 mm CRL), the lip and alveolus, secondary palate, and facial skull are formed.

Development of the forebrain and early face (4-6.5 weeks gestation)

It is important to realize that the ultimate width of the face is determined by the width of the forebrain (prosencephalon). In short, its development involves the following steps. The initial morphological structures of the developing prosencephalon are formed by the cephalic or neural folds (walls), which caudally continue as the neural plate (1.5 mm CRL).[56] These neural walls grow out in a lateral direction and are then transformed into the prosencephalon of the neural tube due to fusion of the walls in the midline. During this outgrowth process, the optic primordia develop bilaterally by a local thickening of both neural walls. Within both primordia, a progressively deepening groove, called the optic sulcus, is formed.[57] As both sulci expand, the fusion of the neural walls is completed and the sulci are transformed into optic vesicles. The final point of neural wall closure—the rostral neuropore—is located between two areas of specific surface ectoderm (epithelium) covering the prosencephalon, i.e., the nasal fields.[56, 58] Within each laterally located nasal field, a lens placode (optic primordium) and nasal placode develop during 6-7 weeks gestation (5-6.5 mm CRL).[58-60]

Development of the nose and primary palate (6.5-9 weeks gestation)

After closure of the rostral neuropore, the development of the nose holds a key position with regard to facial morphogenesis. The nose develops from two widely separated nasal placodes, with the interplacodal area in between (6.5 weeks gestation; 6.5 mm CRL).[10] Therefore, the presumptive nose can be considered as two separate organs, which can develop asymmetrically. First, the three facial swellings (processes) around each placode grow out (the maxillary process and lateral nasal process at the lateral side, and medial nasal process at the medial side), resulting in nasal grooves and tubes, respectively (Figure 1:1-3).[10, 11, 59] Subsequently, the three facial swellings fuse in an occipito-frontal direction, resulting in the formation of the primary palate, i.e., the presumptive lip and alveolus (9 weeks gestation, 17 mm CRL). Figure 1:1-3 demonstrates that the fusion process starts with adherence and fusion of the most occipital parts of the maxillary process and medial nasal process, and that it ends with fusion of the most frontal parts of the lateral and medial nasal processes. As a consequence, both nasal processes surround the nostril. Figure 1:3-4 shows the residual shallow grooves on the fusion lines of the primary palate.

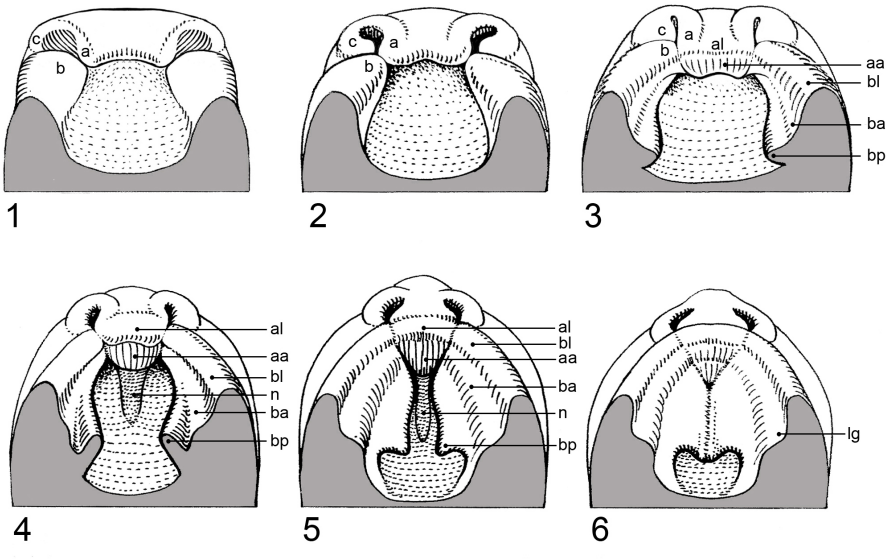

Figure 1. The six successive stages of facial embryonic development viewed from the oral side: the respective fusion processes of the primary palate (1-3) and secondary palate (3-6), and outgrowth of the lip and alveolus (3-6; Adapted from Ten Donkelaar et al.[76]).

1. Two nasal grooves, widely separated, surrounded by the facial processes/swellings (a-c) at seven weeks gestation;
2. Outgrowth and fusion of two (a-b) of the three facial swellings in occipito-frontal direction forming the nasal tubes at eight weeks;
3. Further outgrowth and fusion of the three swellings (a-c), resulting in the formation of the primary palate and the nostril at about nine weeks (note that the swellings are separated by grooves), and the beginning of outgrowth of the lip (al+bl), alveolus (aa+ba) and processes/shelves of the secondary palate; these swellings and shelves exist of mesenchyme covered by ectoderm;
4. Outgrowth of the nasal septum and palatal shelves in fronto-caudal and vertical direction, respectively, and further outgrowth of the lip and alveolus in caudal direction forming the presumptive labial groove at ten weeks;
5. Elevation and outgrowth of the palatal shelves in horizontal position, and start of the fusion of the shelves with the primary palate at 10-11 weeks;
6. Completed fusion of the shelves with the primary palate and nasal septum, as well as with each other, and completion of the lip, alveolus and labial groove at 12-14 weeks. The fusion lines of the primary and secondary palates are striped (3-6).

Abbreviations: a = medial nasal process; b = maxillary process; c = lateral nasal process; al = lip developed from a (prolabium); bl = lip developed from b; aa = alveolus (premaxillae) developed from a; ba = alveolus developed from b; bp = palatal shelves developed from b; if = incisive foramen; lg = labial groove; n = nasal septum; * = Internasal groove.

Development of the lip/alveolus and secondary palate (9-14 weeks gestation)

After fusion of the facial swellings, the residual grooves between the swellings are eliminated by a process called merging.[11, 59] The internasal groove disappears by outgrowth of the presumptive nasal septum in a fronto-caudal direction (17-27 mm CRL; Figure 1:4-6), and not—as is frequently assumed—by fusion of the medial nasal processes in the midline.[11, 59] As a result, the tip and dorsum of the nose, and the nasal septum, columella, and prolabium/philtrum are formed, and the distance between the nostrils/presumptive nasal cavities and between the presumptive eyes/orbits decreases relatively. Simultaneously, the lip and alveolar process of the upper jaw grow out in a caudal direction, thereby forming the labial sulcus (groove) between the lip and alveolus (Figure 1:3-6). During the same period, the palatal shelves (processes) grow out in the oral cavity at either side of the tongue (27-30 mm CRL). Subsequently, they shift from a vertical into a horizontal position above the tongue and fuse with the primary palate, with each other, and with the nasal septum in a fronto-occipital direction. As a result, the secondary palate (the presumptive hard and soft palates including the uvula) is formed (30-50 mm CRL; Figure 1:3-6).[11, 59]

Development of the facial skull (9-14 weeks gestation)

During the late embryonic period, the mesenchyme of the fused facial swellings and palatal shelves differentiates into musculature and bones of the facial skull, starting with the formation of the maxillary bone center in the maxillary process (9 weeks gestation, 17 mm CRL). The number of bone centers developing within one bone, the timing of their development, and the outgrowth of these bone centers followed by fusion or suture formation are crucial factors that determine skull development.[11] In the context of oral and craniofacial clefts, the formation of the premaxillae, maxillae, and palatine bones are explained. Initially, each half of the upper jaw is formed out of three separate bone centers: two centers of the premaxilla (development at 23 and 50 mm CRL) developing within the medial nasal process and bearing two incisor teeth, and one single maxillary bone center. These bone centers grow out and fuse with each other at the original fusion line of the medial nasal and maxillary processes, thereby forming the definitive maxilla, including the alveolar process; the intermaxillary suture develops in the midline, between both halves of the upper jaw. The hard palate develops from the maxillary bone center and palatine bone center at either side. These bilateral bone centers grow out to each other and to those of the premaxilla, thereby forming the incisive, medial, and transverse palatine sutures. Besides these "normal" sutures, additional sutures have been found in the above-mentioned bones and other facial/skull bones (e.g., zygomatic or occipital bone) in adult normal skulls,[10, 11, 32] indicating that the number of bone centers might vary.

NEW ULTRASOUND CLASSIFICATION OF ORAL AND CRANIOFACIAL CLEFTS

To be feasible for clinical as well as research purposes, prenatal classification of clefts should be based on recent scientific insights as well as on modern ultrasound techniques. Due to a more focused and routine approach using high-resolution ultrasound techniques, complete and incomplete clefts of the lip, alveolus (located between the premaxilla and maxilla), and hard and soft palates (including the uvula) can be properly detected nowadays.[31, 37, 41, 61-63] With regard to craniofacial clefts, routine ultrasound can identify agenesis (absence) of the prolabium and premaxilla, hypertelorism, hypotelorism, and atypical clefts.[30, 33-35, 64] However, subtle features—such as subcutaneous and submucous clefts or hypoplasia—can not be visualized prenatally. Additionally, the outcome and prognosis of the various cleft sub-phenotypes may differ from the postnatal setting, as cases that are more likely to be prenatally diagnosed tend to be the more severe cases with associated defects.[39] As a consequence, most postnatal recording and classification systems may not be sufficient and properly applied in the prenatal setting. Therefore, we have developed a new prenatal ultrasound classification of oral and craniofacial clefts (Figure 2).

Oral clefts

Prenatally, it is important to subdivide oral clefts into three main categories: I) *cleft lip/alveolus only*, II) *cleft lip/alveolus and palate*, and III) *cleft palate only* (Figure 2:I-III). Besides their different embryological and etiological features,[2, 3, 7, 48] these categories are differently associated with accompanying defects,[9, 49] and therefore have a different prognosis. As was recently reported,[9] clefts with palatal involvement (II and III) are more frequently associated with additional structural anomalies and underlying chromosomal defects. For example, clefts of the palate only (III) are often seen in association with specific syndromes, such as Treacher-Collins, Stickler, and the velo-cardio-facial syndrome (22q11.2 deletion). Consequently, they require specific prenatal management, including genetic counseling and invasive genetic testing (preferably by array-comparative genomic hybridization, array CGH).[9] By contrast, clefts of the lip/alveolus only (I) are less frequently associated with additional anomalies, and thus have the most favorable prognosis. These cases should be counseled by specialized multidisciplinary cleft palate teams, and prenatal genetic counseling and testing is recommended only when associated anomalies are found.[9]

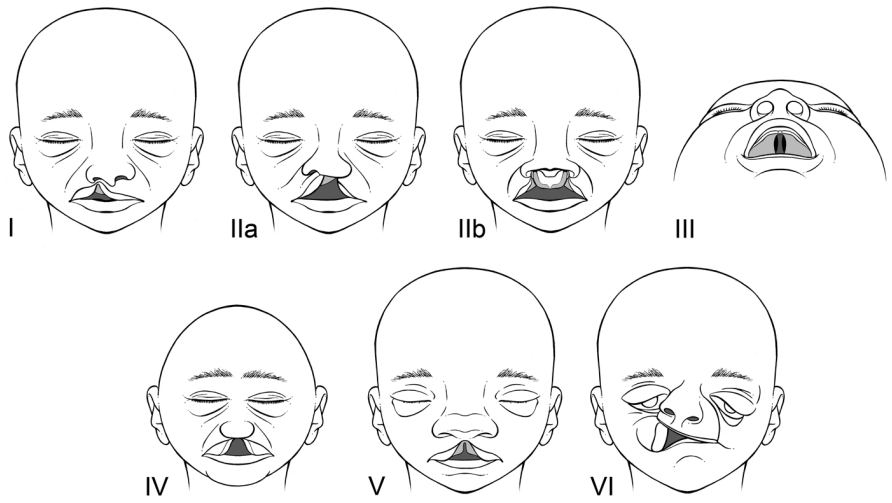

Figure 2. Ultrasound classification of oral (I-III) and craniofacial clefts (IV-VI) in fetuses of 20 weeks gestation. The most frequent sub-phenotypes are underlined within the categories:

I. Unilateral or bilateral complete or incomplete cleft lip/alveolus;
IIa. Unilateral complete or incomplete cleft lip/alveolus and palate (including bifid uvula, not shown);
IIb. Bilateral complete or incomplete cleft lip/alveolus and palate (including bifid uvula; and if complete, with protrusion of the prolabium and premaxillae);
III. Complete or incomplete cleft palate including bifid uvula;
IV. Complete median cleft lip and palate with hypotelorism (that is, agenesis of the premaxillae, prolabium, and nasal septum combined with holoprosencephaly; these cases are always microcephalic);
V. Incomplete median cleft lip (alveolus) with or without hypertelorism (combined with a flat bifid nose, and the alveolar cleft is located at the absent intermaxillary suture);
VI. Atypical facial clefts (located at extra sutures of facial bones, such as the maxilla; in these cases, the nose is normal and not affected by the cleft).

Figure 3 presents the various sub-phenotypes within the three oral cleft categories and Figure 4 shows the categories on 2D ultrasound scan. The most common type within the first category (I) is the *unilateral incomplete cleft lip/alveolus* (Figures 3:I and 4:I),[7] which results from defective outgrowth of the lip/alveolus or from disturbed differentiation of its mesenchyme into bone and musculature (9-14 weeks gestation, ≥17 mm CRL).[1, 4] As the fusion process of the primary palate is completed at this stage, the incomplete cleft lip/alveolus always shows a tissue bridge at the base of the nostril (Figure 2:I and 4:I; Vermeij-Keers et al. submitted).[1, 4] In the second category (II), the *unilateral or bilateral complete cleft lip/alveolus and palate* (Figures 3:II and 4:IIa and IIb) is the most frequently observed sub-phenotype.[7] This type is caused by defective fusion of the facial swellings (6.5-9 weeks gestation, ≤17 mm CRL) and subsequent defective fusion of the palatal shelves (9-14 weeks gestation, ≥17 mm CRL).[1, 4, 11] In contrast to the incomplete cleft lip/alveolus (I), these complete sub-phenotypes do not show a tissue

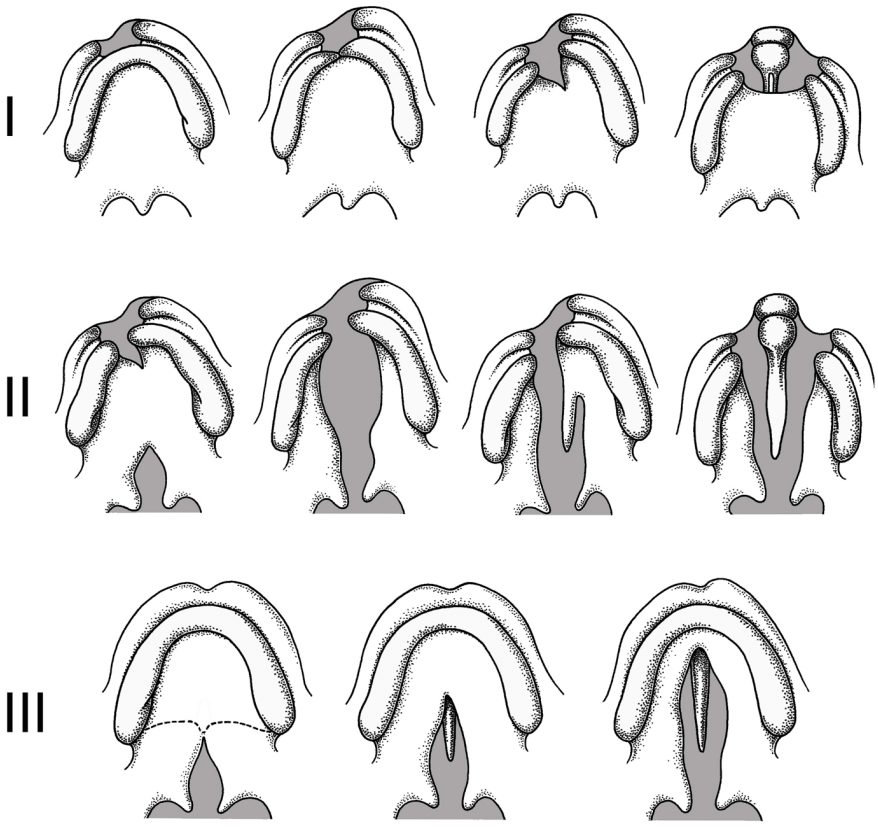

Figure 3. Various cleft sub-phenotypes within the three oral cleft categories: I. cleft lip/alveolus only, II. cleft lip/alveolus and palate, and III. cleft palate only, viewed from the oral side (based on Van der Meulen et al., 1990).[6] The most frequent sub-phenotypes are underlined. From left to right:

I. Unilateral complete or incomplete cleft lip; unilateral complete or incomplete cleft lip with incomplete cleft alveolus; unilateral complete cleft lip and alveolus; bilateral complete cleft lip and alveolus (with protrusion of the prolabium and premaxillae);

II. Unilateral complete cleft lip and alveolus with complete cleft soft palate (including a bifid uvula); unilateral complete cleft lip, alveolus, hard palate, and soft palate; unilateral complete cleft lip, alveolus, hard palate, and soft palate with contra-lateral incomplete cleft hard palate; bilateral complete cleft lip, alveolus, hard palate, and soft palate (with protrusion of the prolabium and premaxillae);

III. Complete cleft soft palate (including a bifid uvula; the dotted line indicates the border between the hard and soft palates); complete cleft soft palate with incomplete cleft hard palate; complete cleft soft and hard palates.

bridge at the base of the nostril and the alveolar deformity generally extends to the incisive foramen (Vermeij-Keers et al. submitted).[4, 11] Although the unilateral and bilateral forms of this cleft type result from similar embryonic processes, it is clinically relevant to further distinguish them (Figure 2:IIa and IIb), because the bilateral forms are more frequently associated with other anomalies than unilateral forms.[9] In the third main category (III), the *complete cleft soft palate* and *complete cleft hard and soft* (including the uvula; Figure 3:III) are most commonly observed.[7] Both sub-phenotypes result from disrupted fusion of the palatal shelves. As these shelves fuse in a fronto-occipital direction, starting at the incisive foramen and ending at the uvula, the time of disruption determines whether there will be a complete or incomplete cleft of the hard and/or soft palate including the uvula (Vermeij-Keers et al. submitted).[4] In other words, if the fusion process is disrupted at a later stage, more of the hard/soft palate will be intact. Consequently, a complete cleft hard and soft palate precedes a complete/incomplete cleft of the soft palate only. Furthermore, palatal clefts always show a bifid uvula (Figure 3:III).[4, 6] Using this latter structure, Wilhelm and Borgers[37] recently developed a new technique to improve the prenatal detection of isolated cleft palates. They found that an intact uvula can be visualized as the 'equals sign' and that absence of this typical presentation indicates a cleft palate.

Craniofacial clefts

With respect to prenatal outcome and counseling, it is crucial to distinguish craniofacial clefts from oral clefts, because they are associated with other (more severe) congenital anomalies and almost always have underlying chromosomal abnormalities, and thus have a different prognosis.[19, 30] Consequently, these cases require invasive prenatal testing and specific counseling, focusing on parent's options, termination of pregnancy being one of them. Based on their different underlying embryonic mechanisms and associations with other anomalies, craniofacial clefts should be further divided into three categories: IV) *complete median cleft lip and palate with hypotelorism*, V) *incomplete median cleft lip (alveolus) with or without hypertelorism*, and VI) *atypical facial cleft* (Figure 2:IV-VI). The first category (IV) is the most commonly described craniofacial cleft and is also known as 'midline cleft lip and palate', which is part of the holoprosencephaly series.[33, 58, 65] This craniofacial anomaly is prenatally characterized by microcephaly as well as agenesis of both premaxillae, the prolabium, and the nasal septum combined with cleft palate and hypotelorism (Figures 2:IV and 4:IV) and originates from early embryological stages (4-5.5 weeks gestation, 1-3 mm CRL). Holoprosencephaly in category IV concerns the semilobar or incomplete form and is caused by insufficient outgrowth of the neural walls and consequently of the telencephalic hemispheres,[66] leading to agenesis of the olfactory bulbs, agenesis or hypoplasia of the corpus callosum, and undivided thalami in later developmental stages.[58] Additionally, both medial nasal processes do not develop and there is no interplacodal area. In the embryonic face, both nasal placodes fuse to one single placode.[10, 11, 58] It is important to realize that these cases always have associated anomalies (chromosomal

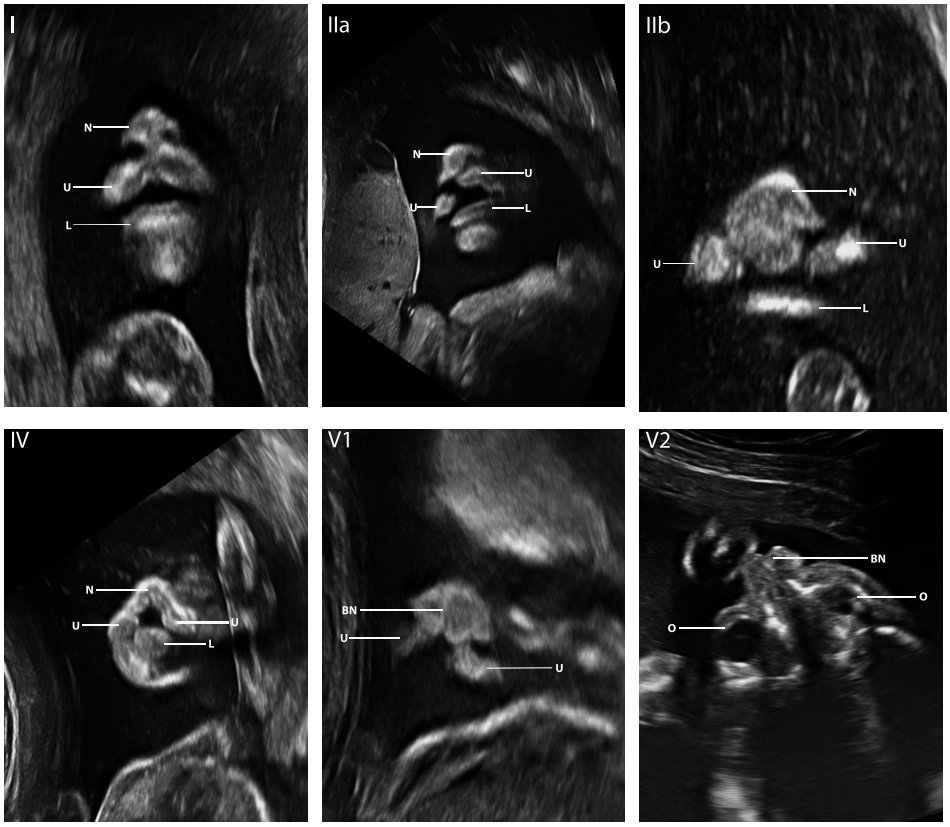

Figure 4. Two-dimensional (2D) ultrasound images of fetuses showing the different categories of oral (I-III) and craniofacial (IV-VI) clefts according to the new ultrasound classification. U = upper lip; L = lower lip; N = nose; BN = bifid nose; O = orbit.

I. Right-sided unilateral incomplete cleft lip and alveolus with deviation of the nasal septum to the contra-lateral side (coronal view) at 20 weeks, 3 days of gestation;

IIa. Right-sided unilateral complete cleft lip, alveolus, and palate with deviation of the nasal septum to the contra-lateral side (coronal view) at 23 weeks, 6 days of gestation;

IIb. Bilateral complete cleft lip, alveolus, and palate with protrusion of the prolabium and premaxillae (coronal view) at 19 weeks, 6 days of gestation;

III. Because detection of isolated cleft palate by 2D ultrasound is still challenging and generally done by 3D ultrasound, 2D images were not available for this category;

IV. Complete median cleft lip and palate with absent prolabium, premaxillae, and nasal septum as well as hypotelorism (coronal view) at 22 weeks, 1 day of gestation;

V.1. Incomplete median cleft lip and alveolus (coronal view) combined with 2. a flat bifid nose without hypertelorism (coronal/axial view) at 24 weeks, 3 days of gestation;

VI. Atypical facial clefts are very rare, and consequently an ultrasound image of this category could not be provided.

defects in 61%-82%), resulting in 100% mortality.[19, 30] Therefore, future parents require specific prenatal counseling and care, including termination of the pregnancy.

The *incomplete median cleft lip with or without hypertelorism* (Figures 2:V and 4:V) is a rare malformation and also known as 'median cleft face syndrome', 'frontonasal dysplasia or malformation', 'bifid nose with median cleft lip', or cleft no. 0.[5, 6, 34, 42, 53] Note that this incomplete cleft condition is a normal feature in rhodents.[67] In case of hypertelorism, the ultrasound shows a bony distance between the orbits that is too wide,[34] which is caused by insufficient frontocaudal outgrowth of the nasal septum during embryogenesis (9-14 weeks gestation, ≥17 mm CRL). The bifid nose and median cleft lip can be considered as remnants of the internasal groove that has not fully disappeared, and the cleft between both premaxillae, if present, as absence of the intermaxillary suture.[11] While these cases are mentally less affected compared to those of category IV, mental retardation may be present, especially in cases with agenesis of the corpus callosum.[34, 42] Besides the frequent association with anomalies of the central nervous system, various other accompanying defects and related syndromes have been described.[34, 42] However, due to its rarity, exact numbers on associated findings are not available. Nevertheless, awareness of these associated anomalies is vital for optimal prenatal care, which should include genetic counseling as well as consultation of a specialized multidisciplinary craniofacial team.

The final category (VI) comprises the various *atypical facial clefts* (Figure 2:VI), which also are very rare malformations.[5, 6, 35, 53, 68] Most of these atypical clefts are caused during late embryogenesis (9-14 weeks gestation) due to the development of additional bone centers and consequently extra sutures.[11] These additional centers can develop within the maxilla and have also been found within other bones of the facial skeleton, such as the zygomatic bone (bipartite os zygomaticum).[6, 10, 11, 68] While most of the skulls with extra bone centers and sutures develop normally, defective differentiation at these extra sutures can rarely result in atypical facial clefts. It should be realized that in cases with atypical clefts, the nose is not affected by the cleft, which makes them easy to distinguish from oral clefts. Similar to category V, these cases should be counseled prenatally by specialized multidisciplinary craniofacial teams.

Compared to Nyberg's classification

In several aspects, this new classification is different from Nyberg's ultrasound classification of facial clefts (Table 1).[30] First, oral clefts are distinguished from craniofacial clefts, while Nyberg et al. considered them to be all facial clefts. Second, the alveolus is not described in their classification. From an embryological point of view, however, it is essential to analyze and describe the alveolus separately,[31] as its deformity is not related to that of the lip and has unique underlying embryonic processes (Vermeij-Keers et al., submitted). Another important point missing is that, although displayed, the differences between complete and incomplete clefts of the lip/alveolus are not explained by Nyberg et al. Prenatally, it is important to distinguish these two groups, given that—in contrast to complete cleft lip—the palate is significantly less involved in incomplete clefts of the lip,[7] and the latter thus has a more favorable prognosis, especially when

Table 1. New ultrasound classification of oral and craniofacial clefts versus Nyberg's ultrasound classification of facial clefts

New Prenatal Ultrasound Classification*		**Nyberg Classification[30]**	
Oral clefts		*Facial clefts*	
Category I	Unilateral or bilateral complete or incomplete cleft lip/alveolus	Type 1	Cleft lip without cleft palate
Category IIa	Unilateral complete or incomplete cleft lip/alveolus and palate	Type 2	Unilateral cleft lip and palate
Category IIb	Bilateral complete or incomplete cleft lip/alveolus and palate	Type 3 3a 3b	Bilateral cleft lip and palate with premaxillary protrusion with hypoplastic midface
Category III	Cleft palate only	—	
Craniofacial clefts			
Category IV	Complete median cleft lip and palate with hypotelorism	Type 4	Midline cleft lip and palate
Category V	Incomplete median cleft lip (alveolus) with or without hypertelorism	—	
Category VI	Atypical facial clefts	Type 5	Facial cleft associated with amniotic bands or limb-body-wall complex

* The most commonly observed morphologic features are underlined

it comes to associated defects.[9] Another difference is that Nyberg et al. did not include clefts of the palate only, as this category was hardly prenatally detected at that time. However, because of recent and future advances in ultrasound techniques and experience,[37, 41] an update is needed and therefore, this category is included in our classification. With regard to craniofacial clefts, Nyberg et al. did not describe or include the incomplete median cleft lip (alveolus) with or without hypertelorism (category V). Given its different patho-embryogenesis and associated anomalies,[5, 6, 10, 11] this craniofacial anomaly should be distinguished from complete median cleft lip and palate with hypotelorism (category IV). Finally, Nyberg et al. presented atypical facial clefts as anomalies associated with amniotic bands or the limb-body-wall complex. However, in line with previous studies,[5, 6, 11, 35, 68, 69] these anomalies should be explained differently and not by amniotic bands. Therefore, these bands were not included in our classification.

CONCLUSIONS

With regard to prenatal care and future research, it is vital to differentiate between oral and craniofacial clefts and between their embryologically different sub-categories, given their varying etiopathological features and accompanying defects, resulting in a different prognosis and clinical outcome. Clefts of the lip/alveolus without palatal involvement—mainly being unilateral incomplete clefts—have the most favorable prognosis because of their relatively low rates of associated structural and chromosomal defects. In contrast, complete clefts of the

lip/alveolus have significantly higher risks of palatal involvement, and thus of accompanying defects. The bilateral complete clefts are more frequently associated with other anomalies than unilateral clefts. Therefore prenatal ultrasound screening should be more focused on the morphological severity of the lip/alveolus. When the palate is involved, specific prenatal management, including genetic counseling and invasive genetic testing, is recommended. In case of craniofacial clefts, prenatal management should always include invasive genetic testing given the frequent association of (more severe) congenital and chromosomal anomalies, and parents' options, including termination of pregnancy, should be discussed. Additionally, referral of future parents to a specialized multidisciplinary cleft palate team or craniofacial team for counseling is advised.

Finally, it is crucial to obtain further clinical experience with new ultrasound techniques, such as the 'equal sign' marker, and other more sophisticated 3D techniques or fetal MRI.

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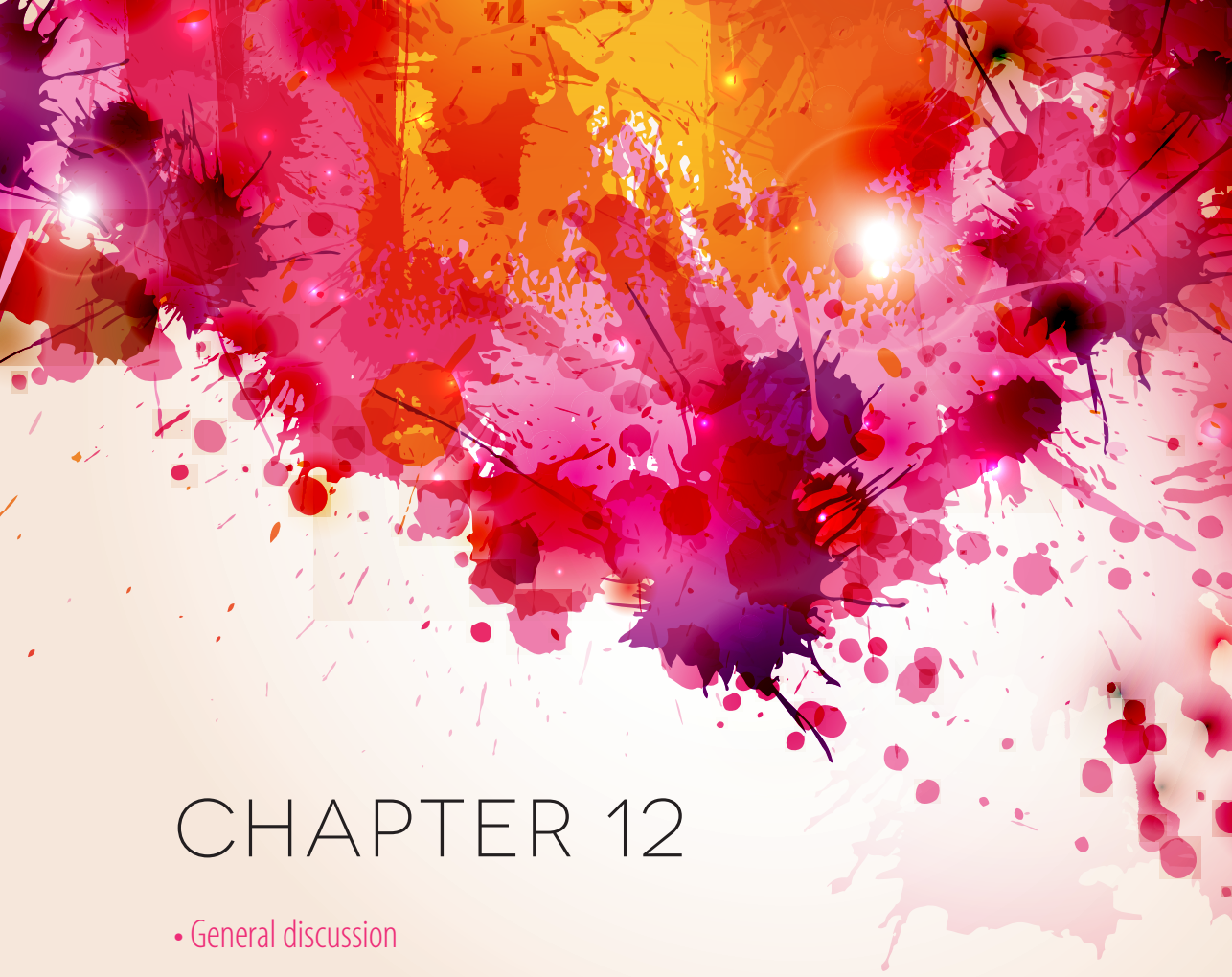

CHAPTER 12

- General discussion
- Summary | Nederlandse samenvatting

General discussion

Oral clefts impose a large burden on the health, quality of life, and socio-economic well-being of affected individuals and their families. They also represent a significant public health burden in terms of immediate and long-term medical costs.[1] Although access to care has increased around the world in recent years, the quality of care still varies substantially.[2] Prevention is the ultimate objective for these anomalies, and understanding of their causes is a condition *sine qua non* for this aim. However, despite extensive research representing a wide variation of designs, methods, and data, the causal factors and mechanisms underlying oral clefts remain largely unrevealed. This thesis is aimed at defining a prenatal and postnatal approach to further our understanding of these factors and mechanisms and to optimize the overall management of oral clefts.

MAIN FINDINGS, LIMITATIONS, AND POSSIBLE EXPLANATIONS

Part I. Descriptive registration and validation

To enable clinical, epidemiological, and fundamental research, the Dutch cleft palate teams—united in the "Dutch Association for Cleft Palate and Craniofacial Anomalies" (NVSCA)—have reported their new patients to the NVSCA registry for over 15 years, with an average of 351 patients per year.[3] Among the objectives are surveillance for changes in frequencies and distributions of specific cleft phenotypes as well as of their influencing factors.[4, 5] Additionally, consistent description is vital to evaluate any treatment strategies, to compare with other registries and studies, and to improve interdisciplinary and inter-center communication. To meet these requirements in an non-time consuming way, the NVSCA uses a unique recording system that easily allows description of all individual anomalies that form the oral cleft.[3, 6]

To investigate the feasibility of this system and ensure that the data provided are valid, a national validation project was conducted. We assessed the quality of registered data from all Dutch teams through extensive medical data review over a 7-year period (1997-2003). The main strengths of this project are the national distribution of the sampling frame—including large urban teaching hospitals and regional ones—as well as the successful retrieval of medical records (96%). Additionally, the postnatal follow-up (median of 5 years) allowed us to include associated anomalies detected later in infancy. However, the use of medical data also has its limitations, as their quality varied by team. Consequently, these data can never be 100% equal to the presentation in the outpatient clinical setting. As described in **Chapter 2**, our project showed that, while the quality of general infant and parental information varied by item, data were accurate and complete for the three commonly studied cleft categories (CL, CLP, and CP), making the NVSCA data highly suitable for comparison with other registries and studies.

Analysis of the anatomical structures and morphological severity revealed that the data quality of the various sub-phenotypes within these categories was generally satisfactory, supporting the clinical feasibility of this descriptive system (**Chapter 3**). However, externally visible (lip/alveolus) and severe (complete) clefts were generally more adequately diagnosed and recorded than less recognizable (hard and soft palate including the uvula) and mild (incomplete or subcutaneous/submucous) defects. These results might be explained by incomplete clinical examination. As reported earlier, this has mainly been a problem in newborn routine examination,[7-9] but it might also happen among the more experienced examiners.[8] Another possible explanation for the underreporting of mild features is that greater severity might encourage physicians to report better.

With regard to associated congenital anomalies, the proportion of individuals affected with these anomalies varies greatly between studies and appears to be related to the time of registration and how data have been collected.[10] Not all anomalies are detectable at birth or in the neonatal period, and early registration might cause underreporting of these anomalies, in particular of those that require specific diagnostic procedures (e.g., chromosomal defects or developmental disorders).[10, 11] In **Chapter 4** we evaluated both major and minor (minimal) congenital anomalies, resulting in a relatively high proportion of cases with associated anomalies (61%) compared to other studies (3% to 63%).[10, 12-15] Craniofacial anomalies were most frequently diagnosed, followed by defects of the central nervous system, skin, upper limbs, and lower limbs. Subsequent validation showed that—in contrast to the oral cleft features—the data quality on associated anomalies is moderate to poor according to Landis and Koch's classification.[16] Given that cases could have more than one associated anomaly, we found an underreporting of approximately 80% of the defects in cases with additional craniofacial anomalies or with additional anomalies of other organ systems, and 54% of the final diagnoses. Two-phased medical data review revealed that underreporting was caused rather equally by delayed diagnoses and deficient recording. Our rates are consistent with those of other studies evaluating underreporting of congenital anomalies during the neonatal period (37% to 86%),[11, 17-20] while registration after longer follow-up periods showed considerably lower rates (7% to 21%).[21-24] In line with this contrast, we assume that a part of the delayed diagnoses are explained by the early registration in the NVSCA. However, our results also show that obvious external defects (such as craniofacial and limb anomalies) were missed during intake. This might partly have been caused by the fact that patients are initially seen and recorded by plastic surgeons, orthodontists, or pediatricians, who are usually not fully trained in dysmorphology and syndromology.

Part II. Prevalence in the Netherlands

Since 2003, the number of new oral cleft patients reported to the NVSCA has fallen.[4, 5] This decline is unlikely to be explained by underreporting or misclassification, as these factors were minimized by standard and extra case-ascertainment activities. Therefore, other influencing

factors were searched, especially in the field of primary prevention, such as periconceptional folic acid supplementation, and the "so called" secondary prevention by prenatal screening.[25] As a result we hypothesized that the prevalence might have been affected by the increased correct periconceptional use of folic acid supplements among expecting Dutch mothers after a government-sponsored mass media campaign in 1995 and the proactive intervention by Dutch pharmacies since 2004.[26-28] In the Netherlands, folic acid supplement use is recommended from 4 weeks before conception until 8 weeks after it.[26, 27, 29] Because this recommended period covers only the developmental period of most CL±P sub-phenotypes and not of CP,[6, 30-32] more adequate use during this periconceptional period might have mainly affected CL±P. This theory is supported by findings that, after discontinuation of supplementation, the folate concentration in serum immediately decreases and the plasma total homocysteine level immediately increases.[33] Given the possible dose-response relationship between folic acid and clefts as well as the possible indirect effects through homocysteine metabolism,[34-36] supplementation until 8 weeks postconception might be too short to prevent CP. Although the evidence on the role of folic acid in oral clefts is largely inconclusive, our hypothesis would be consistent with several studies reporting that folic acid or multivitamin use during the same periconceptional supplementation period is associated with a decreased CL±P risk only,[37-40] while countries with compulsory fortification (United States and Canada) have shown a decline in both CL±P and CP.[35]

Another influencing factor might be the greater prenatal detection of oral clefts and their associated anomalies in the Netherlands. While routine ultrasound screening during 18-22 weeks of gestation was nationally implemented in 2007, the performance of 2D ultrasound scans during this gestational period started to increase as early as the 1990s.[41] As it did elsewhere,[42-46] the rise in prenatally detected anomalies may have led more affected pregnancies to be terminated. This is supported by national data on TOP provided by the annual reports of the Dutch Termination of Pregnancy Act (WAZ). They have shown that the number of second-trimester terminations, especially those performed in the hospitals, have increased since 2003, implying a rise in TOP affected with congenital anomalies.[47, 48] If pregnancies have been terminated because of the presence of an oral cleft with or without associated anomalies, again the CL±P prevalence would have been affected most. Unlike CP, this category can be easily detected by routine 2D ultrasound.[45, 49, 50]

In **Chapter 5** we used NVSCA data to establish the rates of oral clefts among live births in the Netherlands from 1997 to 2006, resulting in an average oral cleft prevalence of 16.8 per 10,000 live births. Time-trend analyses showed that the live-birth prevalence decreased significantly during this period. Additionally, stratification revealed a similar trend for CL±P, while no significant trend for CP was found, supporting that the higher periconceptional folic acid use and/or the greater prenatal detection of clefts and their associated anomalies might have caused the decline in prevalence. As stillbirths and neonatal deaths were not included in our analysis, a change in perinatal or neonatal mortality could theoretically also have affected our rates.

However, we assume this is of minor importance, given that the Dutch perinatal and neonatal mortality decreased during this specific period.[51] Moreover, this would have mainly affected CP, as this category is more frequently associated with severe further defects than CL±P.[2, 10] Other environmental or lifestyle factors changing over time may also account for the decrease in cleft prevalence. While specific data on these factors are not available for oral clefts in the Netherlands, data based on the general Dutch population have shown a decrease in maternal smoking and alcohol consumption during the study period.[52] Given their suggested association with cleft risk,[2, 53] these factors may have played contributory roles in the detected trends of oral clefts.

Unfortunately, comparison of our findings with those of other studies is restricted, particularly due to the great differences between data sources, sample sizes, inclusion and exclusion criteria, times of diagnosis, classifications, and population characteristics.[2, 10, 46, 54, 55] Regional Eurocat data have often been used to describe clefts from the Netherlands in a European or international context,[55-58] and they have occasionally been extrapolated to the whole of the Netherlands.[59] However, as its cleft prevalence seems to differ from national (LVR/LNR)[60] and other European registries,[56, 58] the Northern Netherlands might not contain a representative sample of the Dutch oral cleft population. Therefore, NVSCA data over 1997-2007 were compared with national data from the LVR/LNR and regional data from Eurocat, thereby verifying the detected decreasing trends and investigating whether the prevalence varies within the Netherlands (**Chapter 6**). We found that the overall live-birth prevalence of oral clefts is significantly higher in the Northern Netherlands (15.1 to 21.4 per 10,000) than in the rest of the Netherlands (13.2 to 16.1 per 10,000). Additionally, time-trend analyses confirmed the significant decreasing trend in CL±P for the rest of the Netherlands, but not for the Northern Netherlands. By comparing the rates between registries, we found that the NVSCA and Eurocat have rather similar rates for the Northern Netherlands, while the LVR/LNR has significantly lower rates for both regions, most possibly due to its incomplete coverage.[61] Unfortunately, none of the registries could give complete and reliable national data on associated anomalies, stillbirths, and pregnancy terminations to provide more insight into the causes of regional differences and trends.

Our results of relatively high rates for the Northern Netherlands are in line with previous findings[46, 56, 58] and thus seem to have already existed for a long time and to be fairly constant. Regional differences in epidemiological patterns may be due to variations in genetic and environmental risk factors, and in gene-environment interactions as well.[2, 32, 62] For example, our Northern population consisted of more Caucasian infants than the rest of the Netherlands. As populations of Dutch origin have higher cleft risks than other ethnic groups,[62] our findings may be partly explained by ethnic differences. Additionally, there may be a greater genetic predisposition in the Northern Netherlands due to a lower migration compared to the rest of the Netherlands.[63] This is supported by the higher cleft prevalence in Northern European countries that have relatively homogeneous populations and high quality registrations.[46, 55, 57, 58, 64] The regional differences in trends might be explained by differences in the impact of

prenatal ultrasound screening. While the rise in prenatally detected anomalies may have led more affected pregnancies to be terminated in the rest of the Netherlands, several data suggest that this did not affect the region Northern Netherlands. Eurocat data have shown rather low and stable rates of TOP among clefts in the Northern Netherlands,[44, 46] and all terminated pregnancies affected with clefts were associated with additional anomalies, including chromosomal defects (personal communication M.K. Bakker). Furthermore, in contrast to the rest of the Netherlands, second-trimester terminations have hardly been performed in the Northern hospitals and abortion clinics.[47, 48] It is unknown whether the regional differences in trends can be explained by differences in periconceptional folic acid use, as complete national data on this subject are not available. However, it should be realized that our effect-measures for the Northern Netherlands also indicated decreasing trends and that the absence of a significant effect in the Northern Netherlands could solely be due to sample size.

Part III. Postnatal classification

After complete and detailed description of the various sub-phenotypes of oral clefts, subsequent adequate subdivision according to their related time periods and underlying processes in development is needed to allow linkage to specific cell-biological mechanisms, genes, and environmental factors that are expressed during these periods. Although many systems have been developed to classify clefts,[65-73] none of them are fully based on human embryology of the nose and oral cavity, and infrequent or subclinical features are often not included. Therefore, a new postnatal classification of oral clefts was proposed, dividing broad groups of oral clefts into defects resulting from defective fusion, differentiation, or both.[6] This approach reflects the different underlying patho-embryological processes and timing in development of the primary and secondary palates, and its rationale is in line with the theoretical basis of the NVSCA registry.[3, 6, 30-32, 74-76] After discussing its embryological basis, we tested this new classification on all sub-phenotypes among Dutch newborns registered in the NVSCA (**Chapter 7**). The descriptive data allowed us to classify all different sub-phenotypes—including subclinical features—within the three cleft categories (CL, CLP, and CP) into fusion and/or differentiation defects. In addition, we were able to construct a timetable relating the various observed defects to weeks in embryonic development. For example, a complete cleft of the lip/alveolus arises significantly earlier (by disrupted fusion of the primary palate in the early embryonic period) than an incomplete cleft of the lip/alveolus (by disrupted differentiation of the primary palate during the late embryonic period).[6, 30-32, 75, 76] However, our timetable has some limitations, because over 90% of the observed defects, but not all clefts, could be fitted in. More specifically, some fusion defects of the secondary palate were difficult to place in time. Theoretically, a complete cleft palate can develop relatively early during the late embryonic period (7-9 weeks of development) because of insufficient outgrowth and elevation of the palatal shelves. However, lack of adhesion, apoptosis, or epithelio-mesenchymal transformation (EMT) during the second part of the late embryonic period (9-11 weeks of development) may cause a similar defect.[6,

[30-32, 75, 76] Only when a minimal part or more of the hard palate is fused, insufficient outgrowth and elevation of the palatal shelves can be excluded as underlying mechanisms, and the cleft palate can then be related to one specific timeframe.

While there is general consensus on the embryogenesis of the secondary palate,[2] the developmental processes of the primary palate are complex and have been rather underexposed. Therefore, we tested our classification on adult unoperated patients from Indonesia with clefts of the primary palate only (**Chapter 8**). We were able to classify all their sub-phenotypes—not being influenced by defective development of the secondary palate—into fusion and/or differentiation defects. Additionally, we showed that further morphological grading of incomplete clefts of the lip, as has been proposed by several studies, is not related to the severity of associated alveolar deformities. Analysis of the permanent dentition of these patients revealed that all observed alveolar deformities were located between the central incisor and—often malformed or absent—lateral incisor. As both incisors develop within the premaxilla,[77, 78] our findings imply that the developmental arrest involves the premaxilla.

Part IV. Effects of periconceptional folic acid supplementation

Although multiple (non-randomized) observational studies have suggested a beneficial role of folic acid in supplements in decreasing cleft risk,[35, 79, 80] the evidence remains inconclusive, as many studies—including randomized and cohort controlled trials—identified no significant effects on clefts.[35, 79-83] Results are often mixed in terms of estimated effects, whether they affect certain or all cleft categories, and whether they are attributable to folic acid or other multivitamin components. One of the factors hampering our insights might be that supplementation is often not subdivided by type (folic acid alone or combined with multivitamins) and does not completely cover the embryonic periods of clefts.[35] Also, most studies evaluate heterogeneous cleft groups to reach adequate power, but given their etiologic and genetic heterogeneity this crude approach may just weaken the power to detect effects.[53, 84] Therefore, we analyzed the type, timing, and duration of periconceptional folic acid supplementation in relation to the timing and embryological mechanisms underlying cleft development (**Chapter 9**). This was done by applying our new postnatal classification to combined complementary NVSCA and Eurocat data in a population-based case-control study. By assessing effects on oral clefts relative to other non-folate related congenital anomalies, we unexpectedly found the first evidence that periconceptional folic acid supplementation might be associated with elevated risks for certain types of oral clefts. Defects of the lip/alveolus—mainly resulting from defective differentiation in development—appeared to account for the largest proportion of risk increase, being associated with more than three-fold higher risks. Further analysis systematically revealed two- to three-fold increased risks for differentiation defects developing during the late embryological period, with no associations for fusion defects. Stratum analysis showed similar figures for supplements consisting of folic acid alone, and effects were therefore attributable to folic acid and not to other multivitamin components.

Although our use of malformed controls is widely accepted and beneficial with regard to internal validity,[85-88] some limitations inherent to the observational nature of this study, such as recall/selection bias and confounding, are not completely avoidable. However, our findings are strengthened by their specificity, consistency, systematic pattern, and duration of exposure-response relationship. The deviation of our results from other studies might be explained by our unique design, including the subdivision according to specific supplement and cleft features—an approach not used in earlier studies.[35, 79] Because mechanisms by which folic acid might prevent certain anomalies remains unexplained,[79] a meaningful explanation for our unexpected risk pattern cannot be provided. However, we do know that other aspects surrounding folate metabolism (such as MTHFR gene polymorphisms, folate receptors, and plasma/erythrocyte folate), have also been shown to deviate for clefts.[89-95] Additionally, comparable associations between folate intake and the occurrence of multiple congenital anomalies have been found.[96, 97] Moreover, adverse effects of high folate intake have been established in animal studies.[98-100] In humans, folate fortification and additional supplementation have increased folate intake and blood cell concentrations significantly,[101, 102] but the consequences of long-term high intake are not known yet. Recently, it has been hypothesized that folic acid might lead to changes in epigenetic patterns, thereby altering gene expression.[103-105] This might be an explanation for different health outcomes among those with similar genetic backgrounds.

Part V. Prenatal diagnosis and classification

Oral clefts are being diagnosed prenatally more frequently. Detection rates—predominantly on CL±P—increased from approximately 5% in the early 1980s to over 26% in the late 1990s,[106] and they are as high as 65% today.[107] Consequently, the need for accurate information on the risk of associated anomalies and underlying chromosomal defects is rising to aid in decisions on invasive diagnostics and informing future parents on outcome and prognosis. In **Chapter 10** the frequencies of associated congenital anomalies and chromosomal defects among prenatally and postnatally detected oral clefts were assessed from literature and NVSCA data, providing an inventory of the various detected syndromes and chromosomal defects as well as an algorithm for prenatal diagnostics. We demonstrated that the prevalence of these defects is evidently related to cleft category. Although strongly varying in study characteristics and designs, both prenatal and postnatal studies showed a higher frequency of associated defects in CLP (21% to 66%) and CP (22% to 78%) than in CL (0% to 41%). Furthermore, these frequencies were higher in bilateral (23% to 79%) than in unilateral (16% to 52%) CLP or CL±P. For all categories, chromosomal defects were almost always seen in association with additional congenital anomalies. In the absence of associated anomalies, chromosomal defects were found prenatally in CLP (3.9%), and postnatally in CL (1.8%, 22q11.2 deletions only), CLP (1.0%), and CP (1.6%). However, these results are limited by the great variation in designs and methodologies of the evaluated studies. In line with previous findings,[10] the most important issues were the non-uniform subdivision of oral clefts and the different definitions and classifications

of associated anomalies among studies. Furthermore, chromosomal analysis was mostly performed in associated clefts only, which explains why almost all reported chromosomal defects were accompanied by additional anomalies. As a consequence, the risk of chromosomal anomalies in isolated clefts may be underestimated.

Because the various cleft categories are differently associated with additional anomalies and thus have a different prognosis and outcome, accurate prenatal ultrasound screening and subsequent subdivision of oral clefts will improve counseling and management of the pregnancy significantly. Moreover, it is vital to prenatally distinguish oral clefts from midline and atypical facial clefts. While the latter are often considered to be oral clefts,[65, 67, 70, 108-113] they should be classified as craniofacial clefts given their different patho-embryogenesis and accompanying defects.[30, 31, 114, 115] To classify clefts prenatally, the system of Nyberg is most generally used.[116] While this system has brought structure in the prenatal diagnosis of clefts, it does not incorporate the latest embryological and genetic insights,[30, 31, 53, 74, 84, 114, 115, 117] and it has not been designed for modern ultrasound technologies.[118-129] Therefore, we have developed a new prenatal ultrasound classification of oral and craniofacial clefts considering their underlying embryological processes and associated congenital anomalies as well as advances in ultrasound technology (**Chapter 11**). In contrast to Nyberg et al,[116] we distinguish oral clefts (categories I-III) from craniofacial clefts (categories IV-VI), instead of considering them to be all facial clefts. Another difference is that we have described the alveolus separately because of its unique underlying embryological processes,[6, 31, 32, 75, 76] and we have included clefts of the palate only as these are prenatally detectable nowadays.[123, 125, 126, 128] Besides grouping oral clefts into the three main categories (categories I-III), it is essential to prenatally differentiate between incomplete and complete clefts of the lip/alveolus, because the palate is less frequently involved in incomplete clefts[3], resulting in a better prognosis, especially when it comes to associated defects (chapter 10). Although unilateral and bilateral forms of CLP result from similar embryological processes, it is clinically relevant to distinguish them prenatally, because unilateral forms (category IIa) are less frequently associated with additional anomalies than bilateral (category IIb) forms (chapter 10). With regard to craniofacial clefts, Nyberg et al.[116] described just one type of midline cleft. However, based on difference in embryological processes[30, 31, 75, 76, 114, 115, 130] and associated anomalies,[112, 119, 120, 122, 131, 132] we included two forms in our classification: complete median cleft lip and palate (with absent premaxillae, prolabium, and nasal septum) and hypotelorism (category IV), and incomplete median cleft lip (alveolus) with or without hypertelorism (category V). Furthermore, atypical facial clefts (category VI) are not explained by amniotic bands—as believed by Nyberg et al.[116]— but by different embryological processes.[31, 114, 115, 121, 133, 134]

IMPLICATIONS AND RECOMMENDATIONS

Postnatal management

Clinical outcome and prognosis of patients with oral clefts depend largely on the timely and accurate diagnosis of their cleft phenotype as well as their associated congenital anomalies, including underlying syndromes and chromosomal defects.[2, 10] We found that less visible and mild cleft features are less adequately reported to the NVSCA, possibly due to incomplete examination, and that a considerable part of the associated anomalies, including externally visible anomalies, are missed during the first consultations with the cleft palate teams (Chapters 3 and 4). These findings emphasize the need for early and thorough evaluation of patients with oral clefts. Adequate diagnosis of involvement of the palate is important, not only with regard to associated feeding difficulties, but also because of their relatively high association with additional congenital anomalies.[2, 10] In addition, cleft team members should be trained and focused on the postnatal detection of co-occurring anomalies, especially such as Pierre Robin sequence and cardiovascular/urogenital anomalies. These anomalies are frequently missed during intake (chapter 4) and will change treatment policy as well as the prognosis and clinical outcome of patients. Furthermore, minor defects should also be correctly identified, because they may be recognizable components of specific syndromes or chromosomal defects that significantly affect cleft management.[10] Early genetic counseling seems warranted in most cases to maximize the ascertainment of associated anomalies. Besides the postnatal detection, cleft team members should be more aware of prenatal findings, as a considerable amount of associated anomalies are detected prenatally nowadays.[41, 50, 135]

Postnatal registration

Valid description of cleft sub-phenotypes is vital to investigate their causal factors, evaluate any treatment and preventive strategies, compare with other registries and studies, and improve interdisciplinary and inter-center communication.[53, 84] Despite some challenges described (Chapters 2-4), the NVSCA provides such consistent descriptive data. We demonstrated that the unique NVSCA recording system is clinically feasible and generates overall valid data. However, information on morphologically severe clefts can be interpreted with higher confidence than those on morphologically mild clefts. As these mild (subclinical) features may represent specific genetic or environmental characteristics,[53, 84, 136-138] one should be aware that these characteristics might be underestimated in registry-based studies. The underrepresentation of associated anomalies restricts the use of NVSCA data for research on these anomalies and underlines the need for postnatal follow-up and reregistration at a later age. However, these data can still be valuable, for example in providing low-end estimates of rates, as long as one remains cognizant of the limitations.[19] After these findings, several strategies have been undertaken by the NVSCA Registration working group to improve the completeness of NVSCA registry data. First, the registry form was converted to a digital form in 2008, thereby eliminating paperwork,

reducing transfer errors from paper to database, and allowing obligatory fields for key information.[5] Second, a reregister project has recently been started for 1997 and will be implemented for the subsequent years to complete anomalies and final diagnoses diagnosed after the first cleft palate team consultations.

In contrast to other systems, such as ICD-based registries,[44, 46, 139, 140] the NVSCA data can be fitted into any classification, old or new (Chapters 7 and 9), thereby providing a solid basis—complementary to other registries—for clinical, epidemiological, and fundamental research. Internationally, there is growing awareness that coding oral clefts in a too simplistic way could potentially lead to important information being lost,[53, 84] as well as weakening of the power to detect effects. In line with these studies, registries and future studies should be encouraged to accurately phenotype oral clefts according to standard protocols with data-sharing activities. As many registries and studies are ICD-based, we believe that adjustment of the ICD-10 cleft coding system (Q35-Q37)[141] is required with regard to the anatomical and morphological cleft features, including subcutaneous, incomplete, and complete cleft lip/alveolus and submucous cleft palate. In this way, more accurate international data may become available to facilitate the ongoing identification of causal factors as well as the improvement of overall cleft management.

Postnatal classification

To enable grouping of the detailed descriptive NVSCA data, we have provided a new postnatal classification that is complete and feasible for all sub-phenotypes of the primary and/or secondary palates, including subclinical features (Chapters 6 and 7). Furthermore, with our concept of fusion/differentiation defects, special sub-phenotypes—such as Simonart's bands—can also be explained. Two types of bands have been described: the skin-covered soft tissue bridge located at the base of the nostril with an ipsilateral complete cleft alveolus, and the mucous tissue bridge located between the segmented alveolar process with an ipsilateral complete cleft lip.[142, 143] Although a few mechanisms have been described,[142] the exact developmental processes of these bands have not been identified yet. Our hypothesized mechanisms based on the patho-embryology of the primary palate[6, 31, 144, 145] may contribute to the understanding of such complicated phenotypes. In addition, we demonstrated that whether an incomplete cleft alveolus is a fusion or differentiation defect depends on the morphology of the lip (complete vs. incomplete/subcutaneous cleft), and that the lip should therefore always be evaluated first. As further morphological grading of incomplete cleft lip does not predict the severity of the alveolar deformity, these grades have no therapeutic consequences and thus are neither clinically nor embryologically relevant. These results underline that the lip and alveolar process have independent morphological characteristics and should therefore be evaluated separately in order to have a complete and accurate diagnosis. Our timetable relating the type of clefting to timeframes in development (Chapter 6) can be used as a guideline in research, provided that

one is aware of the limitation that complete clefts of the hard palate have various underlying embryological mechanisms during different specific timeframes in the late embryonic period.

Primary and secondary prevention in the Netherlands

The NVSCA data have shown that the prevalence of oral clefts and its trends among live births varies within the Netherlands (Chapters 5 and 6). Consistent with its deviation from other European regions,[56, 58] the prevalence in the Northern Netherlands is significantly higher than that in the rest of the Netherlands, possibly due to differences in ethnic and genetic population characteristics. Therefore, this region does not contain a representative sample of the overall Dutch oral cleft population. Although regional data have utility for health services, clinicians, and researchers in that specific area and can be compared by global means and trends, our findings underline that extrapolation of regional data to a whole country or larger areas should be made with caution.[55] Further studies investigating etiology and evaluating preventive strategies should consider these geographical differences in cleft sub-phenotypes among live births, stillbirths, and spontaneous/induced abortions, between and within countries, as they may reveal clues to the causal factors of oral clefts.

The detected significant decreasing trend of CL±P among live births in the rest of the Netherlands (Chapters 5 and 6) may have several implications for healthcare and policy makers, partially lying in the possible effects of primary and secondary preventive factors. Firstly, the average 1.9% reduction per year in live-birth prevalence (2.2% for CL±P) we estimate will reduce the psychological burden on patients and their families, as well as the costs associated with the medical care of these patients. The second implication lies in the moral and ethical dilemmas raised by the possible increase in terminations of pregnancies affected with congenital anomalies, since a considerable part of these cases might be non-lethal. Therefore, if oral clefts are detected prenatally, future parents should be counseled by a multidisciplinary cleft palate team that focuses on psychosocial support, genetic counseling, education on cleft management, and parent's options, TOP being one of them.[42, 43, 146, 147] Recently, an evidence-based guideline was developed providing a uniform strategy for prenatal counseling and management of oral clefts in the Netherlands.[148] Future population-based studies also including stillbirths and terminated pregnancies can give more insight into the impact of prenatal screening, especially if prenatal diagnoses are known. However, complete and reliable national data on prenatally diagnosed anomalies and indications of TOP are still lacking. Therefore, uniform national registration of prenatal outcomes as well as implementation of indications for TOP in the WAZ[47, 48] are essential to further evaluate the impact of prenatal screening in the Netherlands. In order to increase our insights, we have conducted an anonymous retrospective (1997-2007) and prospective registration since 2008 to record the number and outcome of prenatally detected oral clefts counseled by the Dutch cleft palate teams. To enable the prospective registration, the NVSCA registry has been expanded with an anonymous digital prenatal form that allows matching to the postnatal form. Although this registration does not provide complete data on prenatally

diagnosed cleft cases, our preliminary data indicate that termination of the pregnancy occurs before and after counseling and in case of additional anomalies, but also when isolated clefts are diagnosed.

With regard to folic acid, it is unknown whether differences in periconceptional supplement use have accounted for the detected regional differences in trends and prevalence, as national data on this topic are lacking and its role in oral cleft risk remains unrevealed.[1, 35, 80] Therefore, future studies should focus on the type, timing, and duration of periconceptional folic acid supplementation[35, 79] in relation to the various cleft sub-phenotypes. This was done with population-based data from the Northern Netherlands in chapter 9. In contrast to our expectations, we found several lines of evidence indicating that periconceptional folic acid use in this region is associated with an increased, instead of decreased, risk of oral clefts, especially of those resulting from defective differentiation during 7-12 weeks of development. Although detected by an observational study, this association is strengthened by the specificity, consistency, systematic pattern, and duration of exposure-response relationship of our findings. Therefore, it is vital to restrict the use of folic acid supplements to the official recommended period for neural tube defects, which is 4 weeks before to 8 weeks after conception. Given the demonstrated duration of exposure-response relationship, minimizing pregnant women's exposure in this way may then reduce the cleft prevalence. In addition, the effect of folic acid on oral clefts is relevant to the ongoing discussions about food fortification, as the folic acid intake and folate blood cell concentrations have been increased significantly by food fortification.[28, 35, 101, 102] Our unexpected results underline the importance of evaluating public health strategies regarding folic acid supplementation, including its timing, duration, and dose, which should be done in the light of potential dietary improvements. Together with other preliminary findings on the potential adverse effects of increased folic acid intake,[98-100, 103-105] our results underscore the need for additional studies on the consequences of increased intake. Large population-based studies using other datasets, but the same approach and methodology, are needed to confirm or refute our findings.

Prenatal counseling and genetic testing

Prenatally, it is crucial to distinguish craniofacial clefts from oral clefts, because they have a different pathogenesis and are associated with other (more severe) congenital anomalies. Therefore, parents expecting a child with a craniofacial cleft require specific prenatal counseling and care, and they should be referred to specialized multidisciplinary craniofacial teams. In case of a complete median cleft lip and palate and hypotelorism—which have high risks of underlying chromosomal defects (61% to 82%) and 100% mortality due to their microcephaly[112, 116]—the option of terminating the pregnancy should be discussed (chapter 11).

With regard to oral clefts, prenatal counseling on the prognosis and risk of chromosomal defects should be tailored to cleft category, and more importantly to the presence or absence of associated anomalies (chapter 10). The demonstrated differences in associations with other

structural anomalies stress the need of accurate prenatal subdivision into three categories (CL, CLP, and CP), and further subdivision of unilateral and bilateral CLP. However, accurate detection of additional anomalies appears to be even more significant to outcome, as the presence of these anomalies is the most important predictor for underlying chromosomal defects. Irrespective of cleft category, clinicians should therefore advise invasive genetic testing to identify chromosomal defects if associated anomalies are seen prenatally. In absence of associated anomalies, CL has the most favorable prognosis when it comes to chromosomal anomalies with a poor outcome. Therefore, if confident in ultrasound findings, prenatal conventional karyotyping is not recommended in CL, but given the few reported 22q11.2 deletions,[149] array comparative genomic hybridization (array CGH) may be considered. In presumed isolated CLP and CP, however, prenatal genetic counseling and invasive testing (preferably by array-based methods) should be considered given their higher risks on underlying chromosomal defects. Especially CP can be associated with specific syndromes, such as the velo-cardio-facial syndrome (VCF, 22q11.2 deletion), Treacher-Collins, and Stickler (Chapter 10). However, when considering invasive testing, the baseline risk of complications (1%) should be weighed against the potential benefits.[150] Additionally, one should be aware of the detection of unexpected or unclassified variants with array-based methods, which should be discussed with future parents.

Because complete clefts of the lip/alveolus have considerably higher risks of palatal involvement than incomplete clefts of the lip/alveolus, they have a less favorable prognosis, especially when it comes to associated defects. Therefore, prenatal ultrasound screening should be more focused on the morphological severity of the lip/alveolus. Furthermore, it is crucial to obtain further clinical experience with new ultrasound techniques, such as the 'equal sign' marker to detect cleft palate,[128] as well as other more sophisticated 3D techniques or fetal MRI.[125] With regard to associated structural and chromosomal defects, follow-up studies are needed to acquire more accurate data on their prevalence and risk factors in prenatal and postnatal oral cleft populations. As array CGH can detect smaller chromosome deletions and duplications compared to conventional karyotyping, performing array CGH in large cohorts of prenatally and postnatally detected clefts would also give us more information about the proportions and types of chromosomal defects that are missed when cases are not genetically tested. This is essential to reach consensus on the role of invasive genetic testing in prenatally detected clefts.

CONCLUDING FUTURE PERSPECTIVES

In this thesis, we demonstrated that adequate and complete description, registration, and subsequent classification are essential in order to further understand oral clefts. When the primary palate is affected, the alveolus should be evaluated and described separately in order to have a complete and accurate diagnosis, as its morphology is not related to that of the lip. Therefore, the commonly used cleft categories should include the alveolus as a separate anatomical

structure, resulting in cleft lip/alveolus (CL/A), cleft lip/alveolus and palate (CL/AP), or cleft lip/alveolus with or without cleft palate (CL/A±P). Furthermore, frequently used classifications, such as the ICD-10 cleft coding system, should be adjusted and also incorporate the different morphological cleft features, thereby generating data on a large scale that can be subdivided according to timing and underlying mechanisms in embryogenesis. The NVSCA recording system provides such detailed data on all sub-phenotypes, which can be fitted into any classification. However, to optimize its quality and value, cleft team members need to be trained and more focused on less visible and mild cleft features as well as on additional congenital anomalies. Furthermore, genetic counseling is warranted in most cases, and reregistration at a later age is strongly recommended to also include certain anomalies that can be detected only later in infancy.

In the prenatal setting, more accurate ultrasound screening will improve counseling, especially regarding palatal involvement. Therefore, pregnant women with a fetus suspected of having an oral cleft should be referred to a tertiary center where more specific ultrasound screening can be performed. Also, it is important to obtain further clinical experience with new ultrasound techniques or fetal MRI. As different cleft categories are variously associated with additional structural and chromosomal anomalies and thus require different approaches in counseling, testing, and management, broad implementation of a uniform prenatal subdivision of clefts is vital. Additionally, follow-up studies, including array CGH, are needed to gain more insight into the proportions and types of structural and chromosomal anomalies missed in associated and presumed isolated clefts and into their risk factors.

Finally, to gain more insight into cleft etiology and effects of primary and secondary preventive strategies, future studies should consider geographical differences in cleft sub-phenotypes and include live births and stillbirths as well as spontaneous and induced abortions. Extrapolation of regional data to larger areas should be made with caution. To evaluate the impact of prenatal screening strategies in the Netherlands, uniform national registration of prenatal screening outcomes as well as implementation of indications for TOP in the WAZ are needed. With regard to folic acid, the increased risk we found for specific sub-phenotypes of oral clefts by an observational study is systematic and specific enough to warrant further evaluation, especially in the light of increased intake due to dietary improvements. Ideally, a randomized controlled trial should be conducted to confirm or refute our findings, but this would be unethical with the knowledge that folic acid can prevent neural tube defects. Therefore, future studies should focus on the type, timing, dose, and duration of folic acid supplementation in relation to embryologically different sub-phenotypes. Until more information is available, prudence is needed and we advise restricting supplementation to the periconceptional period recommended to protect against neural tube defects.

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Summary

Oral clefts—one of the most common congenital anomalies among humans—comprise a wide range of sub-phenotypes affecting the lip, alveolus, and hard and soft palates. While they are often diagnosed immediately after birth, they are increasingly being diagnosed during pregnancy by routine two-dimensional (2D) ultrasonography. Oral clefts may either be isolated or be associated with other congenital anomalies, often as part of a syndrome or chromosomal defect. Although their etiopathogenesis has been widely studied, it is still poorly understood. This thesis is aimed at describing and classifying the various sub-phenotypes of clefts as well as their associated anomalies in both the prenatal and postnatal setting, thereby providing an approach and basis to further understand their etiopathogenesis and optimize their prenatal and postnatal outcome and prognosis.

PART I Descriptive registration and validation

In **chapter 2** we describe the study design and first results of a national validation project evaluating the quality of data recorded in the Dutch registry of patients with oral clefts and craniofacial anomalies, maintained by the Dutch Association of Cleft Palate and Craniofacial Anomalies (NVSCA). We drew a random sample of 250 patients registered with oral clefts in the national NVSCA database from 1997 through 2003 by the Dutch cleft palate teams using a unique descriptive recording system based on the embryology of the head and neck area; of these patients, 13 were excluded because of lacking medical data. After linking registry data to clinical data derived from medical record review, we found that the three cleft categories that are used nowadays to study oral clefts (cleft lip/alveolus = CL, cleft lip/alveolus and palate = CLP, and cleft palate = CP) had been accurately and completely recorded in the NVSCA. All categories showed near-perfect inter-database agreement with a kappa (κ) value of 0.89 and over, a sensitivity of 90% and over, and a specificity of 97% and over. Data quality on associated infant and parental characteristics was reasonable to satisfying, with ranging κ values (0.20 to 0.76), sensitivity (25% to 97%), and specificity (35% to 93%). These findings show that NVSCA data are highly suitable for comparison with other studies and registries.

In **chapter 3** the quality of NVSCA data was further evaluated by analyzing whether the specific features (topography and morphology) of the various sub-phenotypes within the three cleft categories are adequately recorded in clinical practice. Medical data review revealed that the data quality of the various sub-phenotypes was generally satisfactory, but appeared to be related to anatomical location and morphological severity. The topographic anatomical structures (lip, alveolus, and hard and soft palates) showed near-perfect inter-database agreement with a κ value ranging from 0.82 to 0.98, sensitivity of over 87%, and specificity of over 95%. However, when analyzing the morphology of these structures, validity decreased, especially for morphologically mild features. This association was most evident for anomalies of the hard and

soft palates. For example, inter-database agreement was higher for complete/incomplete cleft soft palate (κ value of 0.91 and sensitivity of 96%) than for submucous cleft hard/soft palate (κ value of 0.77 and sensitivity of 69%). Our results support the clinical feasibility of the descriptive NVSCA system and underline its additional value—compared to other registry systems—for fundamental, epidemiological, and clinical research. However, morphologically severe clefts can be interpreted with higher confidence than mild clefts, which might have implications for research on these features and for guidelines on routine neonatal examination.

In **chapter 4** NVSCA data on congenital anomalies, syndromes, and chromosomal defects associated with oral clefts were validated. Through two-phased medical data review, we investigated whether these anomalies are accurately diagnosed and subsequently recorded. We found that the quality on associated anomalies was moderate to poor, with a κ value ranging from 0.59 to 0. Seventy-seven percent of the craniofacial anomalies were underreported in the NVSCA: 30% due to delayed diagnosis and 47% due to deficient recording. Additionally, 80% of the associated anomalies of other organ systems were underreported: 52% due to delayed diagnosis and 28% due to deficient recording. The reporting of final diagnoses was somewhat better; however, 54% were still underreported (24% delayed diagnosis and 30% deficient recording). The rate of overreporting was 1.6% or lower. These results emphasize the need for routine and thorough examination of patients with clefts. Clinicians should be more focused on co-occurring anomalies, and early genetic counseling seems warranted in most cases. Additionally, our findings underline the need for postnatal follow-up and ongoing registration of associated anomalies; reregistration in the NVSCA at a later age is recommended.

PART II Prevalence in the Netherlands

In **chapter 5** we present trends in prevalence of oral cleft live births in the Netherlands over 1997-2006. We performed time-trend analyses on NVSCA data of Dutch infants born alive with oral clefts during the study period. Prevalence rates and the estimated annual percentage change (EAPC) were calculated and stratified into CL±P and CP in order to investigate whether the higher periconceptional use of folic acid supplements or the greater prenatal detection of oral clefts (with or without associated anomalies) followed by pregnancy termination might have affected the prevalence. Both factors would mainly affect CL±P. Unlike CP, this category develops during the period recommended for folic acid use and can be detected prenatally by 2D ultrasound. In the 1997-2006 period, 3,308 infants out of 1,970,872 live births had oral clefts, resulting in an overall prevalence per 10,000 live births of 16.8 (CL±P 11.3; CP 5.5). Time-trend analysis showed that the prevalence of all oral clefts decreased (EAPC –1.8%; 95% confidence interval (CI): –3.0% to –0.6%), as did the CL±P prevalence (EAPC –2.3%; 95% CI: –3.8% to –0.9%). No significant trends were found for the CP prevalence. These findings demonstrate that because the live-birth prevalence of CL±P decreased, that of all oral clefts decreased, suggesting that higher periconceptional folic acid use or greater prenatal detection followed by pregnancy termination might have accounted for the observed decline. While this may have

implications for prenatal counseling and folic acid policy, further studies on these topics are needed first.

In **chapter 6** we compared NVSCA prevalence data over 1997-2007 with national data from the combined National Obstetric and Neonatal Registries (LVR/LNR) and regional data from the population-based registry of Eurocat Northern Netherlands, thereby verifying the detected decreasing trends and investigating whether the prevalence varies within the Netherlands. We found that the overall live-birth prevalence of oral clefts is significantly higher in the Northern Netherlands (15.1 to 21.4 per 10,000) than in the rest of the Netherlands (13.2 to 16.1 per 10,000). Time-trend analyses of both national registries confirmed the significant decreasing trend in CL±P for the rest of the Netherlands, while none of the registries showed significant trends for the Northern Netherlands. Despite some differences in prevalence between registries, they showed similar regional variation in prevalence and trends. In conclusion, our findings show that the prevalence of oral cleft live births varies significantly within the Netherlands, not only between but also within registries. This underlines that extrapolation of regional cleft data should be done with caution. Further studies investigating etiology and evaluating preventive strategies should consider these geographical differences in cleft sub-phenotypes among live births, stillbirths, and spontaneous/induced abortions, between and within countries, as they may reveal clues to the causal factors of oral clefts.

PART III Postnatal classification

In **chapter 7** a new postnatal classification of oral clefts based on the patho-embryology of the primary and secondary palates is described and tested on all sub-phenotypes among Dutch newborns. All unoperated infants registered in the national NVSCA database from 1997 through 2003 were included. Using their descriptive data, we divided the different sub-phenotypes—including subclinical features—within the three cleft categories (CL, CLP, and CP) into fusion defects, differentiation defects, or combinations of these, thereby classifying them according to their underlying patho-embryological processes. In total, 3.512 patients were included, showing a CL in 28%, CLP in 39%, and CP in 33%. Patients with CL showed 22% fusion defects, 75% differentiation defects, and 3% combined fusion and differentiation defects. CLP and CP patients most frequently had fusion defects (70% and 89%, respectively). We were able to construct an embryonic timetable relating almost all observed sub-phenotypes (over 90%) to specific weeks in development. This approach—considering timing and underlying mechanisms in embryogenesis—provides new feasible subgroups for further clinical, epidemiological, and fundamental research.

In **chapter 8** we analyzed adult unoperated patients from Indonesia with clefts of the lip/alveolus only to investigate whether our new classification is complete and feasible for all cleft sub-phenotypes of the primary palate. Compared to the secondary palate, the primary palate has rather complex and underexposed underlying patho-embryological mechanisms. Additionally, we investigate whether further morphological grading of incomplete cleft lips

is clinically and embryologically relevant and should be added to this classification. After local announcements, 108 adult unoperated patients with clefts of the lip/alveolus only were included. Using color photographs, X-rays, and dental casts, clefts were classified as fusion defects, differentiation defects, or combined defects. We further graded the morphology of incomplete cleft lips and analyzed whether these grades were related to the severity of alveolar clefts/hypoplasia. Permanent dentition was analyzed to investigate which alveolar part is deficient in fusion/differentiation defects. All sub-phenotypes—comprising 96 unilateral and 12 bilateral clefts—could be classified into fusion defects (17%), differentiation defects (79%), unilateral fusion-differentiation defects (2%), or bilateral fusion & differentation (2%) defects. We found that the various morphological grades of cleft lip were not related to the associated alveolar clefts/hypoplasia. Additionally, all alveolar and dental deformities were located in the premaxillae. This study demonstrates that this classification is complete and feasible for all clefts of primary palate, that further morphological grading of incomplete cleft lip is neither clinically nor embryologically relevant, and that the premaxilla forms the deficient part in alveolar deformities.

PART IV Effects of periconceptional folic acid supplementation

In **chapter 9** the effects of periconceptional folic acid supplements on the risk of oral clefts relative to other non-folate related congenital anomalies was assessed in a population-based case-control study, using complementary data from the NVSCA and Eurocat databases of children and fetuses born in the Northern Netherlands between 1997 and 2009 inclusive. Cases were live-born infants with non-syndromic clefts (n = 367) and controls were infants or fetuses with chromosomal/syndromal (n = 924) or non-folate related anomalies (n = 2021). We analyzed type, timing, and duration of supplement use related to the three cleft categories as well as to their timing (early/late embryonic periods) and underlying embryological processes (fusion/differentiation defects). Consistent supplement use during the etiologically relevant period (weeks 0 to 12 postconception) was associated with an increased risk of clefts (adjusted odds ratio 1.72, 95% CI 1.19 to 2.49), especially of cleft lip/alveolus (3.16, 1.69 to 5.91). Further analysis systematically showed two- to three-fold increased risks for late differentiation defects—mainly clefts of the lip/alveolus—with no significant associations for early/late fusion defects. Effects were attributable to folic acid and not to other multivitamin components, and inclusion of partial use (not covering the complete etiologically relevant period) generally weakened associations. This study presents several lines of evidence indicating that periconceptional folic acid in the Northern Netherlands might be associated with an increased risk of clefts, in particular of cleft lip/alveolus. This association is strengthened by the specificity, consistency, systematic pattern, and duration of exposure-response relationship of our findings, underlining the need to evaluate public health strategies regarding folic acid and to further investigate potential adverse effects.

PART V Prenatal diagnosis and classification

In **chapter 10** we give an overview of literature and complementary validated NVSCA data on the type and frequency of associated structural and chromosomal anomalies related to oral cleft category in prenatal and postnatal populations. The aim of this study was to provide a basis for prenatal counseling and decisions on prenatal invasive diagnostics. Twenty studies were included: 3 providing prenatal data, 13 providing postnatal data, and 4 providing both. Data from prenatal and postnatal studies showed that the prevalence of associated anomalies is lowest in CL (0 to 20% and 8 to 41%, respectively). For CLP, higher frequencies were found both prenatally (39% to 66%) and postnatally (21% to 61%). Although CP was barely detectable by ultrasound, it was the category most frequently associated with accompanying defects in postnatal studies (22% to 78%). Chromosomal abnormalities were most frequently seen in association with additional anomalies. In the absence of associated anomalies, chromosomal defects were found prenatally in CLP (3.9%) and postnatally in CL (1.8%, 22q11.2 deletions only), in CLP (1.0%), and in CP (1.6%). These findings underline that prenatal counseling regarding prognosis and risk of chromosomal defects should be tailored to cleft category, and more importantly to the presence or absence of associated anomalies. Irrespective of cleft category, clinicians should advise invasive genetic testing if associated anomalies are seen prenatally. In the absence of associated anomalies, prenatal conventional karyotyping is not recommended in CL, although array comparative genomic hybridization should be considered. In presumed isolated CLP or CP, prenatal invasive testing, preferably by array-based methods, is recommended.

In **chapter 11** we present a new prenatal ultrasound classification of oral and craniofacial clefts to aid in prenatal counseling, care, and research. This system is designed for modern ultrasound technologies and subdivides clefts according to their patho-embryological processes, associated congenital anomalies, and recent epidemiological insights. In contrast to most other systems, oral clefts (categories I-III) are distinguished from midline and atypical facial clefts (categories IV-VI), as the latter should be considered as craniofacial clefts because of their different patho-embryogenesis and accompanying defects. Additionally, the alveolus is described separately because of its unique underlying embryological processes, and clefts of the palate only are included as these are prenatally detectable nowadays. Besides grouping oral clefts into the three main categories (categories I-III), it is essential to prenatally differentiate between incomplete and complete clefts of the lip/alveolus, because the palate is less frequently involved in incomplete clefts, resulting in a better prognosis, especially when it comes to associated defects. Although unilateral and bilateral forms of CLP result from similar embryological processes, it is clinically relevant to distinguish them prenatally, because unilateral forms (category IIa) are less frequently associated with additional anomalies than bilateral forms (category IIb). With regard to craniofacial clefts, we distinguish two types of midline clefts based on differences in embryological processes and associated anomalies: complete median cleft lip and palate with hypotelorism (category IV), and incomplete median cleft lip (alveolus)

with or without hypertelorism (category V). The latter category (VI) of our classification comprises atypical facial clefts that are—in contrast to what is generally stated in literature—not explained by amniotic bands, but by different embryological processes.

Nederlandse samenvatting

Schisis is één van de meest voorkomende aangeboren afwijkingen. Er zijn veel verschillende subfenotypen te onderscheiden en afhankelijk van de lokalisatie spreekt men van een lip-, kaak-, of gehemeltespleet, of een combinatie hiervan. Meestal worden deze afwijkingen direct na de geboorte ontdekt, maar door de verbetering in het structurele tweedimensionale (2D) echoscopisch onderzoek worden met name de lip/kaakspleten met of zonder gehemeltespleten toenemend tijdens de zwangerschap gediagnosticeerd. Schisis komt als een geïsoleerde afwijking voor, maar ook met bijkomende aangeboren afwijkingen, meestal als onderdeel van een syndroom of chromosomale afwijking. De ontstaanswijze en oorzakelijke factoren van schisis zijn wereldwijd gedurende vele decennia onderzocht, maar ondanks het grote aantal studies is er nog relatief weinig over bekend. Het doel van dit proefschrift is om zowel de verschillende subfenotypen van schisis en de daarbij voorkomende congenitale afwijkingen te beschrijven en te classificeren, zowel in de prenatale en postnatale setting, om zo een aanpak en basis te bieden voor het verkrijgen van meer kennis over de etiopathogenese van schisis en het optimaliseren van de prenatale en postnatale uitkomst en prognose.

DEEL I Beschrijvende registratie en validatie

In **hoofdstuk 2** hebben we de studieopzet en eerste resultaten beschreven van een nationaal validatieproject waarin de kwaliteit van gegevens werd onderzocht opgenomen in de registratie van patiënten met schisis en craniofaciale afwijkingen van de Nederlandse Vereniging voor Schisis en Craniofaciale Afwijkingen (NVSCA). Hiervoor werd uit de nationale NVSCA-database een random sample genomen van 250 patiënten die gedurende de periode 1997-2003 met een schisis werden geregistreerd door de Nederlandse schisisteams met behulp van een uniek gedetailleerd registratiesysteem gebaseerd op de embryologie van het hoofd/halsgebied; van deze patiënten werden er 13 geëxcludeerd vanwege onvoldoende medische gegevens. Om de kwaliteit van de registratiegegevens te kunnen beoordelen werden deze gegevens vergeleken met een herregistratie van de klinische gegevens verkregen uit de medische status van de desbetreffende patiënten. Hieruit bleek dat de drie grove categorieën die tegenwoordig gebruikt worden om schisis te onderzoeken (lip/kaakspleten = CL, lip/kaak en gehemeltespleten = CLP, en gehemeltespleten = CP), accuraat en compleet worden geregistreerd in de NVSCA. Voor alle categorieën werd een goede inter-database overeenkomst gevonden met een kappa (κ) waarde van 0,89 en hoger, een sensitiviteit van 90% en hoger, en een specificiteit van 97% en hoger. De gegevens met betrekking tot de algemene karakteristieken van het kind en de ouders bleken minder, maar redelijk valide te zijn en toonden een variërende κ-waarde (0,20-0,76), sensitiviteit (25%-97%), en specificiteit (35%-93%). Tezamen laten deze bevindingen zien dat de NVSCA-gegevens uitermate geschikt zijn voor vergelijking en onderzoek met andere studies en registraties van aangeboren afwijkingen, waaronder schisis.

In **hoofdstuk 3** werd de kwaliteit van de NVSCA-gegevens verder geanalyseerd door te onderzoeken of de specifieke kenmerken (topografie en morfologie) van de verschillende subfenotypen binnen de drie schisiscategorieën adequaat zijn geregistreerd in de klinische praktijk. Uit vergelijking van deze gegevens met de herregistratie bleek dat de kwaliteit over het algemeen acceptabel is, maar dat deze varieert met de anatomische lokalisatie en de morfologische ernst van de afwijkingen. De topografische-anatomische structuren (lip, kaak, harde en zachte palatum) toonden een goede inter-database overeenkomst met een κ-waarde variërend van 0,82 tot 0,98, een sensitiviteit van hoger dan 87% en een specificiteit van hoger dan 95%. De validiteit daalde echter bij het analyseren van de morfologie van deze structuren, voornamelijk voor de morfologisch milde kenmerken. Voor het harde en zachte palatum was deze associatie het duidelijkst aanwezig. Zo was bijvoorbeeld de overeenkomst hoger voor de complete/incomplete spleten van het zachte palatum (κ-waarde 0,91 en sensitiviteit 96%) dan voor de submuceuze spleten van het harde/zachte palatum (κ-waarde 0,77 en sensitiviteit 69%). Onze resultaten laten zien dat het unieke NVSCA-registratiesysteem valide en klinisch toepasbaar is en onderstrepen de aanvullende waarde – ten opzichte van andere registratiesystemen – voor verder fundamenteel, epidemiologisch en klinisch onderzoek. De data voor morfologisch ernstigere spleten zijn echter betrouwbaarder dan die voor morfologisch mildere afwijkingen. Dit heeft mogelijk implicaties voor onderzoek naar deze kenmerken alsook voor het verbeteren van richtlijnen op het gebied van routine neonataal onderzoek.

In **hoofdstuk 4** valideerden we de NVSCA-gegevens met betrekking tot geassocieerde congenitale afwijkingen, syndromen en chromosomale defecten. Door middel van herregistratie in twee fasen (eenmaal gebaseerd op de medische gegevens die beschikbaar waren op het oorspronkelijke moment van registratie in de NVSCA, en eenmaal gebaseerd op alle gegevens die beschikbaar waren op het moment van dit onderzoek, dus inclusief postnatale follow-up) werd onderzocht of deze afwijkingen adequaat gediagnosticeerd zijn tijdens het eerste bezoek aan de schisisteams en of ze vervolgens goed geregistreerd zijn. De kwaliteit voor geassocieerde afwijkingen bleek middelmatig tot slecht te zijn, met een κ-waarde variërend van 0,59 tot 0. Van de craniofaciale afwijkingen ontbrak 77% in de NVSCA: 30% door verlate diagnose en 47% door deficiënte registratie. Van de afwijkingen betreffende andere orgaansystemen was 80% niet geregistreerd: 52% door verlate diagnose en 28% door deficiënte registratie. De registratie van de uiteindelijke diagnose (zoals syndromen of chromosomale defecten) was beter, maar nog niet acceptabel, met een onderrapportage van 54%: 24% door verlate diagnose en 30% door deficiënte registratie. De overrapportage was slechts 1,6% of lager. Deze bevindingen benadrukken het belang van uitvoerig routineonderzoek van patiënten met schisis. Hierbij moet meer gefocust worden op bijkomende afwijkingen, en vroege genetische counseling lijkt op zijn plaats in de meeste gevallen. Daarnaast onderstrepen onze resultaten het nut van postnatale follow-up en continue registratie, en herregistratie in de NVSCA op een latere leeftijd wordt dan ook aanbevolen.

DEEL II Prevalentie in Nederland

In **hoofdstuk 5** presenteren we de trends in prevalentie van schisis onder levendgeborenen in Nederland over 1997-2006. Hiervoor verrichtten we tijdtrendanalyses met NVSCA-gegevens van kinderen met schisis die levend geboren werden in Nederland tijdens de studieperiode. De prevalenties en het geschatte percentage jaarlijkse verandering (EAPC) van de prevalenties werden berekend en vervolgens gestratificeerd naar: lip/kaak- met of zonder gehemeltespleten (CL±P); en gehemeltespleten zonder lip/kaakspleten (CP). Op deze manier werd onderzocht of het toegenomen periconceptioneel gebruik van foliumzuursupplementen en/of de toegenomen prenatale detectie van schisis (met of zonder geassocieerde afwijkingen) gevolgd door zwangerschapsafbreking de prevalentie van schisis mogelijk beïnvloed zouden kunnen hebben. Beide factoren zijn voornamelijk van toepassing op CL±P, omdat deze, anders dan CP, ontstaan tijdens de periode die aanbevolen is voor foliumzuurgebruik en deze via de 2D-echoscopisch onderzoek prenataal gediagnosticeerd kunnen worden. Gedurende 1997-2006 hadden 3.308 van de 1.970.872 levend geboren kinderen een schisis, met als resultaat een totale prevalentie per 10.000 levendgeborenen van 16,8 (CL±P 11,3; CP 5,5). Tijdens de studieperiode daalde de prevalentie van schisis significant met 1.8% per jaar (95% betrouwbaarheidsinterval (BI): –3,0% tot –0,6%) doordat de prevalentie van CL±P daalde (EAPC –2,3%; 95% BI: –3,8% tot –0,9%). Er werden geen significante trends voor CP gevonden. Concluderend tonen onze resultaten dat de prevalentiedaling in schisis veroorzaakt is door een daling in CL±P. Deze specifieke daling suggereert dat het toegenomen periconceptioneel foliumzuurgebruik en/of de toegenomen prenatale detectie gevolgd door zwangerschapsafbreking mogelijke oorzaken zouden kunnen zijn. Alhoewel dit implicaties zou moeten hebben voor prenatale counseling en het foliumzuurbeleid, is verder onderzoek naar deze factoren vereist.

In **hoofdstuk 6** worden de prevalentiegegevens van de NVSCA over 1997-2007 vergeleken met de nationale gegevens van de Landelijke Verloskunde en Neonatale Registraties (LVR/LNR) en de regionale gegevens van de Eurocat-registratie in Noord-Nederland. Het doel hiervan was om de gedetecteerde dalende trends te verifiëren en om te onderzoeken of de prevalentie varieert binnen Nederland. Deze vergelijking toonde dat de totale prevalentie van schisis onder levendgeborenen significant hoger is in Noord-Nederland (15,1 tot 21,4 per 10.000) dan in de rest van Nederland (13,2 tot 16,1 per 10.000). Tijdtrendanalyse van beide nationale registraties bevestigden de significant dalende trend in CL±P voor de rest van Nederland, terwijl geen van de registraties significante trends voor Noord-Nederland vertoonde. Ondanks enige verschillen in prevalentie tussen de registraties, lieten ze een vergelijkbare regionale variatie in prevalentie en trends binnen Nederland zien. Concluderend varieert de prevalentie van schisis onder levendgeborenen significant in Nederland, niet alleen tussen maar ook binnen registraties. Dit onderstreept dat men zeer voorzichtig dient te zijn met het extrapoleren van regionale schisisgegevens. Het is van belang dat verdere studies naar de etiologie en naar het effect van preventieve maatregelen rekening houden met geografische verschillen in subfenotypen van schisis onder levend- en doodgeborenen alsmede spontane en geïnduceerde

zwangerschapsafbrekingen, omdat dit kan leiden tot meer inzicht in the oorzakelijke factoren van schisis.

DEEL III Postnatale classificatie

In **hoofdstuk 7** wordt een nieuwe postnatale classificatie van schisis – gebaseerd op de patho-embryologie van het primaire en secundaire palatum – beschreven en getest op alle subfenotypen onder Nederlandse pasgeborenen. Alle ongeopereerde kinderen die geregistreerd werden in de nationale NVSCA-database van 1997 tot en met 2003 werden geïncludeerd. Met behulp van de beschrijvende gegevens van deze kinderen werden de verschillende subfenotypen, inclusief subklinische kenmerken, binnen de drie schisiscategorieën (CL, CLP en CP) ingedeeld in fusiedefecten, differentiatiedefecten, of combinaties hiervan. Op deze manier werden de subfenotypen ingedeeld naar onderliggende patho-embryologische processen. In totaal werden 3.512 patiënten geïncludeerd, waarvan 28% een CL had, 39% een CLP en 33% een CP. Patiënten met CL hadden in 22% van de gevallen een fusiedefect, in 75% een differentiatiedefect en in 3% een gecombineerd fusie-differentiatiedefect. Onder de patiënten met CLP en CP werd in de meeste gevallen een fusiedefect gediagnosticeerd (respectievelijk in 70% en 89%). Daarnaast was het mogelijk om een embryologische tijdstabel te construeren waarbij bijna alle subfenotypen (> 90%) gerelateerd kon worden aan specifieke weken in de ontwikkeling. Deze nieuwe aanpak, waarbij schisis ingedeeld wordt naar de timing en onderliggende mechanismen in embryogenese, is goed toepasbaar en biedt nieuwe subgroepen voor klinisch, epidemiologisch en fundamenteel onderzoek.

In **hoofdstuk 8** hebben we volwassen ongeopereerde patiënten uit Indonesië met een spleet van alleen de lip en/of kaak geanalyseerd om te onderzoeken of de nieuwe postnatale classificatie compleet en toepasbaar is voor alle subfenotypen van het primaire palatum. Vergeleken met die van het secundaire palatum, zijn de onderliggende embryologische processen van het primaire palatum namelijk zeer complex en onderbelicht in de literatuur. Daarnaast onderzochten we of verdere morfologische gradering van incomplete lipspleten zowel klinisch als embryologisch relevant is en toegevoegd dient te worden aan de classificatie. Na lokale aankondigingen van de mogelijkheid tot klinische behandeling, werden 108 volwassen ongeopereerde patiënten met spleten van alleen de lip en/of kaak geïncludeerd. Met behulp van kleurenfoto's, röntgenfoto's en kaakmodellen, classificeerden we de subfenotypen als fusiedefect, differentiatiedefect, of als gecombineerd defect. Vervolgens werden de incomplete lipspleten morfologisch verder gegradeerd en onderzochten we of deze te relateren waren aan de ernst van de geassocieerde alveolaire afwijkingen (spleten of hypoplasie). De permanente dentitie werd geanalyseerd om te onderzoeken welk deel van de alveolus deficiënt is in fusie- en/of differentiatiedefecten. Zesennegentig patiënten toonden een unilaterale en 12 patiënten een bilaterale schisis. Het was mogelijk om alle subfenotypen te classificeren, wat resulteerde in 17% fusiedefecten, 79% differentiatiedefecten, 2% unilaterale fusie-differentiatiedefecten en 2% bilaterale fusie- & differentiatiedefecten. De morfologische gradering van lipspleten bleek

niet gerelateerd te zijn aan de geassocieerde alveolairspleten of -hypoplasie. Daarnaast waren alle alveolaire en dentale afwijkingen gelokaliseerd in de premaxillae. Deze studie demonstreert dat onze nieuwe classificatie compleet en toepasbaar is voor spleten van het primaire palatum, dat verdere morfologische gradering van incomplete lipspleten noch klinisch noch embryologisch relevant is, en dat het deficiënte deel in alveolaire afwijkingen de premaxilla betreft.

DEEL IV Effect van periconceptionele foliumzuursuppletie

In **hoofdstuk 9** onderzochten we de effecten van periconceptionele foliumzuursupplementen op het risico van schisis ten opzichte van andere niet-foliumzuur gerelateerde aangeboren afwijkingen in een populatie-gebaseerde case-controle studie. Hiervoor werden de complementaire gegevens van de NVSCA en Eurocat gebruikt voor kinderen en foetussen geboren in Noord-Nederland van 1997 tot en met 2009. Als cases includeerden we levend geboren kinderen met non-syndromale schisis (n = 367) en als controles kinderen en foetussen met chromosomale of syndromale afwijkingen (n = 924) of met niet-foliumzuur gerelateerde aangeboren afwijkingen (n = 2021). Het type, de timing en de duur van supplementgebruik werd geanalyseerd in relatie tot de drie schisiscategorieën alsook tot de timing (vroege en late embryonale periodes) en onderliggende processen (fusie/differentiatiedefecten) in de embryogenese. Consistent gebruik van supplementen tijdens de etiologisch relevante periode (week 0 tot en met 12 na de conceptie) bleek geassocieerd te zijn met een verhoogd risico voor schisis (aangepaste odds ratio 1,72; 95% BI 1,19 tot 2,49), en in het bijzonder voor lip/kaakspleten (3,16; 95% BI 1,69 tot 5,91). Meer specifiekere analyse toonde twee- tot driemaal verhoogde risico's voor late differentiatiedefecten, welke voornamelijk spleten van de lip/kaak betroffen, zonder significante associaties voor vroege en late fusiedefecten. Effecten waren toe te schrijven aan foliumzuur en niet aan andere componenten van multivitaminen en werden zwakker na inclusie van gedeeltelijk gebruik (gedurende een deel van de etiologisch relevante periode). Deze studie presenteert verschillende lijnen van bewijs die suggereren dat periconceptioneel gebruik van foliumzuur in Noord-Nederland geassocieerd zou kunnen zijn met een verhoogd risico voor schisis, voornamelijk voor lip/kaakspleten. Deze associatie wordt ondersteund door de specificiteit, de consistentie, het systematische patroon, en de 'duur van blootstelling-respons relatie' in onze resultaten. Dit onderstreept dat de evaluatie van 'public health' strategieën en onderzoek naar de potentiële nadelige effecten van foliumzuur noodzakelijk is.

DEEL V Prenatale diagnose en classificatie

In **hoofdstuk 10** geven we een overzicht van literatuur en complementaire gevalideerde NVSCA-gegevens voor het type en de frequentie van geassocieerde structurele en chromosomale afwijkingen in relatie tot de categorieën schisis in prenatale en postnatale populaties. Het doel hiervan is om een basis te bieden voor prenatale counseling en voor beslissingen op het

gebied van prenatale invasieve diagnostiek. Twintig studies werden geïncludeerd: 3 met prenatale gegevens, 13 met postnatale gegevens en 4 met prenatale en postnatale gegevens. Zowel prenatale als postnatale studies toonden dat de prevalentie van geassocieerde afwijkingen het laagste is in CL (respectievelijk 0 tot 20% en 8 tot 41%). Voor CLP werden hogere frequenties gevonden in prenatale (39% tot 66%) en postnatale (21% tot 61%) studies. CP werd nauwelijks gedetecteerd met het 2D-echoscopisch onderzoek, maar de postnatale studies lieten zien dat deze categorie het vaakst geassocieerd is met bijkomende afwijkingen (22% tot 78%). Chromosomale afwijkingen werden het meest gezien wanneer er sprake was van geassocieerde afwijkingen. In de afwezigheid van geassocieerde afwijkingen werden chromosomale defecten prenataal gediagnosticeerd in alleen CLP (3,9%) en postnataal in CL (1,8%, alleen 22q11.2 deleties), in CLP (1,0%) en in CP (1,6%). Deze bevindingen benadrukken dat prenatale counseling met betrekking tot prognose en het risico op chromosomale afwijkingen zou moeten worden afgestemd op de schisiscategorie, en in nog hogere mate op de aan- of afwezigheid van geassocieerde afwijkingen. Daarnaast wordt in het geval van prenatale geassocieerde afwijkingen invasief genetisch onderzoek geadviseerd. Bij de afwezigheid van geassocieerde afwijkingen in CL wordt prenatale conventionele karyotypering niet aanbevolen, maar zou "array comparative genomic hybridization" overwogen kunnen worden. Indien verondersteld wordt dat CLP of CP geïsoleerd voorkomt, is prenataal invasief onderzoek aan te bevelen, bij voorkeur met array-gebaseerde methoden.

In **hoofdstuk 11** presenteren we een nieuwe prenatale echoclassificatie van schisis en craniofaciale spleten met als doel prenatale counseling, zorg en onderzoek te optimaliseren. Dit systeem is ontwikkeld voor moderne echotechnieken en deelt de afwijkingen in volgens onderliggende patho-embryologische processen, bijkomende congenitale afwijkingen en recente epidemiologische inzichten. In tegenstelling tot de meeste systemen, onderscheiden wij schisis (categorieën I-III) van mediane en atypische aangezichtsspleten (categorieën IV-VI). Deze laatste groepen dienen beschouwd te worden als craniofaciale spleten vanwege de andere patho-embryogenese en bijkomende defecten. Daarnaast wordt de alveolus apart beschreven gezien de unieke onderliggende embryologische processen, en zijn de gehemeltespleten zonder lip/kaakspleten ook opgenomen in de classificatie omdat deze steeds beter prenataal te diagnosticeren zijn. Naast de groepering van schisis in drie categorieën is het belangrijk om incomplete van complete lip/kaakspleten te onderscheiden, omdat het palatum minder vaak is aangedaan bij incomplete spleten, wat resulteert in een betere prognose, voornamelijk als het gaat om geassocieerde afwijkingen. Hoewel unilaterale en bilaterale lip/kaak- en gehemeltespleten dezelfde onderliggende embryologische processen hebben, is het klinisch relevant om deze groepen te onderscheiden omdat unilaterale vormen (categorie IIa) minder vaak geassocieerd zijn met bijkomende afwijkingen dan bilaterale vormen (categorie IIb). Wat betreft de craniofaciale spleten zijn er twee aparte categorieën mediane aangezichtsspleten te onderscheiden gezien hun verschillen in embryologische processen en bijkomende afwijkingen: complete mediane lip/kaak- en gehemeltespleet met hypotelorisme (categorie IV) en

incomplete mediane lip(kaak)spleet met of zonder hypertelorisme (categorie V). De laatste categorie (VI) van de classificatie bestaat uit atypische aangezichtsspleten die – in tegenstelling tot wat over het algemeen beweerd wordt in de literatuur – niet verklaard kunnen worden door amnionstrengen, maar door andere embryologische processen.

APPENDICES

Abbreviations

List of publications

PhD portfolio

Curriculum Vitae

Dankwoord

Abbreviations

2D	two-dimensional
3D	three-dimensional
array CGH	array-comparative genomic hybridization
BI	betrouwbaarheids interval
BPA	British Pediatric Association Classification of Diseases
CCL	complete cleft lip
CCLA	complete cleft lip + complete cleft alveolus
CCL+ICA	complete cleft lip + incomplete cleft alveolus
CCLA;ICL	complete cleft lip + complete cleft alveolus combined with a contralateral incomplete cleft lip
CCLA;ICLA	complete cleft lip + complete cleft alveolus combined with a contralateral incomplete cleft lip + incomplete cleft alveolus
CCLA; ICL+CCA	complete cleft lip + complete cleft alveolus combined with a contralateral incomplete cleft lip + complete cleft alveolus
CCP	complete cleft palate
CCSP	complete cleft of the soft palate
CI	confidence interval
CL	cleft lip
CL/A	cleft lip/alveolus
CL/AP	cleft lip/alveolus and palate
CL/A±P	cleft lip/alveolus with or without cleft palate
CLP	cleft lip and palate
CL±P	cleft lip with or without cleft palate
CP	cleft palate
CRL	crown-rump length
D	differentiation defect
EAPC	estimated annual percentage change
ECLAM	Latin American Collaborative Study of Congenital Malformations
EMT	epitheliomesenchymal transformation
EUROCAT	European Registry of Congenital Anomalies and Twins
F	fusion defect
FD	fusion and differentiation defect
FISH	fluorescence in situ hybridization
Fro.	os frontale
HH/SP	hypoplastic hard and/or soft palate
ICD	International Classification of Diseases

ICHP	incomplete cleft of the hard palate
ICL	incomplete cleft lip
ICLA	incomplete cleft lip + incomplete cleft alveolus
ICL+CCA	incomplete cleft lip + complete cleft alveolus
ICSP	incomplete cleft of the soft palate
I.o.d.	interorbital distance
IQR	interquartile range
IUCR	intrauterine growth retardation
I/CCU	(in)complete cleft of the uvula
K	kappa
LNR	Landelijke Neonatologie Registratie (National Neonatal Registry)
LVR	Landelijke Verloskunde Registratie (National Obstetric Registry)
Mand.	mandible
Max.	maxilla
MCA	multiple congenital anomalies
Nas.	os nasale
NNL	Northern Netherlands
NVSCA	Nederlandse Vereniging voor Schisis en Craniofaciale Afwijkingen (Dutch Association for Cleft Palate and Craniofacial Anomalies)
OC	common oral cleft
Occ.	os occipitale
Pal.dur.	palatum durum
Pal.mol.	palatum molle
Par.	os parietale
PPR	prevalence proportion ratio
Pre.	premaxilla
Pre./Max.	premaxilla – maxilla
PRS	Pierre Robin Sequence
SCHP	submucous cleft of the hard palate
SCH/SP	submucous cleft of the hard and/or soft palate
SCL	submucous cleft lip
SCSP	submucous cleft of the soft palate
SD	standard deviation
Temp.	os temporale
Ton.	tongue
TOP	termination of pregnancy
US	ultrasound

VCF	velo-cardio-facial syndrome (22q11.2 deletion)
WAZ	Wet Afbreking Zwangerschap (Termination of Pregnancy Act)
Zyg.	zygoma

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** Joined first authorship: these authors contributed equally to this work.*

PhD portfolio

Summary of PhD Training and Teaching Activities

Name PhD student:	Annemarie Rozendaal
Erasmus MC Department:	Plastic and Reconstructive Surgery
PhD period:	2008 – 2012
Promotor:	Prof. dr. S.E.R. Hovius
Supervisor:	Dr. C. Vermeij-Keers

	Year	Workload (Hours/ ECTS)
1. PhD training		
General academic skills		
- English Course Proficiency A	2008	30 hours
- Biomedical English Writing and Communication	2009	4 ECTS
Research skills		
- Principles of Research in Medicine and Epidemiology, NIHES	2008	0.7 ECTS
- Biostatistics for Clinicians, NIHES	2009	1 ECTS
In-depth courses (e.g. medical training)		
- Microsurgery training Skillslab, Erasmus MC Rotterdam	2008 & 2009	150 hours
Oral presentations		
- Validatie van de NVSCA-registratie Schisis: studieopzet en eerste resultaten. 23e Wetenschappelijke Vergadering NVSCA. Nijmegen, Nederland	2008	1 ECTS
- Drie registraties: prevalentie van schisis in Nederland en Noord-Nederland, een nationale daling 1997-2006. Commissie Registratie Aangeboren Afwijkingen, Ministerie van Volksgezondheid, Welzijn en Sport. Den Haag, Nederland	2009	1 ECTS
- Validation of the NVSCA Registry Common Oral Clefts: study design and first results. 11th International Congress on Cleft lip and Palate and Related Craniofacial Anomalies. Fortaleza, Brazil	2009	1 ECTS
- Ten years registration of common oral clefts: decrease of prevalence in the Netherlands, 1997-2006. 11th International Congress on Cleft lip and Palate and Related Craniofacial Anomalies. Fortaleza, Brazil	2009	1 ECTS
- Tien jaar schisisregistratie: daling prevalentie van schisis in Nederland, 1997-2006. 24e Wetenschappelijke Vergadering NVSCA. Tilburg, Nederland	2009	1 ECTS
- Validatie van de NVSCA-registratie Schisis: kwaliteit van registratie van de individuele schisisafwijkingen. 25e Wetenschappelijke Vergadering NVSCA. Den Haag, Nederland	2010	1 ECTS
- Regionale verschillen in de prevalentie van schisis onder levendgeborenen in Nederland 1997-2007: trendanalyse van gegevens uit drie Nederlandse registraties. 25e Wetenschappelijke Vergadering NVSCA. Den Haag, Nederland	2010	1 ECTS

-	Oral Clefts: Registration, classification, and epidemiology of prenatal and postnatal phenotypes. PhD-weekend afdeling Dermatologie – Erasmus MC Rotterdam. Zuid-Limburg, Nederland	2012	1 ECTS
International and national conferences			
-	Symposium Perinatologie in beeld. Rotterdam, Nederland	2008	1 ECTS
-	23e Wetenschappelijke Vergadering NVSCA. Nijmegen, Nederland	2008	1 ECTS
-	Voorjaarsvergadering Nederlandse Vereniging voor Plastische Chirurgie. Utrecht, Nederland	2009	1 ECTS
-	11th International Congress on Cleft Lip and Palate and Related Craniofacial Anomalies. Fortaleza, Brazil	2009	1 ECTS
-	24e Wetenschappelijke Vergadering NVSCA. Tilburg, Nederland	2009	1 ECTS
-	25e Wetenschappelijke Vergadering NVSCA. Den Haag, Nederland	2010	1 ECTS
Seminars and workshops			
-	Kortjakje, Zondagsschool voor Plastische Chirurgie. Zeist, Nederland	2008	7 hours
-	Training in hechttechnieken zenuwen, pezen en flexoren, Skillslab, Erasmus MC Rotterdam, Rotterdam, Nederland	2008	20 hours
-	PhD-weekend afdeling Dermatologie – Erasmus MC Rotterdam. Zuid-Limburg, Nederland	2012	48 hours
Other			
-	Session chair "Junior Investigations". 11th International Congress on Cleft lip and Palate and Related Craniofacial Anomalies. Fortaleza, Brazil	2009	
-	Member Working Group NVSCA registry	2008 – 2012	120 hours
-	Member Working Group NVSCA website	2008 – 2012	120 hours
Awards			
-	"The best Junior Investigation – Dr. Cassio M. Raposo do Amaral Award" 11th International Congress on Cleft Lip and Palate and Related Craniofacial Anomalies. Fortaleza, Brazil	2009	
2. Teaching activities			
Lecturing			
-	Vierdejaars vaardigheidsonderwijs anatomie en functie van de hand, curriculum geneeskunde, Erasmus Universiteit Rotterdam (EUR), Nederland	2008	6 hours
-	Keuzeonderwijs craniofaciale chirurgie, embryologie en registratie van schisis en andere hoofd/halsafwijkingen, 3e jaars keuzeonderwijsstudenten, EUR, Nederland	2009 & 2010	20 hours
-	Seminar embryologie en registratie van schisis en andere hoofd/halsafwijkingen, orthodontisten in opleiding, Academisch Centrum Tandheelkunde Amsterdam (ACTA)	2009 & 2011	10 hours
Supervising practicals and excursions			
-	Supervisie Microchirurgiecursus voor AIOS Obstetrie en Gynaecologie, Skillslab, Erasmus MC Rotterdam, Nederland	2008	5 hours
-	Supervisie Microchirurgiecursus voor specialisten en specialisten in opleiding, Skillslab, Erasmus MC Rotterdam, Nederland	2008 & 2009	28 hours

Curriculum Vitae

Annemarie Rozendaal werd op 11 december 1981 geboren in Rotterdam. Zij doorliep het lager onderwijs op de Rehobothschool te Ridderkerk. In dezelfde plaats behaalde zij in 2000 haar VWO-diploma aan het Farel College. Aansluitend begon zij haar studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. Tijdens deze studie was zij actief bij de Medische Faculteits Vereniging Rotterdam (MFVR), onder andere als bestuurslid en als voorzitter van de Facultaire Introductie Commissie. Daarnaast was zij werkzaam in het Studententeam Thoraxchirurgie van het Erasmus MC Rotterdam. In 2004-2005 verrichtte zij in dit ziekenhuis haar afstudeeronderzoek op de afdelingen Plastische en Reconstructieve Chirurgie en Orthodontie. Hiervoor deed zij – onder supervisie van Dr. C. Vermeij-Keers en Dr. J.W. van Neck – onderzoek naar de betrouwbaarheid en toepasbaarheid van het landelijk registratiesysteem van de Nederlandse Vereniging voor Schisis en Craniofaciale Afwijkingen (NVSCA) voor patiënten met schisis met of zonder geassocieerde afwijkingen. Als student-assistent continueerde zij tijdens haar co-assistentschappen deze onderzoeksactiviteiten en ontstond de basis voor dit proefschrift. Na haar keuze- en oudste co-assistentschap verricht te hebben op dezelfde afdeling behaalde zij haar artsexamen in 2008. Hierna werd zij als arts-onderzoeker op de afdelingen Plastische en Reconstructieve Chirurgie en Orthodontie aangesteld, onder supervisie van Prof. Dr. S.E.R. Hovius en Dr. C. Vermeij-Keers, met dit proefschrift als resultaat. Tijdens haar promotietraject deed zij tevens diensten in de kliniek en volgde zij trainingen microchirurgie. In 2011 besloot zij zich te specialiseren in de Dermatologie en Venerologie. Ter voorbereiding op deze opleiding volgde zij gedurende 4 maanden een klinische meeloopstage op de afdeling Dermatologie van het Sint Fransiscus Gasthuis te Rotterdam (Dr. M.C.G. van Praag en Drs. D.G.C.T.M. Snels). Hierna werd zij aangenomen voor de opleiding tot dermatoloog in het Erasmus MC (Prof. H.A.M. Neumann en Dr. B.H. Thio), waarmee zij in juli 2012 begon. Gedurende het eerste half jaar liep zij een perifere stage in het Catharina Ziekenhuis te Eindhoven (Dr. G.A.M. Krekels) en per 1 juli 2013 is ze gestart met een tweede perifere stage van een jaar in het Amphia Ziekenhuis te Breda (Dr. A. Erceg).

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In de eerste plaats wil ik graag mijn co-promotor, *dr. C. Vermeij-Keers*, bedanken. Christl, zonder jouw uitgebreide wetenschappelijke kennis, ervaring en expertise op embryologisch gebied en je inspanningen voor de landelijke NVSCA-registratie Schisis had dit promotieonderzoek nooit plaats kunnen vinden. Je bevlogenheid, uithoudingsvermogen en precisie zijn bewonderenswaardig. We hebben heel intensief samengewerkt, en ik ben je dan ook bijzonder dankbaar voor al je tijd en energie. Geen vraag was je te veel, en je was altijd bereid om op korte termijn stukken te beoordelen. Ik heb je kritische blik en volhardendheid zeer gewaardeerd en ontzettend veel geleerd van onze discussies en je manier van denken. Bedankt voor het vertrouwen dat je in me hebt gehad.

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- LEO Pharma B.V.
- Oldekamp Medisch B.V.
- Abbvie B.V.
- Fagron B.V.
- ChipSoft B.V.
- Medizorg B.V.

SINDS 1995
MediZorg

- Dentsply Lomberg B.V.
- Tandtechnisch Laboratorium Laverman
- Orthoproof B.V.
- KINDERTAND, Praktijk voor kindertandheelkunde

Oral clefts are one of the most common congenital anomalies among humans, comprising a wide range of sub-phenotypes affecting the lip, alveolus, and hard and soft palates. They may either be isolated or be associated with other congenital anomalies, often as part of a syndrome or chromosomal defect. Although their etiopathogenesis has been widely studied, it is still poorly understood.

This thesis is aimed at describing and classifying the various sub-phenotypes of oral clefts as well as their associated anomalies in both the prenatal and postnatal setting, thereby providing an approach and basis to further understand their etiopathogenesis and optimize their outcome and prognosis. In part I of this thesis, we validate a unique recording system of oral clefts, based on the embryology of the head and neck area. In part II, the prevalence of oral clefts in the Netherlands, including its differences between regions and registries, is investigated. Part III describes a new postnatal embryological classification of oral clefts, providing subgroups related to specific time periods and underlying embryological processes in development. In the case-control study of part IV, we assess the effects of periconceptional folic acid supplement use on the risk of oral clefts. Finally in part V, we analyze the type and prevalence of associated structural anomalies and chromosomal defects in prenatal and postnatal oral cleft populations and present a new prenatal ultrasound classification of clefts aiding in prenatal counseling and care.