

Characteristics of Oral Cleft Phenotypes

Epidemiological and Genetic Studies in Norway

Åse Sivertsen



Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

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LIST OF PAPERS

This thesis is based on the following four papers. The papers will be referred to by their Roman numerals.

- I Sivertsen Å, Wilcox AJ, Johnson GE, Åbyholm FE, Vindenes HA, Lie RT. **“Prevalence of major anatomic variations in oral clefts.”** *Plastic and Reconstructive Surgery*, accepted for publication, November 2006.
- II Kubon C, Sivertsen Å, Vindenes HA, Åbyholm FE, Wilcox AJ, Lie RT. **“Completeness of Registration of Oral Clefts in a Medical Birth Registry: A Population Based Study.”** *Acta Obstetrica et gynecologica* 2007 Sep 11:1-5
- III Sivertsen Å, Wilcox AJ, Skjærven R, Vindenes HA, Åbyholm FE, Harville E, Lie RT. **“Familial risk of oral clefts by morphological type and severity.”** Submitted.
- IV Sivertsen Å, Lie RT, Wilcox AJ, Åbyholm FE, Vindenes HA, Haukanes BI, Houge G. **“Prevalence of Duplications and Deletions of the 22q11 DiGeorge syndrome region in a Population-based Sample of Infants with Cleft Palate.”** *American Journal of Medical Genetics A*. 2007 Jan 15;143 (2):129-34.

ABBREVIATIONS AND ACRONYMS

BCLP	Bilateral Cleft lip and palate. Bilateral clefts of the primary palate and cleft of the secondary palate.
CLO	Cleft lip only. Cleft of the primary palate, cleft anterior to the incisive foramen, without secondary palate involvement
CPO	Cleft palate only. Cleft of the secondary palate, cleft posterior to the incisive foramen, without primary palate involvement
CLP	Cleft lip and palate. Cleft of the primary and secondary palate
CL	Cleft lip. Cleft of the primary palate with or without secondary palate involvement
CT	Computed tomography
EUROCAT	European network of population-based registries for the epidemiologic surveillance of congenital anomalies
FISH	Fluorescence <i>in situ</i> hybridization
ICBD	International Clearinghouse for Birth Defects
ICD	International classification of diseases and causes of death
MLPA	Multiplex ligation-dependent probe amplification
MBRN	Medical Birth Registry of Norway
MRI	Magnetic resonance imaging
PCR	Polymerase chain reaction
RPL	Right lip, Palate, Left lip numerical coding system

SUMMARY

Characteristics of Oral Cleft Phenotypes *Epidemiological and Genetic studies in Norway*

The surgical treatment of oral clefts in Norway has been centralized to two plastic surgery departments that have had well-organized data sets on their cleft patients since the early 1960's. Since 1967, it has been compulsory to register all births and congenital defects in the Medical Birth Registry of Norway (MBRN). A comprehensive classification of the cleft cases was required in order to make better use of the information in these data sets for clinical studies and epidemiological and genetic research. We worked out a modification of Kernahan's striped-Y diagram and of Schwartz's three-digit numerical coding in order to classify the morphological variation in oral clefts based on nine anatomical focal areas in the primary and secondary palate, anterior and posterior to the incisive foramen. The classification is well suited to providing a population-based reference for 63 common and rare cleft variants and to characterizing the three commonly used categories of clefts (CLO cleft lip only, CLP cleft lip and palate, CPO cleft palate only). This classification is the basis for all our papers *I-IV*. When we present the ascertainment of oral clefts in the MBRN and when estimating the familial risk of cleft recurrence we relate our analyses to this classification. Moreover, we demonstrate the use of molecular genetic analyses to characterize two subgroups of cases. Virtually all patients operated for oral clefts in Norway from 1967 to 1998 (3616 cases) are included in our clinical data.

The distribution of 63 subgroups of clefts in the population (1.9 million births) as a whole is presented, and stratified by the baby's sex and the presence of accompanying malformations (*Paper I*). Clefts of the primary or secondary palate were more severe when both types of clefts are present. The more severe the cleft lip, the more likely that the baby had an accompanying cleft palate. Girls were more likely to have severe clefts, as were cases that had other birth defects. Although cleft lip was more frequent on the left side, clefts were not more severe on the left side.

Based on the completeness of our clinical data we calculated the proportion of clinically verified cases reported to the MBRN (*Paper II*). We described the morphological characteristics of the reported and non-reported cases. The registration was far better when cleft palate was accompanied by a cleft lip. 83% of CLO-cases, 94% of CLP-cases and 57% of CPO-cases were reported to the Registry. The ascertainment was also clearly related to the

severity of the defect. The substantially reduced reporting for mild clefts is essential to understanding the reliability and use of registry data for disease surveillance and research. The large proportion of cleft palate cases not reported indicates that better routines are required for the detection of cleft palate at the neonatal clinical examination.

We also explored whether cleft cases with severe cleft morphology have a higher risk of familial recurrence than the mild cases (*Paper III*). A linkage of clinical data and data from the MBRN (1967 to 2001) allowed us to combine high-quality clinical information on cleft morphology with virtually-complete ascertainment of biological family members for the whole country over a 35-year period. Index cleft cases with non-cleft birth defects were not included when we estimated the recurrence risk of clefts from parent to child and in full-sibships. We found no higher risk of clefts in children of affected mothers than in children of affected fathers. This indicates no major effect of maternal genes and the absence of sex-specific genomic imprinting. The overall risk of clefts was similar in sibships and from parent to child and so was cleft type specificity in the recurrence for CLO, CLP and CPO. The recurrence risks of cleft lip cases were similar regardless of whether cleft palate was present. The recurrence risk of CPO was 56-fold and significantly different from the recurrence risk of CL (31-fold). We found no effect of cleft severity on recurrence risk. The multifactorial threshold model as a model of inheritance of oral clefts is questioned by these findings and indicates that severity is independent of genes predisposing for oral clefting.

As a demonstration of molecular characterization of clefts we studied the prevalence of duplications and deletions in the 22q11.2 region among newborns with open cleft palate without cleft lip, two of the subgroups within the CPO category (*Paper IV*). We selected the study cases (191 cases) from a larger population-based case-control study of oral clefts (573 cases) in Norway (1996-2001). DNA was available from 174 cases and the DNA copy number was analyzed using multiplex ligation-dependent probe amplification technique (MLPA). We found no 22q11.2 duplications and three cases with 22q11.2 deletions, corresponding to a prevalence of 1.8 % (1 of 57). All three del22q11-syndrome cases also had congenital heart defects. They represent one-third of the ten babies with congenital heart malformations in our study population. We conclude that neither del22q11 nor dup22q11 testing is warranted in babies when open cleft palate is the only indication.

BACKGROUND TO THE STUDY

ORAL CLEFTS

Cleft lip and palate is among the most common congenital malformations of the head and neck. The birth prevalence in Norway is about 2 per 1000 live births, which represents one of the highest rates of clefts in the Western world (Abyholm 1978; ICBD 1998). About 120 newborns with oral clefts are referred for surgical treatment in Norway every year. It is a heterogenous group of birth defects with regard to both morphology and etiology. A cleft can involve the upper lip alone or it can extend to involve the alveolar ridge and the palate. A cleft in the palate can be submucous or be more extensive, including both the soft and hard palate. A combination of cleft lip and cleft palate is the most common clinical manifestation.

Embryology

Oral cleft arises when the normal medial growth and fusion of the facial swellings is disturbed during the early weeks after conception (Sperber 2002; Sperber 2002).

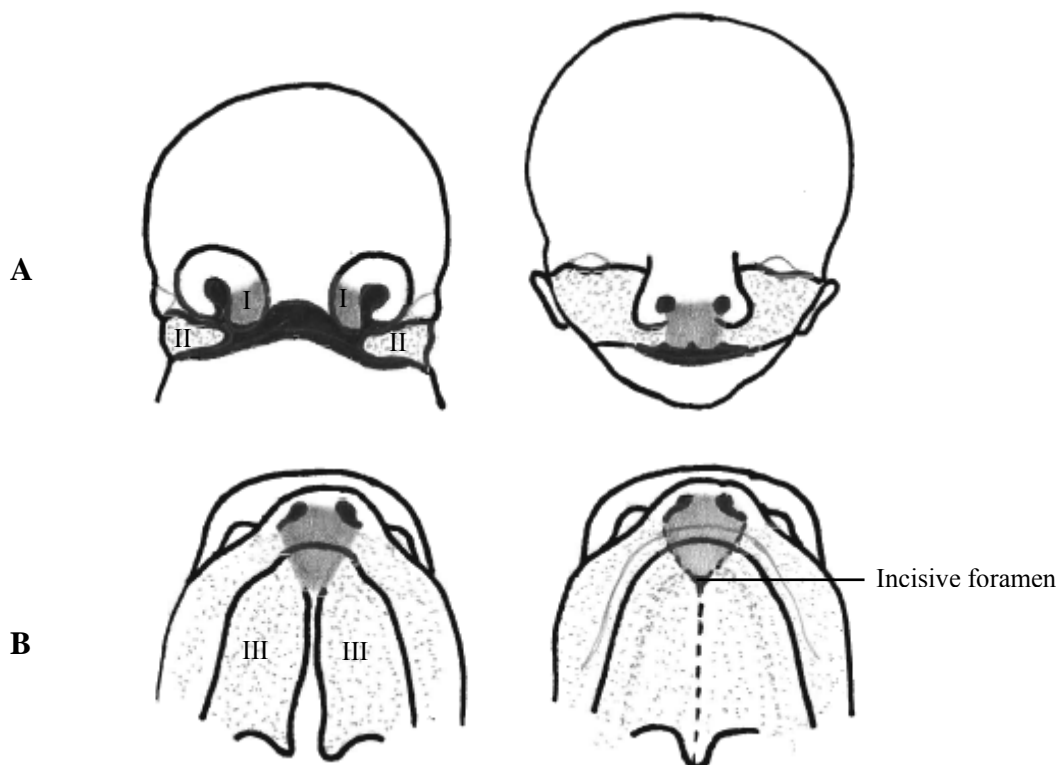


Figure 1. A, The medial nasal swellings (I) fuse with the maxillary swellings (II) to form the primary palate (lip, alveolus, hard palate anterior to the incisive foramen). B, The palatine shelves (III) of the maxillary swellings fuse to form the secondary palate (posterior to the incisive foramen). Drawn after illustrations by J.Leland (Langman 1981).

Disturbances in the development of the primary palate between the fifth and seventh gestational week can result in cleft lip, alveolus and hard palate anterior to the incisive foramen (Figure 1). The fusion of the shelves of the secondary palate forms the roof of the mouth and the nasal floor between the seventh and twelfth gestational week. Failures in this development can result in cleft of varying extent in the palate posterior to the incisive foramen (Figure 1).

Cleft of the primary palate is commonly located laterally in the lip, alveolus and hard palate, while clefting of the secondary palate is always in the midline. Clefts of the primary palate are unilateral or bilateral, located on the right and / or left side (Figure 2).

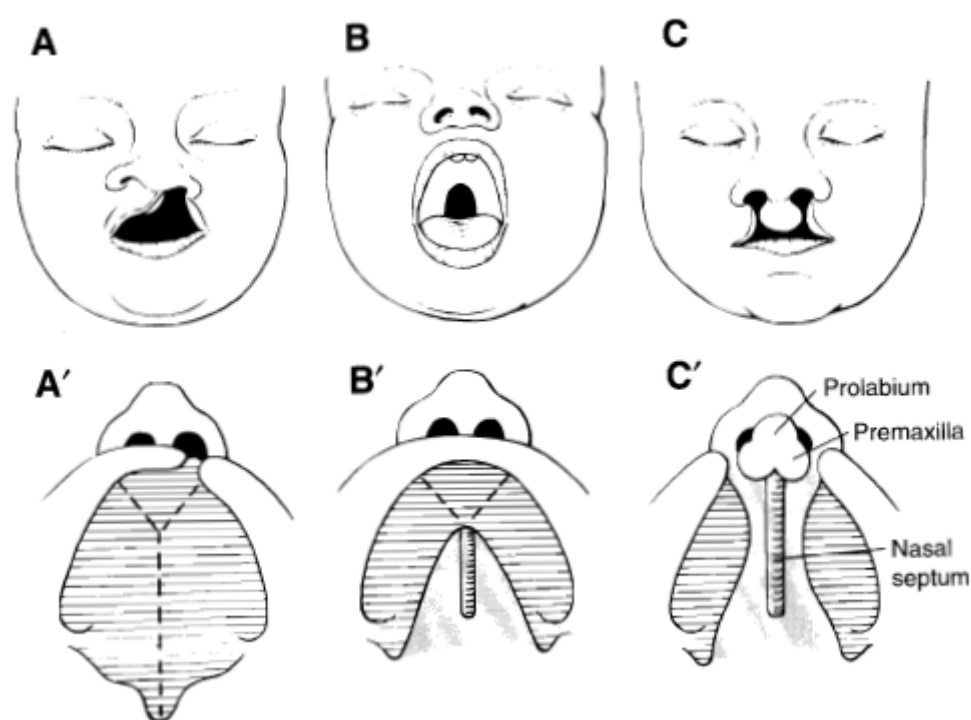


Figure 2. A and A', unilateral left-sided cleft lip. B and B', cleft palate only. C and C' bilateral cleft lip and palate. Source: (O'Rahilly and Muller 1992)

A complex trait

Understanding of the underlying causes of oral clefting is still fragmentary. The etiology is thought to be multifactorial in nature, including genes, environmental factors and their interaction effects.

Genetic research has identified several genetic aberrations that can cause clefting (reviewed in (Jugessur and Murray 2005)). However, the genetic background of the vast majority of cases is unexplained. The Online Mendelian Inheritance in Man database

(www.ncbi.nlm.nih.gov/Omim/) lists over 430 Mendelian disorders associated with oral clefts. The majority of oral clefts, however, appear without accompanying birth defects or other signs of malformation syndromes. Most couples who have a newborn child with oral cleft are unfamiliar with the condition, and they do not know of any relatives with clefting.

An indication of a strong genetic influence is the observation that a mother or father with cleft lip has a thirty-fold higher risk of the same condition in their child, than a parent without clefting. The fact that, in twins with oral clefting, concordance is far greater for monozygotic twins (35-40%) than dizygotic twins (4-8%) (Gorlin, Cervenka et al. 1971) also strongly supports a major genetic component. Concurrently, the modulation of the genetic expression is considerable; otherwise the concordance in monozygotic twins would be expected to be even higher. Some degree of heterogeneity *in utero* environment or genetic non-penetrance might underlie this discordance in monozygotic twins (Murray 2002).

Major environmental risk factors have been difficult to detect. This might be due to a genetic modulation of the effects of exposures during pregnancy, either through the fetus or the mother. Several rare exposures, such as certain medications against epilepsy (American Academy of Pediatrics Committee on Drugs 1979) and severe acne (Rosa, Wilk et al. 1986), are associated with increased risk of oral clefts. Among the more common exposures associated with increased risk of clefts, cigarette smoking is the most consistent across studies (Wyszynski, Duffy et al. 1997; Christensen, Olsen et al. 1999). On the other hand, there are a growing number of studies that have documented the protective effect of folic acid supplementation on the risk of clefts (Wilcox, Lie et al. 2007).

Individuals without cleft features may carry genes that are associated with increased risk of oral clefts in more susceptible individuals. This reflects the possibility that the expression of genes may be modulated by other genes in the fetus or the mother, or through certain environmental exposures during pregnancy. In order to understand the variation in liability of clefting, current research is directed towards identifying genetic aberrations in multiple genes, and the likely interactions between these genes and the environment.

Outline of treatment procedures

The multidisciplinary approach taken in modern treatment involves plastic surgeons, orthodontists, speech therapists and otolaryngologists. Specialists in medical genetics,

psychology/psychiatry, oral and maxillofacial surgery and prosthodontics may also be involved.

The strategy of cleft surgery is to achieve “maximum results with minimal surgery”, which means that the surgeons focus on the negative effect on growth of scarring resulting from surgery. In order to optimize growth conditions, plastic surgery applies the important principles of handling tissue gently and limiting the number of operations.

Most newborns with oral clefts have problems achieving negative oral pressure, and the mothers need help to optimize feeding conditions. The arrangement of treatment, the timing of and decision on the best surgical approach depend on the child’s cleft phenotype (see Treatment protocol, Bergen in Appendix). Shortly after birth, a long-term treatment plan is worked out for the baby by the cleft team members. Children with cleft lip and palate will have a cleft lip closure operation at the age of three months and palate closure at about 12 months. Speech therapy is given at an early stage in order to avoid negative speech patterns. Orthodontic treatment and an operation for secondary bone tissue transfer to the alveolar cleft (at the age between 7 and 11 years) has made non-prosthodontic rehabilitation possible in cleft lip and palate patients. Some patients need reconstruction of the sulcus or a correction of the lip prior to bone grafting. Patients with cleft palate are vulnerable to unremitting otitis media that requires ventilation tube insertion.

Whether further surgery is required depends on the patient’s functional result. Hyper nasality due to velopharyngeal insufficiency may need surgical correction during early school-age. Poor symmetry of the nose or poor projection of the midface may require surgery. It is preferable to postpone such major corrections until the end of adolescence in order not to disturb the growth potential of the tissue.

Burdens in childhood and adolescence

In Norway, 4.6% of all children born in 2002 (live, stillborn, and terminated pregnancies) had major or minor birth defects registered in the Medical Birth Registry of Norway (MBRN) (MBRN 2004).

The list below presents the prevalence of a few birth defects and chronic medical conditions of particular interest to plastic surgeons (Table 1). The literature references also include children who have the condition diagnosed later in childhood.

Condition	Prevalence (per 1000 live born)	References
Oral cleft	2	(Abyholm 1978)
Rare orofacial clefts (Median, Oblique, Lateral)	<0.02	(Gorlin, Cohen et al. 2001; Eppley, van Aalst et al. 2005)
Hemifacial microsomia	0.2	(Gorlin, Cohen et al. 2001)
Craniosynostosis	0.4	(Gorlin, Cohen et al. 2001)
Congenital upper limb anomalies	2	(Giele, Giele et al. 2001)
Hypospadia (treated)	3 (boys)	(Abdullah, Pearce et al. 2006)
Clubfoot	1.2	(Krogsgaard, Jensen et al. 2006)
Spina bifida	0.3	(Nikkila, Rydhstrom et al. 2006)
Congenital heart disease	12 (0-18 years)	(Marelli, Mackie et al. 2007)
Developmental dislocation of hip (treated)	6	(Duppe and Danielsson 2002)
Profund permanent hearing impairment	0.4 (0-5 years)	(Parving and Stephens 1997)
Diabetes mellitus, insulin dependent	0.1 (1-5 years)	(Wadsworth, Shield et al. 1995)
Combination of asthma, eczema and rhinitis	52 (6-7 years)	(Shamssain and Shamsian 1999)
Epilepsy	3.4 (0-16 years)	(Larsson and Eeg-Olofsson 2006)

Table 1. Prevalence of a few birth defects and chronic diseases in childhood and adolescence.

The total prevalence of congenital malformations reported (including live births, stillbirths and terminations of pregnancy after prenatal diagnosis) has increased during the last 25 years

(EUROCAT 2002). The background rate of major birth defects in the MBRN has increased from 2.1 percent in 1967-1982 to 2.5 percent in 1983-1997, most likely because of improved survival, better diagnostics and improved ascertainment (Skjaerven, Wilcox et al. 1999). The age at diagnosis of many internal anomalies, such as certain cardiac and urinary system anomalies, has been brought forward to the prenatal or early postnatal period. These anomalies therefore end up being included in the information system of registries. Another likely reason for the increased prevalence of congenital malformations is because prenatal diagnosis followed by termination of pregnancy brings cases of congenital anomaly into the information system that would not otherwise have been diagnosed or reported among spontaneous abortions (Dolk 2005). The European birth defect monitoring system, EUROCAT, reported no significant time trend in the prevalence of oral clefts for the period 1980-1998 in 30 European Regions (EUROCAT 2002).

Consequences for individuals with oral cleft

Nowadays, children with oral clefts in Norway undergo a well-organized system of treatment. Surgical treatment and the handling of patients have improved dramatically during the course of the last century (Sæther 1996). The improvement of anesthetics (Jones 1971), surgical procedures and handling of infections have transformed palate closure from being a life-threatening procedure to one associated with very low mortality rates.

Still, the burden of being born with an oral cleft is considerable. Affected children have to undergo several surgical interventions that can bring substantial distress and pain into their lives. Parents, too, have to cope with additional worries from the time of birth, including feeding problems, concerns regarding facial appearance and speech articulation of their baby, as well as unavoidable reactions from other people. Many mothers blame themselves and reflect extensively on what they might have done wrong, especially during the first trimester of pregnancy. In spite of being in this vulnerable situation, the family is suddenly expected to relate to medical personnel and adjust to hospital routines for treatment. The children experience a significant number of psychosocial risks, including multiple medical appointments and evaluations, which may result in stigmatization. It is particularly upsetting when a defect happens to be in the middle of the face, where it is inevitably visible to everybody. Both the parents and the child need to be able to cope with this and not be overwhelmed by reactions in their surroundings.

Most individuals with cleft lip and palate are ultimately successful in coping with these difficulties. However, in a Norwegian study conducted in the 1980's increased frequencies of anxiety and depression were found among adult cleft cases. These symptoms were strongly correlated with concern about facial appearance, speech and the wish for further treatment (Ramstad, Ottem et al. 1995). In a recent study of psychosocial functioning in children and young adults, participants with clefts reported significantly more behavioral problems, symptoms of depression and more problems of being teased. No significant difference was reported in terms of anxiety and self-esteem (Hunt, Burden et al. 2006).

Children with clefts are at risk of social withdrawal and reduced ability to make friends. In adult life, facial attractiveness and speech articulation might influence the choice of partner, as well as marriage and employment. In the above-mentioned Norwegian study, individuals with clefts were slightly better educated but had lower income than the controls. They were less likely to be married, and those who did marry were older and had fewer children than the controls (Ramstad, Ottem et al. 1995). In data from the MBRN, males with clefts were less likely to reproduce than females with oral clefts. The females with clefts did not differ significantly in their chance of childbearing than women without clefting (Skjaerven, Wilcox et al. 1999; Lie, Wilcox et al. 2001).

In Denmark, oral clefts have been found to be associated with an increased risk of hospitalization due to mental illness, increased substance abuse and increased mortality from suicide (Christensen and Mortensen 2002; Christensen, Juel et al. 2004). Whether this is specifically due to social or biologic factors is not known. These findings emphasize the need for special follow-up of children with oral clefts.

Surgical treatment of cleft has improved over time and resulted in better functional and cosmetic results. It will be interesting to see whether this will improve the psycho-social conditions for children with clefts. At the same time, the focus on looks and attractiveness is particularly strong in modern society. The desire to be perfect is reflected, for example, in the extensiveness of cosmetic plastic surgery.

The different outcomes after treatment have been, and still are, the greatest concern to professionals involved in the treatment of patients with oral clefts. One of the main challenges is to identify patients who are more prone to poor cosmetic and functional results. The identification of factors predicting the cosmetic and functional outcome of surgery is central to clinical follow-up and to studies evaluating treatment methods and results. It is a

fundamental principle that the patient's preoperative cleft morphology and her / his other medical conditions are all related to the final outcome of the treatment.

EPIDEMIOLOGICAL STUDIES OF ORAL CLEFTS

Although oral clefts are easily diagnosed and described compared to many other birth defects, the methodological issues in epidemiological studies of clefts are still riddled with challenges.

Subdivision of oral clefts

A main challenge in studies of oral clefts is to achieve an adequate number of cases to attain statistical power to detect real differences. The commonly used subdivision into cleft lip with or without cleft palate (CL) and cleft palate without cleft lip (cleft palate only, CPO) makes even greater demand on the number of cases. Splitting of clefts into CL and CPO is mainly motivated by genetic and embryologic findings. CL and CPO rarely segregate in the same family (Fogh-Andersen 1942), and the formation of the lip and palate appear to be developmentally and temporally distinct processes (Sperber 2002; Sperber 2002).

A further subdivision into syndromic and non-syndromic cases is commonly used. This subdivision is based on the observation that a substantial proportion of syndromic cases is associated with specific genetic mutations. Other subdivisions have been conducted in studies that regard bilateral CL as a more severe form than unilateral CL and in studies that suggest CPO to consist of two etiologically different subtypes: clefts affecting the soft palate and clefts affecting both the hard and soft palate (Christensen and Fogh-Andersen 1994; Clementi, Tenconi et al. 1997).

Risk of oral clefts

In epidemiology, incidence and prevalence are two different measures of disease risk. The prevalence at birth, or the proportion of newborns that have an oral cleft, is the most accepted way of reporting the frequency of clefts (Rothman and Greenland 1998). The term "at birth" needs to be more precisely defined, especially with regard to whether or not stillborns and abortions are included in the term. The incidence of clefts is not obtainable, because it is not

possible to ascertain all conceptuses that occur or the conceptuses that survive until lip and palate formation are completed in the first trimester of pregnancy.

Most countries in the Western world have experienced an overall improvement in their standard of living during the 20th century. Since genes change slowly with time, temporal trends in disease prevalence are a strong indication of the presence of environmental determinants (Khoury, Beaty et al. 1993). In Norway and Denmark, the prevalence at birth of oral cleft has been fairly constant in the past decades (Bille, Knudsen et al. 2005; Harville, Wilcox et al. 2005). In Denmark, an increase in the prevalence of clefts was reported during the period 1936 through 1961. This was explained by improved survival among newborns, especially those with associated malformations, and a better ascertainment of syndromic and small clefts. The same study reported a nearly constant prevalence of the most extensive clefts (bilateral CL and CPO including the hard palate) over a period of 52 years (Christensen 1999).

Geographic origin, ethnic background and socio-economic status are some of the factors that may account for the wide variability seen in clefting rates (Vanderas 1987; Murray, Daack-Hirsch et al. 1997; Croen, Shaw et al. 1998; Tolarova and Cervenka 1998). Native Americans have one of the highest birth prevalences of CL (3.6/1000 births) followed by Asians (1.7/1000 in Chinese, 2.1/1000 in Japanese births) and African-derived populations (0.3/1000 births) (18). Caucasian populations have birth prevalence of CL around 1/1000 while the rates in Scandinavian countries tends to be higher (Mossey and Little 2002; WHO 2002). Norway and Denmark have CL rates of around 1.5/1000 (Bille, Knudsen et al. 2005; Harville, Wilcox et al. 2005). The prevalence of CPO, however, shows less variation by race and ethnicity (Abyholm 1978; Vanderas 1987; Gorlin, Cohen et al. 2001; Mossey and Little 2002).

Accompanying defects

The range of reported frequencies of accompanying defects in clefting is typically wide (CL 2%-30% and CPO 10-50%). This might be due to differences in the definition of accompanying defects, how long after birth and how carefully babies are examined, and the selection of cleft patients (Fraser 1970; Shprintzen, Siegel-Sadewitz et al. 1985). Cleft patients referred for treatment have been found to have a lower frequency of accompanying defects than non-operated cases. A possible explanation is that the non-operated cases are

mainly stillbirths and infants who die before the time of operation, and they are known to be more often combined with congenital malformations (Christensen, Holm et al. 1992).

It is considerably more difficult to make a uniform ascertainment and classification of minor and more subtle associated anomalies. Christensen uses the 22q11-deletion syndrome as an illustration of this problem; many patients with 22q11 deletion syndrome and CPO have the deletion 22q11 undiagnosed because they often display moderate symptoms: moderate learning disabilities, moderate speech-problems and variable insufficiency in different internal organs (heart, thymus, kidneys). The inclusion of cases with 22q11 deletion syndrome in a study of non-syndromic (isolated) CPO could reduce the power of the study if the risk factors for CPO in this syndrome are different from the risk factors for CPO without accompanying defects (Christensen 2002).

A consistent finding is that CPO is more frequently associated with other defects than CL (Gorlin, Cohen et al. 2001). Laterality also appears to be an important consideration: more malformations have been found in infants with bilateral than with unilateral CL (Milerad, Larson et al. 1997).

Attempts have been made to catalogue the type of anomalies expressed along with oral clefts (Shprintzen, Siegel-Sadewitz et al. 1985; Cohen and Bankier 1991). Congenital heart anomalies and neural tube defects have been found more frequently associated with oral clefts cases than would have been expected by chance alone. Other possible associated anomalies include urinary tract anomalies, esophageal atresia, vertebral anomalies and short stature (Gorlin, Cohen et al. 2001; Cohen 2002).

Sources of ascertainment

A variety of sources of ascertainment are available for epidemiological studies of oral clefts, including population-based clinical records, medical records from clinical centers (surgery, orthodontic treatment, and speech therapy), registries of congenital malformations, birth certificates and death certificates. All sources are subject to bias, some perhaps more than others. The efficiency of record systems greatly depends on local practice and organization. Hospital registries often depend on one or a few enthusiastic clinicians. The evaluation of different ascertainment sources is dependent on highly reliable identification of individuals. In Norway, the National Population Registry was set up from 1964 to 1966 and the 11-digit

national identity number was introduced as mandatory identification. This is a unique individual identification number, which includes the date of birth and sex of the individual. Surgical files have played a major role in oral cleft research (Christensen 2002). Surgical files do not, for example, include stillborns and children dead before referral, and they therefore give an underestimated basis for prevalence at birth. The completeness of various ascertainment sources was studied in Denmark for the period 1983-87 (Christensen, Holm et al. 1992). Based on the comparison of three nationwide ascertainment resources (not birth certificates) and a smaller autopsy study, they found that 95% ascertainment was obtained by means of surgical files for CL without accompanying defects. However, surgical files included only 60% of the 232 CPO cases ascertained in one of the other resources. Forty six of the 97 unoperated cases that were not included in the surgical files had submucous cleft palate, and 24 were stillborns or newborns that died before the time of surgery.

Cleft treatment centers draw their cases from different populations that are often not well defined. In Norway, the national health authorities have authorized two centers to treat all cleft patients in the country. In other countries without centralized treatment, the specialized centers would attract the more severe and complicated cases and thereby introduce an ascertainment bias (Fraser 1970; Shprintzen, Siegel-Sadewitz et al. 1985).

The international practice of birth certificate recording is highly variable. Most countries in the Western world have compulsory registration of newborns. Different variables are registered, however, and some registries include birth defect registration, whereas others do not. Birth defect registration in MBRN can be viewed as a specialized type of birth certificate-based system, where all births, not only those with birth defects, are registered. Some other countries have separated birth certificate recording and the surveillance of congenital birth defects into two separate registrations made at different times. Others surveillance systems collect data from several sources and conduct active “case-finding” on the basis of specific indications. The problem associated with systems that utilize multiple sources of information is both the increased cost and the increased time lag from birth to surveillance (Kallen, Hay et al. 1984; Lie, Irgens et al. 1992).

CLINICAL CLASSIFICATION OF ORAL CLEFTS

The clinical classifications of oral clefts have largely been based on anatomical and embryological considerations and they have become accepted for their practical surgical

value. An early and precise anatomical diagnosis is clearly advantageous to the surgeon in order to decide the best approach and timing for the surgical treatment. An accurate morphological assessment of the cleft in the newborn is a prerequisite for planning the long-term treatment of the cleft. Other medical conditions that are not directly related to the cleft have not been included in existing clinical classifications of oral clefts. The coexistence of oral cleft with other birth defects or medical conditions that are thought to share a common etiology is referred to as a syndrome.

The changes in clinical classification of oral clefts reflect both increased knowledge of embryology and the historical development of the treatment of clefts. The examples of classification systems given below have been important to the clinical treatment of clefts during the 20th century. The first generally accepted classification was developed by Davis and Ritchie in 1922 (Davis and Ritchie 1922). This is a three-group classification of clefts, with the alveolus as the demarcation point between cleft lip and cleft palate, as opposed to today's incisive foramen

- I Cleft lip (unilateral, median or bilateral)
- II Cleft palate (soft palate, hard palate)
- III Cleft lip and palate (unilateral, median and bilateral)

As the surgical techniques of cleft palate closure improved, it made greater sense to focus on the importance of a better functioning soft palate and an adequate velopharyngeal space to ensure adequate speech. Veau wrote a classic treatise on V-Y cleft palate closure in 1930, and, in the following year, he proposed a four-group classification of clefts (Veau 1931; Millard 1977):

- I Cleft of the soft palate
- II Cleft of the soft and hard palate
- III Unilateral complete cleft of the alveolus, hard and soft palate
- IV Bilateral complete clefts of the alveolus, hard and soft palate

The fact that Veau's classification ignored clefts of the lip and alveolus fully illustrates the problem that clinical classifications are often limited or focused on the special interests of the clinician.

Fogh-Andersen differentiated between three cleft types (Fogh-Andersen 1942):

I Cleft lip (unilateral or bilateral), “as far as the incisive foramen”

II Cleft lip and palate (unilateral or bilateral)

III Cleft palate (submucous, soft or hard) “never further than the incisive foramen”

He argued that a more detailed classification was impossible to use for the classification of cases that had had their first operation elsewhere. Fogh-Andersen’s observations of a distinction between clefts anterior and posterior to the incisive foramen agreed remarkably well with later studies of embryological development.

In 1958, Kernahan and Stark (Kernahan and Stark 1958) based their classification on the early studies of the movements and closure of the soft tissue palatal shelves during the relevant embryological periods (Stark 1954; Stark and Ehrmann 1958) (Figure 1 and 2). This classification emphasized the embryological basis of the incisive foramen as the boundary marker between the anterior and posterior fusion processes in the primary and secondary palate. A further description of the cleft, such as left or right-sided and complete or incomplete made this classification in reality identical to Fogh-Andersen’s classification. At its congress in 1967 the International Confederation for Plastic and Reconstructive Surgery established a classification that combined Fogh-Andersen’s and Kernahan & Stark’s classifications and terminologies (Millard 1977):

Group 1. Clefts of the primary palate

a. Lip

b. Alveolus

Group 2. Clefts of the primary and secondary palate

a. Lip

b. Alveolus

c. Hard palate (secondary palate)

Group 3. Clefts of the Secondary palate

a. Hard palate

b. Soft palate

The popularity of this classification was later reduced because lip was not mentioned in the terminology (Millard 1977).

In 1971, Kernahan introduced the striped-Y diagram (Kernahan 1971).

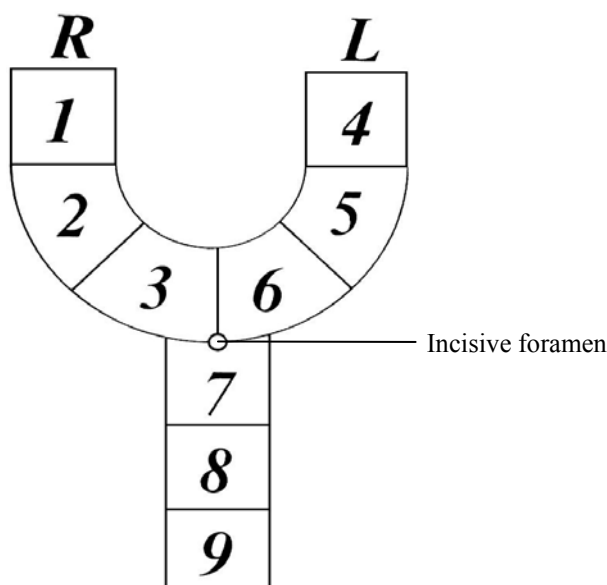


Figure 3. Kernahan's striped-Y diagram (Kernahan 1971)

Kernahan pointed out the problem of using too wordy descriptions of cleft morphology in the extensive medical records of the cleft patient. In daily clinical practice, this increased the amount of time spent on obtaining histories and delayed proper identification of the specific type of cleft. By introducing the Y-diagram, the morphological description was more uniform and comprehensive and this facilitated communication between cleft team members. The right (R) and left (L) limbs of the Y were divided into three sections: the anterior portion = lip (1 and 4), the middle = alveolus (2 and 5) and the posterior = the area of the hard palate from the alveolus back to the incisive foramen (3 and 6). The palate posterior to the incisive foramen is divided into three sections: the hard (7 and 8) and soft (9) palate. The cleft area of each patient was indicated by stippling the respective segment and was used as an initial visual record of the primary diagnosis in every patient. This symbolic classification system allows members of the cleft team to quickly assess the nature of the deformity. Many cleft treatment centers all over the world were attracted to this neat, symbolic, and highly intuitive visual rendering of the cleft. By the use of additional symbols in the segments, surgeons could add information and adapt the method to their own needs.

Along with improved surgical techniques and results, more detailed and complex striped-Y diagrams developed (Elsahy 1973; Millard 1977; Smith, Khoo et al. 1998). Clinically

interesting morphological characteristics, such as the amount of premaxillary protrusion and rotation, associated nasal deformities, and the presence of velopharyngeal incompetence were given additional segments and numbers in these later classifications.

The changes in clinical classifications have often followed the introduction of new principles of treatment, motivated by the clinicians' awareness that certain cleft morphology has a better or poorer outcome of treatment. The basic accuracy and simplicity of the original striped-Y, together with its being adaptable to surgeons' individual preferences, have made the striped-Y model a long-lived and well-known classification method (Millard 1977).

In clinical practice, numerous classification systems have been used and no unique system has been universally accepted. The complexity of nomenclature and confusing terminology have been the major arguments against several of the classifications. Most of the above-mentioned classification systems have been used in clinical studies. In epidemiological studies however, Fogh-Andersen's classification is the most frequently used system.

Epidemiological and clinical studies are dependent on the number of study cases if they are to detect real differences. Given a prevalence of two newborns with oral clefts per 1000 live births, the recruitment of study cases needs to proceed over many years and through collaboration between treatment centers in order to achieve large enough numbers. Having an ethnically homogeneous population is advantageous with regard to minimizing the effects of population substructure. To describe the characteristics in subgroups and detect real differences, an even larger number of cases is required.

The clinical classification systems are very well-suited to individual patient recording but poorly suited to computerized data entry and data processing of many patients. The "RPL" (Right lip, Palate, Left lip) numerical coding of the visualized data in the original striped-Y overcame these difficulties. The RPL system comprised an accurate and systematic numerical recording of the Y-diagram, that was well-suited to computerization (Schwartz, Kapala et al. 1993).

IMPLICATIONS OF EPIDEMIOLOGICAL STUDIES

Descriptive and genetic epidemiological studies of oral clefts during the last decades form the basis for current research into the complex interplay between multiple genes and environmental factors that lead to the development of clefts. Searches for genetic

predisposition to oral clefting have resulted in a long list of candidate genes that are potentially involved in the embryologic development of oral clefts (Jugessur and Murray 2005). The Pregnancy Heredity and Environment -project conducted in Norway from 1996 to 2001 was designed to participate in the worldwide genetic mapping of potential genes implicated in oral clefts and their interaction with environmental factors.

Special knowledge of descriptive characteristics, such as cleft phenotypes, their prevalence in the population and their distribution in the sexes, is needed in order to conduct genetic studies. Molecular analyses may also be used to describe the characteristics in subgroups of clefts.

We decided to perform a detailed mapping of the heterogeneous cleft morphology in a large population-based sample. When genes or loci linked to the development of clefts are identified, knowledge of their prevalence in the general population and in cleft patients is mandatory for further research and clinical use. As the genetic background of oral clefts gradually becomes clearer, it will be necessary to try to connect genes and cleft phenotypes. Some genes and loci might be more involved in certain cleft types. The relationship between specific genes and cleft phenotypes might not be possible to unravel unless there is a way to clearly dissect and define the observed cleft morphology.

AIMS OF THE STUDY

An overall motivation has been to use the extensive morphological documentation of oral clefts during thirty years of clinical practice in Norway, for the benefit of research and cleft management. We wanted to use the morphological heterogeneity within the commonly used categories of clefts, Cleft lip only (CLO), Cleft lip and palate (CLP) and Cleft palate only (CPO) to explore characteristics of these categories.

Virtually all patients with oral clefts, 3616 patients treated in Norway from 1967 to 1998, were linked to the MBRN in order to:

- 1) give a population-based prevalence of major morphological characteristics in an ethnically homogenous cleft population
- 2) evaluate the ascertainment of the cleft registration in the MBRN

All patients with oral clefts treated in Norway from 1967 to 2001 (4138 cases) were linked to the MBRN (2.1 million births) in order to:

- 3) estimate the familial risk of cleft recurrence based on the extent of clefting in parents and first-registered siblings.

We selected a subgroup of cleft palate, cases with overt cleft palate (191 cases), from a larger population-based case-control study of oral clefts (587 cases) in Norway (1996-2001) in order to:

- 4) estimate the prevalence of a 22q11.2 deletion or a 22q11.2 duplication in individuals with open cleft palate.

MATERIALS AND METHODS

Clinical data from the departments of plastic surgery at Haukeland University Hospital and Rikshospitalet University Hospital are the main data source in *Papers I, II and III* together with data from the MBRN.

The work in *Paper IV* draws upon the resources from the project “Pregnancy, Heredity and Environment”, which is a population-based, case-control study of all Norwegian babies with oral clefts born live from 1996 to 2001.

STUDY DESIGN AND STUDY POPULATION

All four papers in this thesis are based on data for patients with oral cleft treated in the Departments of Plastic Surgery at Haukeland University Hospital and Rikshospitalet University Hospital. All four studies are population-based in that the clinical data are linked with all live births and stillbirths registered in the MBRN.

In *Paper I*, we focus on the heterogenic morphology in oral clefts in a population-based descriptive study of 3616 cleft cases referred for surgery from 1967 to 1998. The linking of the clinical data to 1.869.382 births in the MBRN adds information about accompanying defects to the cases and gives a population-based prevalence at birth of the different cleft types.

In *Paper II*, we evaluate the ascertainment of oral cleft registration in the MBRN. The study population is the same as in *Paper I*. We use the clinical diagnosis as the answer and evaluate the proportion of clinical CLO cases that is registered in the MBRN (ICD-8) as either CLO or CLP, the proportion of clinical CLP cases that is registered as CLP, and the proportion of clinical CPO cases that is registered as CPO. Clinical cases that were coded differently from this in the MBRN were not included in the study.

Paper III is a population-based historical cohort study of 4138 cleft cases surgically treated from 1967-2001 and all non-affected babies from the same period. Using these data we estimate the familial risk of recurrence. The analytical files consist of a “sibship-file” and a “generation-file” of data from the MBRN linked with the clinical data. The “sibship-file” takes the mother and her successive births (1967-2001) as the unit of analysis. 572.772 children in the file have younger siblings and all together they have a total of 804.286 younger siblings. In the “generation-file”, the newborn and her/his mother and father is the unit of

analysis. 944.908 children born from 1967 to 1983 later become parents to 703.131 children registered in the MBRN until 2001. We extended the period of study by 3 years compared to *Papers I and II*, from 1998 to 2001, in order to include as many newborns as possible of parents who had their own birth registered in the MBRN (born after 1966).

In *Paper IV*, we study the population-based prevalence of 22q11.2 deletion and 22q11.2 duplications in newborn children with open cleft palate. 191 cases with overt cleft palate from a large population-based case-control study of newborns with oral clefts were analyzed. The case-control study, which was conducted in Norway from 1996 to 2001, enrolled 573 of 676 newborns with cleft. The controls were not used in this study.

THE CLINICAL DATA

Virtually all patients treated for an oral cleft birth defect in Norway from 1967 to 2001 are included in the data from Haukeland University Hospital and Rikshospitalet University Hospital. The two cleft teams have collaborated closely in treatment and follow-up. When sketching the history of cleft-treatment, I mainly refer to the surgical treatment because the surgeons' documentation forms the basis for the clinical data in this thesis. I mainly refer to the treatment in Bergen because the team in Oslo has its history documented in several publications and because of my own attachment to the plastic surgery department in Bergen.

Cleft treatment in Norway over more than 30 years

The two pioneers of Norwegian plastic surgery, Halfdan Schjelderup and Wilhelm Loenneken were trained in England and returned to Norway in 1948. Mr. Schjelderup settled in Bergen and Mr. Loenneken in Oslo and they soon took over the cleft surgery (Sæther 1996). At that time, cleft lip was operated by general surgeons in many hospitals while the alveolar and palatal clefts were left un-operated. A few palate closure operations were also performed, for example by Mr. Herman Gade at Haukeland Hospital. Some patients were given an obturator to reduce nasal air flow but this option was not offered many patients. Making and adjusting an obturator requires advanced technical dentistry and this was not a priority at a time when there was shortage of dentists.

The local school authorities had no obligation to children with speech difficulties until 1951 (Act of 23 November 1951 relating to specialist schools). A year earlier, free dental care was

introduced for school children. In the 1950's teachers and dentists paid more attention to children with oral clefts and enforced better treatment for these children (Sæther 1996). Mr. Schjelderup's precise notes in the medical records of patients with oral clefts can be traced back to his start at the Betanien Hospital in Bergen in 1953. When the third plastic surgeon in Norway, Gunnar E. Johnson, was permanently appointed to the Betanien Hospital in 1964, the number of new patients with oral clefts had reach 30-40 per year. Mr. Johnson had a special interest in cleft surgery, and he implemented the idea of multidisciplinary treatment (Schjelderup and Kvinnsland 1967). Frank Åbyholm, leader of the cleft team in Oslo from 1990, joined the team in Bergen from 1985 to 1990, thus strengthening the interconnection between the two teams. Hallvard Vindenes, with a background of oral and maxillofacial surgery and plastic surgery, joined the cleft team in 1990. A few years later, Paul Egil Gravem was attached to the team. They realized the importance of continuing the close collaboration with the cleft team in Oslo. The benefit of a common system of classification was perceived and, during the 1990's, the two teams completed a retrospective systematic morphological description of the patients dating back to 1967, the year the MBRN was established.

The completeness of the data

Our classification of study cases is mainly based on the detailed morphological description given by the surgeon in the primary operation, in addition to photos and study casts. The completeness of cleft patient registration in Norway over more than 30 years is unique internationally.

Modern cleft treatment is built on knowledge gradually acquired by several professions working together. Norway has taken part in the great international advances in cleft treatment during the last decades. Good quality documentation forms the basis for the multidisciplinary treatment and long-term treatment planning that have been important principles in Norwegian cleft treatment (Bohn 1973; Tindlund 1987). Being a small country that has experienced positive economic development has given our country favorable conditions:

Centralization

The context of a multi-specialty healthcare team was established in Oslo and Bergen during the 1960's, partly on the initiative of national socio-political structures that defined the resources, and partly on the initiative of the dentists, speech therapists, teachers and doctors

involved with the patients. In 1982, the Government formalized centralization of cleft treatment to the clinics in Bergen and Oslo (Sæther 1996). The centralization of cleft treatment in two centers has been important in terms of the clinicians gaining sufficient experience. About 120 children with oral cleft are born in Norway every year. One-third has been treated in Bergen and two-thirds in Oslo.

Centralized treatment has resulted in routine referral of newborn babies with cleft from the maternity unit to the treatment centers. Centralization has probably also reduced the loss to follow-up because the centers established procedures for calling in patients at certain intervals. Co-operation with the local child health center, in order to help families in need of special follow-up, has prevented further drop-out. The formalized centralization in 1982 secured the financial support of the national health authorities and defined the resources needed for this group of patients. In 2005, thirty man-labour years were attached to the cleft team in Bergen.

Before 1982, some patients with oral cleft were operated in three other surgical departments (Bodø, Stavanger and Skien), sometimes by a trained plastic surgeon. When we linked the clinical data for cases operated at Haukeland University Hospital or Rikshospitalet University Hospital during the period 1967-1998 with the MBRN, 639 babies with a cleft diagnosis in the MBRN were not included in the clinical data set. 151 of them were registered in the MBRN as stillborn, 195 as dead during the first year of life and 293 could not be accounted for. Some of them have probably been operated elsewhere in Norway or abroad, and some of them are probably false positives. We wanted to include all cases operated in Norway in our clinical data and also tried to collect data on patients operated in Stavanger, Bodø and Skien. These departments were contacted. All together, 320 cleft-patients had been operated in these three hospitals: 112 patients in Skien, 71 in Bodø and 137 in Stavanger. Because of incomplete documentation of the cleft morphology and the laboriousness of collecting these data, we decided not to include them in the data set. We found, however, that many of the patients had already been referred to Haukeland University Hospital or Rikshospitalet University Hospital in 1982 and therefore already included in the data set.

Travel distances in Norway are great for many patients referred to the cleft teams. Because of the communication along the coast and the traditional trade between Bergen and Northern Norway, most patients living along the coast all the way north to Finnmark have been treated in Bergen. Rikshospitalet University Hospital has recruited the patients from the more densely populated area in South-Eastern Norway. The plastic surgeons in Bergen were very capable to

adjust to patients needs. Twice a year they travelled by costal-steamer, seeing patients in out-patient clinics in several small places along the coast (Tindlund 1995). This modern way of meeting patients was stopped in the 1980's by the local financial authorities in Hordaland County who paid the surgeons' expenses. They thus failed to realize the great benefit this system had in terms of recruiting patients for treatment and follow-up and reduced travel expenses for all patients and their parents.

Economic compensation to families

The reimbursement of families by the Government for all expenses associated with cleft conditions in children was necessary in order to offer adequate treatment to all patients, irrespective of the family's finances and the travel distance involved. Since 1946, the Government has covered expenses for the surgical treatment of congenital defects. The family's travel expenses, loss of income to the parents in connection with hospital stay, and all costs relating to speech therapy, dental and orthodontic treatment were partly covered from 1947 and fully compensated from January 1971 (Sæther 1996). The Norwegian national authorities' have focused on enabling child health centers and schools to help families in connection with their need for special follow-up.

Continuity

The strength of both the Norwegian cleft teams has been their continuity and stability in all professions. The early establishment of cleft teams consisting of permanently employed professionals ensured professional and economic resources for this group of patients (Sæther 1996). Patients with clefts have been a priority in plastic surgery and within the special fields of orthodontic treatment and speech therapy. Since 1967, operations on cleft patients have mainly been restricted to four surgeons in each of the two clinics. The surgical techniques have been thoroughly documented, and the changes in technique relatively few and well-considered and based on consensus among colleges.

Multidisciplinary long-term treatment planning

The final results of surgical treatment in the newborn are not seen until adulthood. The long-term follow-up of cleft patients by the same clinicians who saw the newborn baby has been an important principle in Norwegian cleft treatment (Schjelderup and Kvinnsland 1967; Bohn

1973; Tindlund 1987; Tindlund and Holmefjord 1997). The correct timing of surgery - for example, when not to operate just as much as when to operate - is based on this experience. Systematic multidisciplinary follow-up in out-patient clinics, Clinical Conference Days (Fellesklinikkene), was established in Bergen in 1967 (Tindlund 1987). This system of follow-up depends on a well-organized program involving all cleft team members. All patients with clefts are seen by an orthodontist, speech therapist, plastic surgeon, ear-specialist and psychiatrist by the age of six. The patients and their parents are seen individually by all of the cleft team members during a session. The conclusion drawn from these five different consultations is given to the family after the cleft team members have met a few days later to agree on long-term treatment planning for each patient. The timing of the Clinical Conference Days has changed, but in principle it remains otherwise unchanged since its inception in 1967. Since 1994, all newborns with oral clefts and their parents have been invited to a one-day information course within the first weeks of life, organized by the cleft teams. Depending on the cleft type, as seen in the treatment protocol in Bergen (in Appendix), the procedure of the Clinical Conference Days today is that the children are seen by all cleft team members as newborns, at 6 years, 15 years and 20-30 years of age. The patients' conditions are documented by pictures, study casts, X-rays and written information provided by each of the cleft team members.

Clinical documentation

Clinical documentation is the foundation of multidisciplinary treatment, long-term treatment planning and the development of improved medical treatment. Documentation of cleft morphology in the newborn is important because it is often impossible to recognize the original condition after the primary operation.

An example of the Norwegian contribution to the international specialist environment and development of universally accepted principles in cleft treatment is the pioneering work done by the orthodontist Egil Harvold at the School of Dentistry at the University of Oslo. His work highlighted secondary scarring of traumatized tissue in cleft operations and he was able to show the association between scarring and underdeveloped upper jaw (Harvold 1954). Harvold found that jaw expansion instead of extracting teeth was necessary in order to achieve a normal bite and growth of the middle face.

Harvold's work and the continuous documentation by the cleft teams have formed the basis for other important clinical research projects in Norway: a secondary bone tissue transfer operation to the alveolar cleft that made secondary tooth eruption possible and non-prosthetic rehabilitation in cleft lip and palate patients (Abyholm, Bergland et al. 1981; Bergland, Semb et al. 1986) and a systematic evaluation of orthopedic protraction of the upper jaw in cleft patients (Tindlund 1995).

The documentation of favorable and negative results of different surgical techniques has been continuous. That is how knowledge of surgery's long-term influence on soft tissue as well as on bone development has been built, step by step. Most patients have been followed by the same plastic surgeon who performed the primary operation through to adolescence. The routine of clinical evaluation at regular intervals and the fact that the surgeon planned to see the patient through to adolescence has probably inspired the surgeons to provide high quality documentation.

Multidisciplinary cleft teams introduced effective and systematic documentation. The diagnosis used among the cleft team members was the quite brief and precise description given by the surgeon in the primary surgical report. Together with X-rays since the late 1970's, photos and study casts, this documentation forms the basis for our retrospective classification of cleft morphology.

In addition, the medical records for every cleft-case describe the presence or absence of non-cleft congenital malformations and chronic medical disorders. The use of supplementary examinations and diagnostics of accompanying conditions is very variable, however. More general descriptions, such as "delayed motor development" and "attention deficit", are characteristics quite commonly used in the medical records without further diagnostics. We therefore had to carry out a relatively rough sorting of cleft cases that had a medical condition of a chronic nature recorded in their files. In our studies, the collective term "with accompanying defects" includes cases that had accompanying congenital malformations, chronic medical conditions or vaguely described dysfunctions registered in their medical records.

Classification of cleft morphology

The retrospective classification of the cleft cases treated at Haukeland University Hospital was performed by a senior plastic surgeon, Gunnar E. Johnsson. A few other plastic surgeons

including myself were involved in the coding supervised by Mr. Johnsson. At Rikshospitalet University Hospital, too, only one clinician was responsible for the coding, and the clinician was exempted from other work during the retrospective classification. The coding was filled in a form and thereafter punched into a database.

The system of coding is slightly different, however, at the two hospitals. In Oslo, the presence or absence of cleft in the alveolar ridge was coded separately, and in Bergen the extent of tissue deficit was an additional registration. It was, however, straight forward to combine the two clinics' systems of classification in our modification of the Y-diagram presented here (Figure 4).

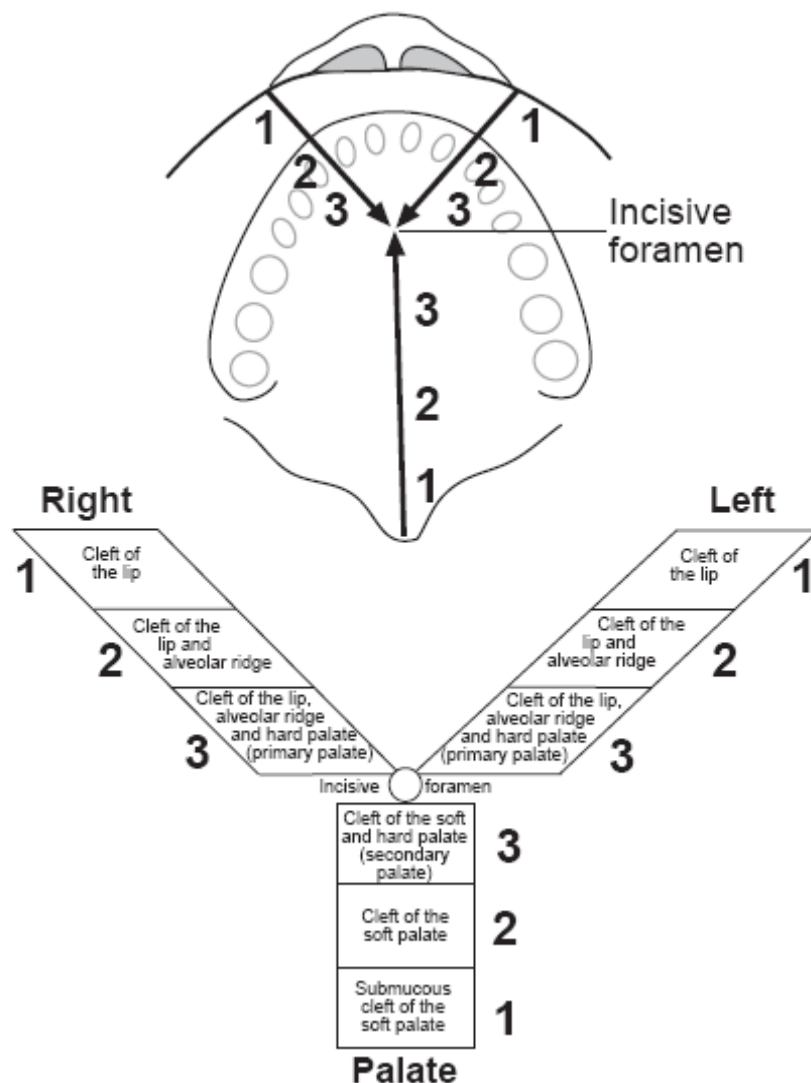


Figure 4. Our classification of the cleft morphology, a modification of Kernahan's striped-Y diagram (*Paper I*).

Definitions in the modified Y- diagram:

Cleft of the lip

Cleft lip is defined by everything from minor scarring to a well-defined cleft.

Cleft of the lip and alveolar ridge

Cleft in the alveolar ridge is defined by indentation in the alveolar ridge at birth. Since the late 1970's the diagnostics was supplemented by X-rays taken the year before secondary teeth eruption in order to detect bone tissue deficit. The milder alveolar clefts without alveolar indentation in newborns, but with bone tissue deficit at the age of ten years have therefore been included since the late 1970's.

Cleft of the lip, alveolar ridge and hard palate (primary palate)

Cleft of the hard palate is defined by everything from a small to a complete cleft in the hard palate anterior to the incisive foramen.

Submucous cleft palate

Submucous cleft palate is defined by the presence of hyper nasality and at least two of the following criteria: bifid uvula, muscular diastases in the midline and a bony notch in the posterior edge of the hard palate.

Cleft of the soft palate

Cleft of the soft palate is defined by a visible open cleft in the soft palate. Bifid uvula alone is not included.

Cleft of the soft and hard palate (secondary palate)

Cleft of the hard palate is everything from a small palpable cleft to a complete cleft in the hard palate posterior to the incisive foramen.

Severity

In our papers we describe "severity" by grading the anatomical completeness of the clefts. Severe cleft lip is defined as a cleft that extends into the hard palate (primary palate) (3 in the RPL system). In *Paper I*, mild cleft lip is defined as a cleft of the lip and of the alveolar ridge (1 or 2). Similarly, in *Paper I* severe cleft palate implies an affection of the hard palate (secondary palate) (3) and mild cleft palate affection only of the soft palate (1 or 2). In *Papers II* and *III*, we use a threefold grading of severity: mild, moderate and severe (1, 2 and 3, respectively) of cleft lip and of cleft palate. This use of the word "severity" differs from the

clinicians' use in that it does not take into account the result of treatment or risk of treatment failure.

MEDICAL BIRTH REGISTRY OF NORWAY

The MBRN was established in 1967 by the Directorate of Health in order to monitor maternal and perinatal health problems and to contribute to the clarification of their causes (Irgens 1998). Based on compulsory notification, the Registry comprises all live births and stillbirths with a gestational age of 16 weeks or more. Since 1998, the reporting of all induced abortions has become compulsory.

Almost all births (>99%) in Norway take place in a hospital (Nilsen, Daltveit et al. 2001). During the stay in the maternity ward, all newborns are examined by a physician, usually a pediatrician, and routine blood tests (screening for phenylketonuria) are taken. The diagnoses of birth defects recorded in the MBRN are based on these examinations, as well as on any additional diagnostic procedures during the stay in the maternity unit, and for some babies in the pediatric intensive care unit. As a standardized procedure, the midwife attending the birth clears the mouth of the newborn with her finger, and at the same time she palpates the palate for clefting. A visual examination of the palate is repeated by the physician, usually the day after birth. The midwife and / or the physician attending the birth are responsible for completing the MBRN notification form when the infant is discharged from the maternity unit. Stillbirths and deaths are recorded directly in the MBRN notification form. For neonates referred to the intensive care unit or to a pediatric ward, the MBRN notification form is completed by the doctors there. The standardized notification form was unchanged until 1998, and the coding practice has remained unchanged since the start of the Registry. The notification comprises demographic variables as well as data on maternal health before and during pregnancy, previous reproductive history, complications during pregnancy and delivery, and pregnancy outcome.

Classification of birth defects

The MBRN use the International Classification of Diseases and Causes of Death (ICD-classification) for the classification of birth defects (<http://www.who.int/classifications/icd/en/>). This is a classification that groups the

malformations on the basis of which organ(s) is / are involved. There may be different causal pathways for different types of birth defects within the same organ group (causal heterogeneity). Until 1998 the 740.0 – 759.9 codes in ICD-8 were used to register the birth defects in the MBRN, and from 1999 the Q00 - Q99 codes in ICD-10 have been used (table 2). We have not used the details of cleft types given in the ICD classification in *Paper I*. In *Paper II* and *Paper III*, we used the ICD's specification of CLO, CLP and CPO in the MBRN-data, but we chose the clinical diagnosis, when available, as the answer.

Congenital malformations, deformations and chromosomal abnormalities	ICD-8	ICD-10
CPO	749.0	Q35
CLO	749.1	Q36
CLP	749.2	Q37
Non-cleft birth defects	> 739.9 and < 749.0 or > 749.9 and < 760.0 or 524.9, 551.2, 551.3, 551.4	> Q00 and < Q35 or > Q37 and ≤ Q99

Table 2. ICD coding of oral clefts and non-cleft birth defects the MBRN used in our studies.

In *Paper IV*, we use the ICD-10 coding to specify the accompanying birth defects. In the other papers we give no organ specification of the non-cleft birth defects. We include all other birth defects recorded in the above-mentioned ICD codes and in the clinical data to describe the presence or absence of non-cleft birth defects (table 2). We use the collective terms “with or without accompanying defects”.

PREGNANCY HEREDITY AND ENVIRONMENT - project

Because virtually every Norwegian infant with clefts is treated at either Haukeland University Hospital in Bergen or Rikshospitalet University Hospital in Oslo, it was possible to conduct a

population-based study of oral clefts with a high degree of case ascertainment. This population-based study, “Pregnancy, Heredity and Environment” was conducted from 1996 to 2001, and the cases were recruited through the treatment centers for clefts. Funding was provided by the National Institute of Environmental Health Sciences and the Norwegian Research Council. This study was designed as a case-control study to study gene-environmental interaction. Our study of the prevalence of 22q11.2 deletion and duplication in newborns with open cleft palate draws upon resources from this study. It is restricted to the study cases with open cleft palate.

Every family with a child with oral cleft born in Norway during the period 1996 -2001 and registered at either of the two cleft centers was invited to take part in the study. The mothers received a letter from the surgery clinic approximately one month after the baby’s birth. This invitation contained a detailed description of the study. The consent assumed that all data would be anonymized and allowed “research on genetic factors of cleft lip and palate”. After the consent form was returned, the mothers received self-administered questionnaires with questions about their reproductive history, maternal health, medication and other treatments, family history of clefts and a spectrum of environmental exposures including smoking, alcohol, vitamin supplementation, food frequency, occupational exposures and stress during the periconceptional period and in early pregnancy. An English translation of the questionnaires is available at

http://dir.niehs.nih.gov/direb/studies/ncl/question/ncl_pregnancy_en.pdf and

http://dir.niehs.nih.gov/direb/studies/ncl/question/ncl_nutrition_en.pdf.

Nearly 300.000 women gave birth in Norway between 1996 and 2001, of whom 676 mothers had a baby with an oral cleft referred for corrective surgery. 24 mothers who did not speak Norwegian, or whose baby died after birth, were excluded, leaving 652 eligible case mothers. Of these, 573 agreed to participate (88%). 196 of the babies had cleft palate only. Five of them had submucous cleft palate and were therefore excluded from our study. DNA was extracted from full blood samples taken from the case babies (n=191) under general anesthesia during the first operation and from the samples used to screen the newborns for phenylketonuria. Clinical data was collected prospectively and made possible a detailed classification of cleft-types and added information on accompanying birth defects to our study.

Genotyping

In *Paper IV*, the multiplex ligation-dependent probe amplification technique (MLPA) was used for genotyping (Schouten, McElgunn et al. 2002). This method of gene dosage analysis is based on polymerase chain reaction (PCR) and is commercially available. The SALSA P023 MLPA-DiGeorge syndrome test kit developed and manufactured by MRC-Holland (Amsterdam, the Netherlands) that we used has 39 specific nucleic acid sequences that are amplified simultaneously using a single PCR primer-pair. Figure 5 illustrates how this method works.

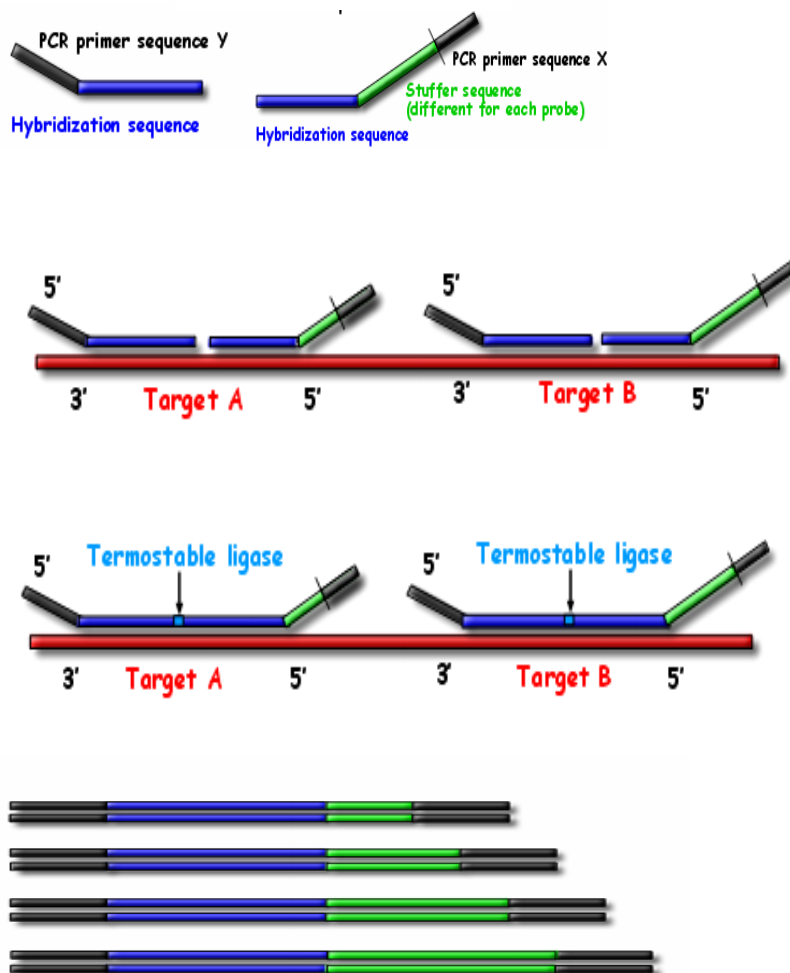


Figure 5. The multiplex ligation-dependent probe amplification technique. Source: http://www.mrc-holland.com/pages/support_mlpa_infopag.html

MLPA probe mix is added to denatured genomic DNA. Each MLPA probe consists of two different oligonucleotides that both contain one of the PCR primer sequences. If these two probes successfully hybridize to their adjacent target sequences, they can be ligated by a thermostable ligase. Only ligated probes are amplified in the PCR reaction. The amplification products are separated by capillary electrophoresis. The length of the amplification product of two probes that have hybridized and united is unique. Compared to a similar electrophoresis profile obtained from a DNA control sample, the relative peak area of each amplification product reflects the relative copy number of the target sequence of that probe in the analyzed sample.

An ordinary multiplex PCR requires one pair of primers for each fragment to be amplified. The presence of these primers results in various technical problems. In MLPA the use of a single primer is possible because no sample DNA is amplified, only the probes that are added to the sample.

RECORD LINKAGE

The national identification number is unique to all residents of Norway. The newborn is given a person specific number shortly after birth by Statistics Norway. This 11-digit number is used as the identification of the person in the MBRN and in the clinical records in all Norwegian hospitals. By means of the mother's identification number recorded on the birth notification form, record linkage is established with the Population Registry run by Statistics Norway to obtain the infant's identification number. This procedure ensures near complete ascertainment of births. Very few records are not routinely matched, and the solving of unmatched records has had high priority throughout the history of the MBRN. Non-matches between MBRN and the civil registration of births are mainly due to Norwegian citizens giving birth abroad, around 500 births annually. The person specific identification number was used for MBRN and record linkage with the clinical data. The clinical data was collected prospectively in the data collection of the Pregnancy Heredity and Environment -project.

THE ETHICAL ISSUES

The linking of the clinical data and the data from the Medical Birth Registry in *Papers I, II* and *III* was approved by the Regional Committee on Research Ethics for Western Norway

and the Data Inspectorate. Confidentiality was given highest priority and subject identity was protected by removing personal identification numbers from the data after linking the two data sources.

Our study of the prevalence of 22q11.2 deletion and duplication- syndromes in *Paper IV* was covered by the ethical committee's approval from the Regional Committee on Research Ethics for Western Norway of the mother study (Pregnancy Heredity and Environment-project) and the informed consent of the parents in this study. All data were anonymized in accordance with the consent from the participants.

STATISTICAL ANALYSIS

In *Paper I*, associations were tested using Fisher's Exact test (StatXact-4.0.1).

In *Paper II*, we estimated trends in ascertainment by grade of cleft severity and calculated test-for-trend p-values by log-binomial regression using STATA version 9.2. We also tested for a change in ascertainment between two time periods.

Oral clefts are relatively rare events and relative risks could therefore be approximated by odds ratios. In *Paper III*, the relative risks of recurrence were estimated using logistic regression in STATA 9.2. Correlation between repeated observations within nuclear families was accounted for using robust estimation of variances.

In *Paper IV* we based our estimation of the prevalence of 22q11.2 duplications in patients with cleft palate on an application of Bayes' theorem:

$$\frac{\text{Duplication 22q11.2 syndrome, prevalence in patients with CPO}}{\text{Duplication 22q11.2 syndrome, prevalence at birth}} = \frac{\text{CPO, prevalence in patients with duplication 22q11.2 syndrome}}{\text{CPO, prevalence at birth}}$$

REVIEW OF PAPERS

I Prevalence of major anatomic variations in oral clefts. Sivertsen Å, Wilcox AJ, Johnson GE, Åbyholm FE, Vindenes HA, Lie RT. *Plastic and Reconstructive Surgery*, accepted for publication in November 2006.

We describe morphological variations of oral clefts in a large population-based sample of 3616 cleft cases treated in Norway for oral clefts between 1967 and 1998. Our classification of cleft morphology is based on nine anatomical focal areas located anterior and posterior to incisive foramen, in the primary and secondary palate, respectively. The data provide a population-based reference for 63 common and rare variants of oral clefts. The relative proportion of cleft types is illustrated in modified striped-Y Kernahan diagrams. Their distribution in the population is presented as a whole and stratified by baby's sex and the presence of accompanying malformations. Clefts of the lip or palate are more severe when both types of clefts are present. In the absence of cleft palate, 18% of the babies with cleft lip had a complete cleft in the primary palate (severe cleft lip). Where cleft palate was present, 81% of the babies had severe cleft lip. Similarly, among babies with cleft palate, 40% had complete cleft in the secondary palate (severe) in the absence of cleft lip, while, where cleft lip was present, 93% of the babies had severe cleft palate. The more severe the cleft lip, the more likely that the baby had an accompanying cleft palate. Girls were more likely to have severe clefts, as were cases that had other types of birth defects. Although cleft lip was more frequent on the left side, clefts were not more severe on the left side. In bilateral cleft lip, severity was similar on both sides.

II Completeness of Registration of Oral Clefts in a Medical Birth Registry: A Population Based Study. Kubon C, Sivertsen Å, Vindenes HA, Åbyholm FE, Wilcox AJ, Lie RT. *Acta Obstet et gynecologica*. 2007 Sep; 11:1-5.

The evaluation of sources of ascertainment is an important factor in striving for complete and accurate information on congenital malformations for research and public health purposes. The clinical data from 3616 cleft cases treated in Norway between 1967 and 1998 was linked with data from the MBRN. We calculated the proportion of clinically verified cases reported to the Registry by the anatomical completeness of the cleft within the three commonly used categories of clefts. 94% of *cleft lip and palate*-cases, 83% of *cleft lip only*-cases and 57% of *cleft palate only*-cases were reported to the registry. Reporting to the birth registry strongly

depended on the severity of the cleft. For example, 71 percent of cases with severe CPO were reported, while only 11 percent of cases with mild CPO were reported. The substantially reduced reporting of mild clefts is important to remember when using registry data for disease surveillance and research findings. The registration of cleft palate was far better when the cleft palate was accompanied by cleft lip. The large proportion of non-reported cleft palate cases indicates insufficient cleft palate diagnostics at the neonatal clinical examination.

III Familial risk of oral clefts by morphological type and severity. Sivertsen Å, Wilcox AJ, Skjærven R, Vindenes HA, Åbyholm FE, Harville E, Lie RT. Submitted.

The tendency for clefts to recur in families is high, and it points to an important genetic contribution to the etiology. We wanted to find out whether cleft cases with severe cleft morphology have a higher risk of familial recurrence than mild cases. The data of all patients treated in the two national centers for cleft treatment from 1967 to 2001 were linked to the population-based MBRN (4138 cases and 2.1 million births). This allowed us to combine high-quality clinical information on cleft morphology with virtually complete ascertainment of biological family members for the whole country over a 35-year period. We estimated the recurrence risk of clefts from parent to child and in full-sibships. Index cases with non-cleft birth defects were not included.

We found no higher risk of clefts to the children of affected mothers than to the children of affected fathers. This indicates that genetic causes of oral clefts seem to work mainly through the fetus. The specificity of recurrence risk for the three cleft types, cleft lip with or without cleft palate and cleft palate only (CLO, CLP and CPO), was similar in sibships and from parent to child. The recurrence risks of cleft lip cases were similar regardless of whether cleft palate was present. We estimated a joint recurrence risk for first-degree relatives and found that the recurrence risk of CPO was 56-fold and significantly different from the recurrence risk of CL (31-fold). We found no effect of cleft severity on recurrence risk of either cleft type. This argues against the multifactorial threshold model as a model of inheritance of oral clefts and for a genetic model in which severity is independent of genes predisposing for oral clefting.

IV Prevalence of Duplications and Deletions of the 22q11 DiGeorge Syndrome Region in a Population-based Sample of Infants with Cleft Palate. Sivertsen Å, Lie RT, Wilcox AJ, Åbyholm FE, Vindenes HA, Haukanes BI, Houge G. *American Journal of Medical Genetics A*. 2007 Jan 15;143(2):129-34.

The prevalence of duplications and deletions of the 22q11.2 (DiGeorge syndrome) region was studied among babies born in Norway with open cleft palate and without cleft lip. During a 5-year period (1996-2001) 245 newborns with cleft palate were referred for surgery at the two national centers for cleft treatment. DNA was available from 174 cases with open cleft palate. DNA copy number was analyzed using the multiplex ligation-dependent probe amplification (MLPA) technique, and an unambiguous result was obtained in 169 (97%) of the samples. We found no 22q11.2 duplications and three cases with 22q11.2 deletions, a prevalence of 1 of 57 (1.8%). All three del22q11.2-syndrome cases also had congenital heart disease, which represents one-third of the ten babies with heart malformations in our study population. We conclude that testing for 22q11.2 deletion or duplication is not warranted in babies with open cleft palate as the only finding.

DISCUSSION

GENERAL CONSIDERATIONS

There are several features that make Norway favorable for conducting population-based epidemiological studies of oral clefts. Cleft lip and palate is one of the most common birth defects in Norway, and the frequency in this ethnic homogenous population is among the highest in the world. The ascertainment of cases is high because treatment of children with oral clefts is centralized in two centers. The centers have collaborated closely on treatment and registration. It is also important that all costs relating to treatment are refunded to the families. From 1967 there has been compulsory registration with MBRN of all newborns and stillborns after week 16. Thus the 3616 children referred for oral cleft treatment from 1967 to 1998 can be related to a population of 1.9 million births. The two treatment centers have carried out high quality documentation. This has enabled a detailed retrospective classification to be made of the cleft morphology of all cases referred for treatment since 1967. Since the early 1960's, newborns have been given an eleven-digit unique personal identification number that makes it possible to link MBRN with the patients' medical records. This linking ensures a population reference to the clinical cases and a two-source ascertainment of accompanying birth defects. Together with insignificant emigration, this linking also makes follow-up through generations possible and virtually complete.

METHODOLOGICAL CONSIDERATIONS

Study design

The patients treated by the two cleft teams since 1967 and the births registered in the MBRN can be regarded as population-based historical cohorts.

The two cleft teams' archives of children with oral clefts referred for surgery form the basis of our clinical data. The children are registered in the cleft teams' archives at the time of referral, usually during the stay in the maternity ward. Once included in the cleft teams' archives, they are actively called in at regular intervals, from the newborn period to adolescence. In this system of repetitive consultations the chances of being left out are small. Parents of a child with a minor cleft lip may under certain circumstances regard the newborn as not needing surgical treatment. These children are most likely referred to the cleft team later in childhood. Growth of the facial structures may give rise to a need for surgical treatment or other persons

in contact with the child may insist on referral to a specialist center. Development of speech-difficulties in children may uncover undiagnosed cleft palate. These cases of delayed referral are included in our study-cases.

Much effort is made to ensure that the data collected by the MBRN is of high quality, both by manual and computer quality controls. Solving the problems of non-matching between the medical birth records and the civil registration of births (Statistics Norway) due to registration errors has been given high priority over the years. Residual non-matching between the MBRN and the civil registration of births after manual routines are completed amounts to less than 500 births annually (MBRN 1987). The infant and the father's national identification numbers are provided through the exchange of computer files between MBRN and Statistics Norway. The father's national identification number may be missing if unmarried mothers do not provide information about paternity, or if the pregnancy resulted in a stillbirth. Women tend to marry men older than themselves. A notable proportion of females born after 1967 (and registered in the MBRN) have married men who were born before 1967 (and not registered in the MBRN). This explains the reduced number of fathers relative to the number of mothers in the generation-file of our family study-cohort in *Paper III*.

Internal validity

Clinical cases; ascertainment and classification

Children with oral clefts who are referred for surgery at Haukeland University Hospital or Rikshospitalet University Hospital are included in our studies. Terminated pregnancies, stillbirths and early deaths are therefore missing from our study cases. It is possible that the distribution of the 63 cleft types is different among these groups compared to the cleft cases referred for surgery. Other major birth defects are a major cause of death in these cases. In our data, clefts were more severe in the presence of accompanying defects (*Paper I*).

The risk of misclassification is reduced by the precision of diagnostics ensured by a specialist's examination in favorable working conditions. Our clinical study cases have the cleft morphology confirmed by the plastic surgeon's examination under general anesthesia mainly by the age of three months (lip closure) or 12 months (palate closure) and by X-ray examination of the alveolar ridge at the age of ten (since the late 1970's).

In all classifications there will be some borderline cases where one is in doubt about the class to which the case belongs. Some study-cases are possibly wrongly classified as submucous cleft palate that in addition to have bifid uvula have a small open cleft in the soft palate. Other cases may be wrongly classified as cleft in the soft palate that also have a minor cleft in the hard palate. The defect in the hard palate may not even be palpable, but, in theory, it might be possible to detect by X-ray, MRI scan or high resolution CT examination. A refinement of diagnostics between soft and hard palate has had no consequences in terms of the surgical treatment and has therefore never been applied. For genetic and epidemiologic studies, however, it may be important to know that our clinical grouping of study-cases is not absolute.

Another problem is that cases with minor anomalies in the lip or palate may not be included. Sub-clinical clefts without significant functional or cosmetic loss are probably often overlooked and not recorded in the MBRN, nor referred to the cleft teams. Special characteristics of individuals with sub-clinical clefts are not known, for example, whether they are more frequent in clinical syndromes and prone to accompanying defects. Genetic and epidemiologic studies that miss the sub-clinical cases are prone to lose power and utilitarian value.

Sub-epithelial cleft lip can be manifest by minor anomalies such as philtral flattening and nostril asymmetry (Lehman and Artz 1976). Some of these cases might be referred to the team in later childhood if the defect becomes more striking as a result of growth, and they are then included in our data. Ultrasonography of the upper lip has been used in other studies to detect defects of the orbicularis oris muscle, but with considerable technical uncertainty. These studies have tried to demonstrate that orbicularis oris anomalies may be part of the cleft lip phenotype and more common in first degree relatives of cleft cases (Martin, Hunter et al. 2000; Neiswanger, Deleyiannis et al. 2006; Weinberg, Neiswanger et al. 2006).

In our studies, submucous cleft palate is the mildest form of cleft palate morphology registered. Bifid uvula, a frequent palatal anomaly present in 4 in 1000 (0.4%) of schoolchildren (Chosack and Eidelman 1978) is not included. Nor is occult submucous cleft palate. Individuals with occult submucous cleft palate have hyper nasal speech because of hypoplasia of the musculus uvulae and abnormal insertion of other palatal muscles. Bifid uvula, muscular diastases in the midline or bony notch in the posterior edge of the hard palate (as found in submucous cleft palate) is not present in occult submucous cleft palate (Kaplan

1975; Lewin, Croft et al. 1980). It has been suggested that occult submucous cleft palate can be viewed as a severity subgroup of submucous cleft palate (Sommerlad, Fenn et al. 2004). The frequency of submucous cleft palate based on classic morphologic signs (Calnan 1954) and the presence or absence of hyper nasality is reported to be 0.02-0.08 % (2-8 in 10000) (Weatherley-White, Sakura et al. 1972; Garcia Velasco, Ysunza et al. 1988; McWilliams 1991). These are frequencies based on the screening of large populations of pre-school and school-age children. Up to 90%, of individuals with submucous cleft palate are reported to remain asymptomatic (McWilliams 1991). We have the submucous cleft palate cases treated for hyper nasality included in our studies (prevalence 1 in 10000). They have often had a diagnostic delay until preschool-age (Abyholm 1976). In the newborn and up to the age of speech-articulation, submucous cleft palate is often asymptomatic. An inability to impound negative intraoral pressures for feeding purposes and unremitting ear disease may occur. It is demanding even for a specially trained plastic surgeon to diagnose submucous cleft palate, and therefore unlikely that asymptomatic submucous cleft palate is diagnosed in the routine examination of neonates. In individuals with hyper nasality, videofluoroscopi and nasoendoscopi are required to distinguish between submucous cleft palate, occult submucous cleft palate and the wide range of velopharyngeal disorders.

Medical Birth Registry of Norway; ascertainment and classification of birth defects

The registration in the MBRN is compulsory and the entire population participates. In addition to being a population-based reference for our studies, we use the information about the sex of the child and the presence of congenital malformations recorded in the MBRN.

The ascertainment of pregnancy outcomes typically covered by surveillance, such as congenital malformations, is a great concern for the MBRN. Doubly ascertained data was obtained on oral clefts in the period 1985 to 1988. The overall estimated proportion of cleft cases reported to the MBRN was estimated to 78 percent in comparison with a pediatric database (Lie, Heuch et al. 1994). An under-reporting of the youngest, smallest fetuses in the MBRN is well known, and so is the high proportion of birth defects among these (MBRN 1997).

2.6 percent of live born children in 2002 (live, stillborn, and terminated pregnancies) had major birth defects registered in the MBRN (MBRN 2004), that is diagnosed in their first week of life. This is quite close to the consensus that emerged in the 1980's that

approximately three percent of newborn children are affected by major congenital malformations. To this figure was added a similar proportion with types of anomalies not usually detected in the neonatal period but discovered in the months and years after birth (Kalter and Warkany 1983). The presence of medical symptoms in the newborn is important for the physician to be able to detect congenital malformations in the baby during the first week of life. The diagnostic problems can be illustrated by the most common congenital malformation, congenital heart disease. Several congenital heart defects are asymptomatic until the pulmonary vascular resistance decreases during the first weeks after birth. Some murmurs are not possible to detect by auscultation until then. Delayed symptoms given by the postnatal hemodynamic situation and the later physiologic changes and increasing work load on the heart with age, can explain some of the differences in prevalence reported. Only one in 2000 Norwegian newborns had a congenital heart defect diagnosed in their first week of life while the prevalence of congenital heart disease in children up to 18 years is referred to be 12 in 1000 in a large Canadian study recently published (MBRN 2004; Marelli, Mackie et al. 2007). Better knowledge about diseases and modern diagnostic techniques, for example in prenatal ultrasound screening, has brought forward the age of some diagnosis to the prenatal or early postnatal period. Malformations such as oral clefts, Down syndrome or spina bifida, are clearly visible in the neonates while other congenital malformations are asymptomatic. This means that the registration of some diagnosis in the birth registry is better than others.

An important aspect is the degree to which ascertainment in a birth registry is random. There may be higher ascertainment among siblings following the birth of a malformed infant and among the births to parents with birth defects, because these babies are examined more carefully. It is possible that other parental characteristics, such as the socio-economic level and age, are linked to ascertainment. Ascertainment might also be linked to certain characteristics of the babies. Children with one birth defect are probably more thoroughly examined in order to search for accompanying defects. The recognition of symptoms and structural defects might be more difficult in certain groups of neonates. Immature facial characteristics in preterm born children, for example, might make the diagnosis of oral cleft more difficult and the registration in the MBRN incomplete. Underreporting of congenital malformations in MBRN may also be caused by professionals' fear of wrongly stigmatizing the newborn. A tendency probably exists to regard congenital malformations as normal phenotype variation and this might be caused by a wish not to give concern to the parents and an intentional postponement in order to "wait and see" whether the defect becomes a problem.

The fact that ascertainment in the MBRN also causes a selection of certain phenotypes of a birth defect is studied in *Paper II*. Certain cleft phenotypes are more difficult to detect and we have estimated the difference in ascertainment between mild and severe cleft cases.

Many studies conducted in the USA have documented the inadequacy of birth certificates as a source of case ascertainment for congenital malformations (Hexter and Harris 1991; Watkins, Edmonds et al. 1996). Comparison of birth certificates with medical records has detected false positives as well as false negatives. Compared to ascertainment of birth defects in multiple-source registries, birth certificates had a tendency to register only one malformation and to register only a minor malformation. For this reason, most registries in the USA that use birth certificates also use other sources (Olsen, Polan et al. 1996).

Cleft type misclassification in the MBRN should not bias *Papers I, II* and *IV*. In these papers, we have restricted our study to cases referred for surgical treatment that have the cleft diagnosis recorded in the clinical data. We let the clinical cleft diagnosis count when the category of cleft differs in the MBRN and in the clinical data. In *Paper III*, however, some of the recurrent cases are recorded in the MBRN alone (mostly stillbirths and early deaths). These cases with only one source of ascertainment and without a surgeon's evaluation are more prone to misclassification. In *Paper II*, we evaluate the extent to which the clinical cases are registered in MBRN and not the extent of misclassification in the MBRN.

In *Paper I* and *III*, we do not use organ-specific birth defect categories other than oral clefts in our analyses. Two source registrations of non-cleft birth defects ensured a better ascertainment of non-cleft birth defects in our study-cases with clefting. The problem of misclassification was reduced by summarizing all non-cleft birth defects in the category "with accompanying defects". The only specification of non-cleft birth defects is with or without accompanying defects. In *Paper IV*, however, we used the organ specific birth defect categories in the ICD-10.

Confounding

Previous studies have found a mild association between oral clefts and several different exposures. Rare maternal exposures, such as anticonvulsants and corticosteroid medication can cause oral clefts (American Academy of Pediatrics Committee on Drugs 1979; (Park-Wyllie, Mazzotta et al. 2000; Puho, Szunyogh et al. 2007). More common exposures such as cigarette smoking, alcohol consumption and herbicides have also been linked to higher risks

of clefts (Wyszynski, Duffy et al. 1997; Shaw and Lammer 1999; Lorente, Cordier et al. 2000; Kayano, Suzuki et al. 2004). Maternal nutrition during pregnancy, for example low dietary intake of B- vitamins or deficient or excessive intake of vitamin A, also appears to contribute to the risk (Rothman, Moore et al. 1995; Krapels, van Rooij et al. 2004). Maternal age and paternal age seem to have modest effects on the risk of having a child with oral cleft (Kazaura, Lie et al. 2004; Bille, Skyttthe et al. 2005; Harville, Wilcox et al. 2005; Zhu, Madsen et al. 2005). Several of these exposures are associated with several adverse reproductive outcomes, such as spontaneous abortion, abnormal placenta and low birth weight. The association between severity of clefts and the presence of accompanying defects might be confounded by some of these exposures (*Paper I*). Theoretically, the high risk of recurrence in some families is confounded by high exposure to some of these exposures (*Paper III*). Known environmental factors should not, however, produce very high recurrence risks (Khoury, Beaty et al. 1988). Data on possible exposures in pregnancy are insufficiently recorded in the MBRN and in the medical records. Data on smoking habits are not included in the MBRN until 1999. We have therefore not adjusted for possible confounders in our analyses.

External validity

The higher prevalence of CL in Scandinavian than in most Caucasian populations might be explained by ethnic-genetic factors and / or environmental factors. Another possible explanation is better ascertainment in the Scandinavian countries on the basis of the well organized registration of births and centralized cleft treatment. We cannot know whether the distribution of the 63 subgroups of clefts (*Paper I*), the familial risk of recurrence (*Paper III*) and the prevalence of 22q11.2 deletion and duplication syndrome (*Paper IV*) differ in other Caucasian populations until similar studies are conducted in these populations.

In *Paper II* we find the ascertainment of severe clefts to be better than that of mild ones. It is likely that the mild phenotype of many non-lethal birth defects is more difficult to detect and more prone to under-reporting than the severe phenotype. If so, this has implications for surveillance and epidemiological studies of other birth defects as well. Our study might serve as an example for similar studies of other birth defects.

Our classification and presentation of the data in a numerical coding and in the Y-diagram can serve as an example for conducting similar population-based studies of the morphology of

other birth defects (*Paper I*). In the literature, we have not found studies of familial recurrence risk in relation to a grading of morphology in other birth defects. It would have been interesting to see the effect of severity on familial recurrence risk of other birth defects than oral clefts (*Paper III*).

Syndromic and non-syndromic cleft cases

Clefts are commonly described as syndromic or nonsyndromic (isolated), depending on the presence or absence of non-cleft birth defects (Christensen 2002). We want to emphasize the roughness of this sorting.

The rationale for excluding cleft cases with accompanying defects in etiological studies is to reduce etiological heterogeneity, while the rationale for including them is that there may be common etiological factors of clefts without accompanying defects (nonsyndromic) and of clefts with accompanying defects (syndromic). The tradition of excluding a proportion of cases with a particular phenotype from studies because they happen to have other features is more clinical than epidemiological (Khoury, James et al. 1992; Lie 1995). In clinical practice, families with syndromic cleft cases will be offered genetic counselling on the basis of specific genetics and recurrence rates. The most preferred design of an epidemiological study is to obtain as much information on associated anomalies as possible and thereafter to perform analyses with and without the cleft cases with accompanying defects (Christensen 2002).

The use of supplementary examination has become more extensive during the last decades. The development of modern technology has made great refinements to diagnostics. Technical methods for verifying chromosomal defects and gene mutations have made explanations to a number of simple and complex genetic disorders. Various patterns of multiple birth defects have been explained by gene disorders, chromosomal abnormalities or teratogenic exposures. An infant with a recognized cause of multiple birth defects is labeled “syndromic” by both epidemiologists and clinical geneticists. However, most reported patterns of multiple congenital anomalies have no known cause. In the literature there is a tendency to label cases that share phenotype similarities not previously reported or even a single case as a “new syndrome”. These conditions of unknown cause are by some authors preferably termed by phenotype until a cause for the condition has been identified (Khoury, Moore et al. 1994).

Non-specificity of diagnoses among syndromic clefts is considerable, and the ascertainment probably varies from center to center. Correspondingly, the definition of non-syndromic oral

clefts has considerable lack of specificity. Because the presence of three or more minor anomalies has been found to be strongly associated with the presence of a major anomaly (Leppig, Werler et al. 1987; Leppig, Werler et al. 1988), it has often been common practice to limit non-syndromic cleft cases to those associated with two or fewer minor anomalies. When two or more minor birth defects are found, the risk of also finding a major birth defect is around 10-20% (Mueller and Young 2001). The usual understanding of a major birth defect is a structural abnormality of prenatal origin present at birth, which seriously interferes with viability or physical well-being (Kalter and Warkany 1983). Minor birth defects, i.e. abnormalities that do not interfere with viability or physical well-being, are present in approximately 10% of newborns. However, minor anomalies can be those that distinguish between syndromic and non-syndromic clefts. Cleft cases with van der Woude syndrome, for example, might have the pits in the lower lip as the only additional sign of the syndrome (van den Boogaard, Dorland et al. 2000; Kondo, Schutte et al. 2002).

The signs and symptoms in a syndrome may not be possible to detect in infancy and the right diagnosis can therefore delay until late childhood or adolescence. Our clinical study cases are followed up by the cleft teams from newborn to adolescent. However, the extent to which accompanying defects, chronic diseases and conditions have been reported in the medical record varies during the period of registration. Clinical signs without immediate clinical importance to surgery and other treatment have been at risk of being left out of the medical records. In the last two decades there has been a change towards the cleft team also being more involved in accompanying medical problems. Specialists in medical genetics have been attached to the two teams, and this is a sign that more attention is given to accompanying medical conditions. The medical treatment have become more specialized and centralized during the period of registration and it has become convenient both for the clinicians and for the patients to co-ordinate treatment and follow-up. The registration of accompanying defects and medical conditions in the clinical records has therefore probably more complete in the last half of the study period.

We have characterized non-syndromic and syndromic cases by separating cleft cases with any non-cleft birth defect from those without. We found that 10% of CL cases and 29% of CPO cases had accompanying defects (*Paper I*). Individuals with oral cleft and an accompanying defect, “minor” as well as “major”, registered in the MBRN were defined as “possible-syndromic”. In the non-syndromic cases we have excluded all cleft cases with certain ICD-8 and ICD-10 diagnoses (Table 2). In addition are cleft cases that have birth defects and

“chronic medical conditions” recorded in the medical record defined as “possible-syndromic” also excluded from the “non-syndromic” cases. Dysfunctions imprecisely described in the medical record, such as “delayed motor development” and “attention deficit” have led to us grouping the case as “with accompanying defects”.

Different aspects of “severity”

In clinical practice, a patient with “severe cleft” generally means that the patient has a cleft type that is difficult to treat and that the patient is at higher risk of a poor cosmetic or functional result. Accordingly, in daily clinical use the term “severity” depends on the outcome of treatment. When evaluating the speech of a patient, severe cleft is a cleft that has given a patient a severe speech problem. When evaluating the bite, severe cleft means a cleft that has severely influenced the position of the teeth and the growth of the middle face. At the time of surgery a severe cleft often means the wide or broad cleft that is a technical challenge to close. Specific morphological findings that challenge the operation-technique, such as tissue deficit in the cleft area and rotation of the segments, would probably make the surgeon talk of a severe cleft.

In our papers we use the term “severity” explicitly to describe the anatomical completeness of the cleft. The term is carefully explained in the papers in order to avoid any ambiguity.

Some epidemiological studies have graded bilateral clefts as being more severe than unilateral clefts and cleft lip with cleft palate as more severe than cleft lip without cleft palate. This is based on an acceptance that unilateral and bilateral clefts, and cleft lip with and without cleft palate, are associated conditions with a common etiology. The similar and dissimilar epidemiological characteristics of cleft lip with and without cleft palate have been discussed (Harville, Wilcox et al. 2005). In the literature, we have not found a similar discussion about whether unilateral and bilateral clefts are similar or different entities. Our system of classification creates favorable conditions for characterizing unilateral and bilateral clefts separately.

Paper I

In the interest of both clinicians and epidemiologists, we developed a finer system for the sub-classification of clefts in order to refine the three commonly used main categories of clefts.

We wanted the classification to be user-friendly in daily clinical practice and to improve communication between the cleft team members. At the same time, we wanted to make use of the classification to present a vast amount of data.

Kernahan's and our modified classification are built on the embryologic origin of the lip and palate. The secondary palate forms a few weeks after the formation of the primary palate and thus justifies the terms primary and secondary palate. The incisive foramen is the boundary marker in the midline, between the anterior and posterior fusion processes. The embryological development is however not clearly understood. For example, it is discussed whether the nasopalatine nerve and artery run through the incisive foramen or within a separate canal anterior to the incisive foramen (Radlanski, Emmerich et al. 2004). The formation of the primary palate is also outlined in different ways - whether it develops from the medial nasal swellings (most widely accepted) and / or from the maxillary swellings (O'Rahilly and Muller 1992). Our classification in the Y-diagram is based on major landmarks within the primary and secondary palate. The structures (lip, alveolus, soft and hard palate) are quite specific and knowledge of their morphology is required for treatment planning and follow-up.

We ended up with a comprehensive classification that is adapted to daily clinical practice. However, the classification can easily be extended to include more details in, for example, clinical follow-up studies. The evaluation of surgical results often requires an even more precise description of the cleft morphology than we present in our classification. The surgical treatment of a complete unilateral cleft is far more challenging in the presence of tissue deficit or pronounced rotation of the premaxilla. More morphologic details can be recorded by adding extra boxes to the Y-diagram with numbers or text. In our study, this is possible, on the basis of photos and casts that are systematically stored. Further studies of clinically important variables are interesting, but beyond the scope of this thesis.

We confirm previous findings of CPO being characterized by a higher proportion of females and CL by a higher proportion of males (Gorlin, Cohen et al. 2001). Other studies have a female predominance in operated CPO cases and a male predominance in unoperated CPO cases (Vanderas 1987; Christensen, Holm et al. 1992). We do not have the detailed morphology of unoperated cases and it is therefore not possible for us to reproduce these analyses in our data.

Characteristics of our study cohort were that mild CLO and severe CLP were both common cleft types. Comparison of the epidemiologic features of cleft lip with and without cleft palate raises the question of whether they are distinct defects (Harville, Wilcox et al. 2005). Another

etiologic question is whether unilateral and bilateral clefts are variants of the same defect. There was a striking difference in the number of bilateral clefts between CLO and CLP. A large number of unilateral clefts with severe anatomical involvement seemed to concur with a large number of bilateral clefts. Compared to the large number of mild unilateral CLO cases, the mild bilateral CLO cases were remarkably uncommon. Mild cleft lip was rare in combination with cleft palate. Severe unilateral and bilateral cleft lip seemed to predispose to cleft palate.

Paper II

Assessment of the data quality of population-based registration systems is essential in order to understand the reliability and usefulness of disease surveillance and research findings resulting from the use of registry data. The evaluation of sources of ascertainment is an important factor in efforts to achieve complete and accurate information on congenital malformations for research and public health purposes.

The oral cleft birth defect consists of a wide range of cleft phenotypes, from the very mild to the severe and complete clefts. The overall spectrum of birth defects is similarly wide, varying from mild, minor defects to severe, major defects. Our evaluation of the ascertainment of graded cleft severity in the MBRN maybe comparable with an evaluation of the ascertainment of minor and major birth defects in the Registry. The ascertainment of the mild clefts may have resemblance with the ascertainment of the minor birth defects, and likewise severe clefts resemblance with major birth defects.

Few studies are available on the quality of the registration of cleft defects in birth certificates. In Arkansas, only 65% of children treated for clefts had the cleft recorded on their birth certificates, and as few as 48% had the correct cleft type recorded (Green, Nelson et al. 1979). A similar pattern was observed in Denmark in the 1970's. 75% of clinically verified cleft cases had the cleft recorded on their Danish birth certificates (Olsen 1982).

It is surprising that such serious and clearly visible defects as oral clefts are overlooked or not reported. As many as 1 of 5 of the clinical study cases with mild cleft lip and 1 of 15 with severe cleft lip, with or without cleft palate, were not reported to MBRN. The study of characteristics of the non-reported cases would be interesting, but beyond the scope of this thesis.

The fact that cleft palate has poorer ascertainment than cleft lip made us ascribe this difference to insufficient diagnostics of cleft palate. The diagnostics of cleft palate depends on an active visual inspection of the mouth, while a cleft lip is more easily visible. A visible inspection of the palate using extra light and a tongue depressor is described as favorable to palpation in a recent publication (Habel, Elhadi et al. 2006). We made telephone enquiries to the four largest departments of obstetrics in Norway and revealed that inspection while using extra light and tongue depressor this is the most commonly used examination technique, and after that visual inspection while the child is crying. It is possible that a visual examination while the child is crying is insufficient because the stretch of the soft palate makes the anatomy difficult to recognize and judge.

It is important to be aware of the symptoms of submucous cleft palate in the newborn: problems of obtaining negative intraoral pressure that may lead to feeding difficulties, nasal regurgitation and a secondary “failure to thrive”. The presence of bifid uvula, a high palatal arch or a short soft palate in these children ought to bring forward contact with the cleft team. One in three newborns with open cleft in the soft or soft and hard palate were not registered in the MBRN. A greater proportion of these patients than of those with submucous clefts will have the symptoms mentioned above. A diagnostic delay will mean that the mother and child will miss instructions on how to safely feed the infant. During the babbling period, the excess airflow through the nose results in hyper nasality and in difficulties in saying words with pressure sounds, such as “dada”. The parents will miss out on the instruction in play activities which focus on verbal interaction and appropriate modeling of speech and language. The right timing of surgery is important in order to achieve normal speech. Prolonged hyper nasality may lead to the development of unfortunate compensatory speech patterns. An early cleft diagnosis creates better conditions for detecting other birth defects and medical problems that are more common in children with cleft palate, such as unremitting otitis media.

Our data show that better diagnostics of cleft palate in neonates is necessary. We suggest that physicians examining neonates in maternity units in hospitals need to be regularly reminded about a careful examination of the palate. Personnel working in the child health care centers and general practitioners need to be familiar with the symptoms of cleft palate and know the importance of early diagnostics.

Paper III

Several family studies of the recurrence risk of birth defects in general have been conducted on data from the MBRN. In these studies, twenty-four categories of birth defects were defined on the basis of the ICD-8 classification, and oral clefts had the highest rates of familial recurrence (Lie, Wilcox et al. 1994; Skjaerven, Wilcox et al. 1999; Lie, Wilcox et al. 2001). Children of mothers with birth defects were found to have a significantly higher risk of birth defects than the children of mothers with no birth defect (relative risk 1.6; 95% CI 1.3-2.1), but they had no increased risk of different types of defect than their mothers (Skjaerven, Wilcox et al. 1999). Similar trends were described for the children of fathers with birth defects, with two additional findings: the relative risk of birth defects was higher for children of malformed fathers than for children of malformed mothers (2.4 versus 1.6), and the increased risk was not entirely confined to the specific defects carried by the father (Lie, Wilcox et al. 2001). The mothers of affected first infants were 2.4 times as likely as other mothers to have second infants with any registered defect. This increased risk was primarily due to an increased risk of the same defect (7.6 times higher) in the second infant as in the first (Lie, Wilcox et al. 1994).

The high risk of oral cleft recurrence is striking compared with the familial recurrence risk of birth defects in general, and point to the genetic contribution to oral clefts. The recurrence risk of CL was thirty-fold and of CPO even higher (fifty-fold). Still, it is important to remember that the absolute risk is relatively low. A mother with CPO has a 96% chance of having a child without CPO. The increased risk of birth defects among the children of parents with birth defects account for a negligible share of the birth defects in the next generation (Skjaerven, Wilcox et al. 1999).

We restricted our index study cases to cases without non-cleft birth defects. Still, the etiology of oral clefting in the remaining families is very heterogenic and contributes unevenly to the total risk of recurrence. In the recurrent cases, we included cleft cases with accompanying defects because we wanted the approach to be more clinically relevant. A family with non-syndromic oral cleft present in a family member will ask about the risk of cleft in the next born child and not the risk of cleft specified to the absence or presence of accompanying defects.

If maternal exposures in pregnancy were a major effect on recurrence, we would expect the risk to be higher between siblings than across generations. Maternal exposures are probably

more similar in pregnancies closer in time (1-3 years between siblings vs. 20-30 years across generations). Known environmental factors should not produce very high recurrence risks (Khoury, Beaty et al. 1988). The estimated higher recurrence of CL in siblings than across generations in our data (although not significantly different) may perhaps be produced by an environmental factor. Preliminary analysis of data in the Pregnancy, Heridity and Environment –project shows a moderate effect of maternal smoking on the risk of CL and a less obvious effect on the risk of CPO (RT Lie, personal communication).

In general, the inheritance of oral clefts does not readily conform to a specific Mendelian pattern of inheritance. The prevalence of cleft in children of affected parents is 4% in our study and thus far below the expected incidence in a fully penetrant autosomal dominant disorder (50%). Autosomal recessive disorders normally affect individuals in one generation, and manifest when a child is homozygous or compound heterozygous for a genetic defect predisposing for clefting. In our data, the prevalence of clefts in siblings of an affected child and in children of affected parents are both 4%. The prevalence of clefts in offspring of affected mothers and fathers is so similar that the influence of an X-linked locus is considered unlikely.

Oral clefts are commonly referred to as a multifactorial disorder, similar to several other of the common congenital malformations (neural tube defects, congenital dislocation of the hip, congenital heart disease) and many of the acquired diseases of childhood and adult life (asthma, diabetes mellitus, glaucoma, hypertension). In the context of multifactorial inheritance, a threshold model is usually applied. In the multifactorial disorders model, environmental factors interact with genes at different loci (locus heterogeneity). The threshold is explained by an additive effect for each locus on the liability for disease. The liability is normally distributed in the general population and in the relatives of affected individuals. One consequence of the threshold model is that the risk of the disorder is greatest among the relatives of the most severely affected patients, because they are at the extreme end of the liability curve. In our data, the recurrence of cleft is similar among relatives of either mildly or severely affected index cases. Another consequence of the threshold model is that the relatives of an affected individual of the less frequently affected sex are at higher risk (males with CPO, females with CL). This was not found in our study.

Great variability in the phenotype is a characteristic of many congenital malformations. The morphologic variation of oral clefts can be studied. Phenotypic variation and deviations from Mendelian inheritance may be explained by incomplete penetrance, variable expressivity,

genomic imprinting and allelic heterogeneity. These diverse genetic phenomena are susceptible to influence by environmental exposure, genetic and epigenetic factors. Similar mechanisms probably bring corresponding complexity and phenotype variation to the inheritance of multifactorial disorders.

The effect of genomic imprinting relates to different levels of gene expression depending on which parent has transmitted the determinant. In our data, we find the same prevalence of cleft in offspring of affected mothers and fathers, suggesting no effect of sex-specific genomic imprinting and no major effect of maternal genes or mitochondrial genes. Both penetrance and expressivity are thought to be the result of gene-gene and gene-environment interaction. Penetrance refers to an all-or-none concept, while expressivity is the variable phenotype in people who carry the same disease genotype. A single abnormal gene can produce diverse phenotypic effects in different organs. The presence of multiple risk alleles at a specific locus might result in a correlation between a specific allele and a specific phenotype. The biological explanations are probably many within each of these terms. Changes in the appearance and structure of DNA, as well as changes in the DNA-sequence of a gene, might alter the genetic expression.

In our data we saw no pattern of increased severity in cleft morphology into the next generation. Increased expression of a dominantly inherited disorder in downstream generations (anticipation), as observed for myotonic dystrophy, may serve as an example of delayed biological explanation and a phenomenon that illustrates poor genotype-phenotype correlations across generations (Tramonte and Burns 2005). The underlying reason for this was unknown until it was detected that the myotonic dystrophy mutation is a DNA triplet repeat sequence that expands in successive meioses.

If the prevalence of sub-clinical clefts is more prominent in families with clefts, these families have an even higher relative risk of cleft recurrence. To add substance to this hypothesis, a prevalence study is warranted of sub-clinical clefts in the population and in first-degree relatives of individual with different cleft types.

Paper IV

In the prevalence study of the 22q11.2 deletion and duplication syndromes, the results may be biased because 35 mothers declined to consent (15%). These mothers are more likely to have some kind of disability which is associated with DiGeorge syndrome. Babies with 22q11.2

deletion or duplication syndrome are more likely to have a parent with the same genomic imbalance, as 10-15% of DiGeorge cases are inherited from an affected parent. Some of these parents have learning disabilities or psychiatric problems that make them less likely to consent to an investigation into a dominant condition of this kind.

In the Pregnancy Heredity Environment - project, cases were included from 1996 to 2001 and accompanying birth defects were included for the same period. Most children have had some years of observation, others only a few months. To the extent that defects diagnosed later are different from other defects, the picture of accompanying defects provided in our data may not be complete.

The detection of large (usually 3Mb) deletions flanked by low-copy repeats within 22q11.2 by fluorescence in situ hybridization (FISH) has been available for a long time. The routine FISH method does not detect small deletions or duplications. In addition, a more cost-effective diagnostic technique of deletions has been desirable. The multiplex ligation probe amplification (MLPA) technique was described in 2002. Recent publications have proven MLPA to be highly sensitive and accurate for detecting copy number changes in the 22q11.2 region (Fernandez, Lapunzina et al. 2005; Vorstman, Jalali et al. 2006). With a normal diploid peak area between 0.7 and 1.3, similar to that in our study, the sensitivity was 0.95 (95% CI 0.93-0.97) and the specificity 0.99 (95% CI 0.98-0.99) for any of the 39 probes in the kit (Vorstman, Jalali et al. 2006). The test-kit contains seven DNA sequences within the 22q11.2 subdomain and another four DNA sequences on chromosome 22. Most del22q11-syndrome cases, also the three positive ones in our study, have all seven of the sequences within 22q11.2 deleted. Only few patients have smaller deletions within the same region (Lindsay 2001). We expected a 22q11.2 duplication patient to have the corresponding DNA sequences duplicated, even though some of the dup22q11.2 cases reported in the literature have tended to have larger duplications (Ensenauer, Adeyinka et al. 2003). The four target sequences on chromosome 22 outside the 22q11.2 subdomain makes it possible to differentiate genomic disorders affecting larger regions.

Each of the probe signals in a sample is related to twelve internal controls and to the corresponding probe in the normal controls. The technical quality of an analysis was evaluated by the relative probe signals' deviation from 0.7-1.3. Our criteria for technical acceptance are specified in the paper. 48% of the samples had to be reanalyzed because the technical quality did not meet the standard we had set before the analyses were started. By reducing the amount of DNA from 100 to 50ng in our reanalysis we obtained diagrams that

were easier to interpret. We found that the method required manual dexterity and great precision.

The results of three small studies (a total of 91 newborns with CPO without accompanying conditions, in different European countries) are added in a publication, and a prevalence of 22q11.2 deletion syndrome of 1.1% is presented (Ruiter, Bongers et al. 2003). Their conclusion is similar to our larger, population-based study: that screening for the del22q11 and dup22q11 syndromes is not warranted in neonates with open cleft palate without the presence of other clinical signs of the syndromes.

Advances in genetic medicine and molecular genetic techniques of analysis have made the screening of the human genome possible. During the last 10-15 years, explanations have been found for a number of simple and complex genetic disorders. This has induced high expectations to genetic diagnostics. The challenge in clinical practice is to delimit the diagnostics to disorders relevant to clinical symptoms. Genetic testing must be based on thorough knowledge of clinical signs and symptoms of possible disorders. In diagnostics, “purposeful genetic testing” should be used instead of the term “screening” to emphasize the necessity of being guided by clinical features.

An infant that presents with oral cleft is seen by a pediatrician and sometimes also by a specialist in medical genetics. The procedures for further diagnosis and follow-up depend on the clinical presentation. The procedures are clear and well-defined when accompanying disorders seriously interfere with viability. Accompanying disorders that do not interfere with viability or physical well-being are given less attention and are at risk of being neglected. It is an important principle that any additional clinical symptom can be a possible sign of a more complex condition. Patients with oral clefts have a considerably higher risk of other birth defects and of syndromes that may benefit from early diagnostics and medical treatment.

The rationale for screening is early intervention in order to try to prevent subsequent morbidity. The evaluation of 22q11 screening in all newborns with open CPO has to take into account factors such as the severity of the syndromes, their prevalence in CPO patients, the consequences of diagnostic delay and the possibility of missed medical treatment. The chances of false positive and negative results and the cost of analyses must be considered, together with the knowledge of the prevalence of other clinical signs of the disorder and the subsequent chances of diagnostics in later childhood. Guidelines for genetic testing ensure a system of equal rights to medical treatment. It may prevent the physicians' choice of follow-

up being influenced by random circumstances such as the family's resources and medical knowledge and the physician's interest in syndromes.

The prevalence of 22q11.2 deletion and duplication syndrome in CPO patients and the prevalence of other accompanying defects in CPO patients with 22q11.2 deletion, studied in our work are important in the evaluation. 22q11.2 deletion syndrome is a congenital disorder with several serious implications (disturbed cardiac outflow, impaired parathyroid glands and T-cell function, learning difficulties and psychiatric disorders) and it is important to work for early diagnosis. However, in newborns with open CPO we found neither 22q11.2 deletion nor 22q11.2 duplication in the absence of other clinical features of these conditions. Therefore, it is reasonable to conclude that genetic screening of 22q11.2 deletion in all newborns with open CPO is not warranted. Genetic testing of 22q11.2 deletion should be restricted to patients with additional signs of the disorder.

CONCLUSIONS

Cleft treatment has been centralized and carefully organized in Norway for more than 30 years. Together with the compulsory registration of births in the Medical Birth Registry this has created favorable conditions for conducting epidemiological and genetic studies of oral clefts. Identification of common and uncommon features in the heterogeneous cleft phenotypes is needed both in clinical practice and in etiology research. We find a modification of Kernahan's Y-diagram and Schwartz's three-digit numerical coding well suited to providing a population-based reference distribution for 63 common and rare cleft variants and to characterizing the three commonly used categories of clefts. The classification of cleft morphology serves as a detailed map of phenotypes made ready for further epidemiological and genetic characterization: Clefts of the lip or palate were more severe when both types of clefts were present. Cleft lip was more frequent on the left side, but not more severe on the left than on the right side. Girls were more likely to have severe clefts, as were cases that had non-cleft birth defects.

We continued the characterization of cleft phenotypes in our other three studies:

The reporting of the oral cleft birth defect to the MBRN depended heavily on the severity of the cleft. To the degree that severity is related to the etiologic factors producing the cleft, the systematic under-representation of mild cleft cases may have practical importance both for surveillance and for discovery of preventable causes. The large proportion of non-reported cleft palate cases discloses insufficient cleft palate diagnostics at neonatal clinical examinations.

The recurrence risks of cleft lip (with or without cleft palate) and cleft palate alone were 31-fold and 56-fold, respectively, and significantly different. Cleft lip and cleft palate alone have almost distinct genetic causes which seem to work mainly through the fetal genes. The anatomic severity of a cleft did not affect the recurrence risk of clefts in first degree relatives. This weakens the multifactorial threshold model as a model of cleft inheritance and opens for a genetic model in which severity is independent of genes predisposing for oral clefting.

In a population-based sample of 191 newborns with open cleft palate referred for surgery, we found three cases with 22q11.2 deletion (1 of 57) and no cases with 22q11.2 duplication. All three cases with deletion also had congenital heart defects. We conclude that neither testing for 22q11.2 deletion nor for duplication is warranted in babies when open cleft palate is the only finding.

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Appendix

CLP Treatment Protocol, Check-list

	ORTHODONTICS	PLASTIC SURGERY	SPEECH	EAR, NOSE & THROAT
AFTER BIRTH	Presurgical? Surgeon decides. (Only in few cases: wide clefts or marked asymmetry)			
1 MONTH	Every Months:	INFORMATION SEMINAR FOR PARENTS BY THE COMPLETE TEAM.		
3 MONTHS		Closure of lip and anterior palate. BCLP at 5 weeks interval (Millard technique).	Parent counseling on speech development.	Presurgical control.
12 MONTHS		Closure of hard and soft palate (von Langenbeck technique).	Further parent counseling, contact local speech therapist.	Presurgical control. If necessary, myringotomy with tube insertion. Information to local ENT-specialist.
2 YEARS			Age 2 and 3 years: phone calls to parents.	Recommended controls every 4 months.
4 YEARS	Control.	Control. BCLP: Sulcus plasty, Columella plasty.	Diagnosis of speech development, articulation and nasality. Report to local professionals.	Examination of ears, nose and throat. Hearing test.
5 YEARS			Individual control.	Nasopharyngoscopy in a few cases of VPI.
6 YEARS	CLP CONFERENCE DAY: April or October 1. THE COMPLETE TEAM DIAGNOSES ALL PATIENTS OF THIS AGE GROUP. 2. TEAM ASSEMBLY DISCUSSES ALL INDIVIDUAL TREATMENT PLANS. 3. INDIVIDUAL LETTERS WITH TREATMENT PLAN TO ALL PATIENTS / PARENTS.			
6-7 YEARS	Interceptive ortopedics? (by maxillary deficiency). a. Transverse expansion, b. Protraction, c. Retention (fixed).			
7-8 YEARS	Alignment of upper incisors?			
10 YEARS	Control.	Secondary bone grafting? (8-11 yrs)	Individual controls and treatment if necessary.	Individual controls of ears and hearing status.
11-13 YEARS	Conventional orthodontics			In some cases nasopharyngoscopy or surgical treatment of ears.
14 YEARS	Bonded retainer palatal upper incisors?			
15 YEARS	CLP CONFERENCE DAY: April or October 1. THE COMPLETE TEAM DIAGNOSES ALL PATIENTS OF THIS AGE GROUP. 2. TEAM ASSEMBLY DISCUSSES ALL INDIVIDUAL TREATMENT PLANS. 3. INDIVIDUAL LETTERS WITH TREATMENT PLAN TO ALL PATIENTS..			
16-19 YEARS	Dental adjustments? related to Orthognathic surgery or Prosthodontics.	Adjustment: Lip and nose?	Individual controls and treatment if necessary.	In some cases surgical treatment of nose.

Table 1 in supplementary materials, Paper I

Frequency of sub-categories of 1443 cleft cases in **girls** by grading the degree of clefting in the right lip (R), the palate (P) and the left lip (L) in the RPL coding system: Cleft lip only (352 cases), Cleft lip and palate (405 cases) and Cleft palate only (686 cases).

		<u>010</u>	<u>118</u>	<u>020</u>	<u>278</u>	<u>030</u>	<u>290</u>	CLEFT PALATE ONLY		
<u>000</u>	<u>905703</u>									
<u>001</u>	101	<u>011</u>	1	<u>021</u>	-	<u>031</u>	3			
<u>100</u>	58	<u>110</u>	2	<u>120</u>	-	<u>130</u>	2			
<u>002</u>	66	<u>012</u>	5	<u>022</u>	7	<u>032</u>	20			
<u>200</u>	31	<u>210</u>	2	<u>220</u>	4	<u>230</u>	11			
<u>003</u>	46	<u>013</u>	2	<u>023</u>	-	<u>033</u>	145			
<u>300</u>	19	<u>310</u>	1	<u>320</u>	2	<u>330</u>	84			
<u>102</u>	1	<u>112</u>	-	<u>122</u>	-	<u>132</u>	1			
<u>201</u>	-	<u>211</u>	1	<u>221</u>	-	<u>231</u>	2			
<u>103</u>	2	<u>113</u>	-	<u>123</u>	-	<u>133</u>	7			
<u>301</u>	2	<u>311</u>	-	<u>321</u>	-	<u>331</u>	4			
<u>203</u>	1	<u>213</u>	-	<u>223</u>	-	<u>233</u>	6			
<u>302</u>	4	<u>312</u>	-	<u>322</u>	-	<u>332</u>	6			
<u>101</u>	9	<u>111</u>	-	<u>121</u>	-	<u>131</u>	1			
<u>202</u>	4	<u>212</u>	1	<u>222</u>	-	<u>232</u>	6			
<u>303</u>	8	<u>313</u>	-	<u>323</u>	-	<u>333</u>	79			
									CLEFT LIP AND PALATE	

Table 3 in supplementary materials, Paper I

Frequency of sub-categories of 272 cleft cases in **females with accompanying defects** by grading the degree of clefting in the right lip (R), the palate (P) and the left lip (L) in the RPL coding system: Cleft lip only (19 cases), Cleft lip and palate (65 cases) and Cleft palate only (188 cases).

		<u>010</u>	<u>30</u>	<u>020</u>	<u>70</u>	<u>030</u>	<u>88</u>
<u>000</u>	20917						
<u>001</u>	4	<u>011</u>	-	<u>021</u>	-	<u>031</u>	-
<u>100</u>	4	<u>110</u>	1	<u>120</u>	-	<u>130</u>	-
<u>002</u>	3	<u>012</u>	-	<u>022</u>	-	<u>032</u>	1
<u>200</u>	1	<u>210</u>	-	<u>220</u>	1	<u>230</u>	-
<u>003</u>	2	<u>013</u>	1	<u>023</u>	-	<u>033</u>	17
<u>300</u>	4	<u>310</u>	0	<u>320</u>	-	<u>330</u>	16
<u>102</u>	-	<u>112</u>	-	<u>122</u>	-	<u>132</u>	-
<u>201</u>	-	<u>211</u>	-	<u>221</u>	-	<u>231</u>	-
<u>103</u>	-	<u>113</u>	-	<u>123</u>	-	<u>133</u>	0
<u>301</u>	-	<u>311</u>	-	<u>321</u>	-	<u>331</u>	1
<u>203</u>	-	<u>213</u>	-	<u>223</u>	-	<u>233</u>	1
<u>302</u>	1	<u>312</u>	-	<u>322</u>	-	<u>332</u>	3
<u>101</u>	-	<u>111</u>	-	<u>121</u>	-	<u>131</u>	-
<u>202</u>	-	<u>212</u>	1	<u>222</u>	-	<u>232</u>	2
<u>303</u>	-	<u>313</u>	-	<u>323</u>	-	<u>333</u>	20

CLEFT LIP ONLY

CLEFT LIP AND PALATE

CLEFT PALATE ONLY

Table 4 in supplementary materials, Paper I

Frequency of sub-categories of 330 cleft cases in **males with accompanying defects** by grading the degree of clefting in the right lip (R), the palate (P) and the left lip (L) in the RPL coding system: Cleft lip only (47 cases), Cleft lip and palate (110 cases) and Cleft palate only (173 cases).

	<u>000</u>	<u>25655</u>	<u>010</u>	<u>21</u>	<u>020</u>	<u>63</u>	<u>030</u>	<u>89</u>	CLEFT PALATE ONLY
<u>001</u>	12		<u>011</u>	-	<u>021</u>	1	<u>031</u>	0	
<u>100</u>	4		<u>110</u>	-	<u>120</u>	-	<u>130</u>	-	
<u>002</u>	7		<u>012</u>	-	<u>022</u>	1	<u>032</u>	4	
<u>200</u>	5		<u>210</u>	-	<u>220</u>	-	<u>230</u>	2	
<u>003</u>	10		<u>013</u>	-	<u>023</u>	1	<u>033</u>	31	
<u>300</u>	2		<u>310</u>	1	<u>320</u>	1	<u>330</u>	25	
<u>102</u>	1		<u>112</u>	-	<u>122</u>	-	<u>132</u>	1	
<u>201</u>	-		<u>211</u>	-	<u>221</u>	-	<u>231</u>	-	
<u>103</u>	-		<u>113</u>	-	<u>123</u>	-	<u>133</u>	1	
<u>301</u>	2		<u>311</u>	-	<u>321</u>	-	<u>331</u>	5	
<u>203</u>	-		<u>213</u>	-	<u>223</u>	-	<u>233</u>	-	
<u>302</u>	-		<u>312</u>	-	<u>322</u>	-	<u>332</u>	2	
<u>101</u>	3		<u>111</u>	-	<u>121</u>	-	<u>131</u>	1	
<u>202</u>	1		<u>212</u>	-	<u>222</u>	-	<u>232</u>	-	
<u>303</u>	-		<u>313</u>	-	<u>323</u>	-	<u>333</u>	33	

CLEFT LIP ONLY

CLEFT LIP AND PALATE

Paper I

Sivertsen, Å; Wilcox, A. J.; Johnson, G. E.; Åbyholm, F. E.; Vindenes, H. A.; Lie, R. T.,
Prevalence of major anatomic variations in oral clefts. Preprint. To be published as Major
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Lippincott Williams & Wilkins.

Prevalence of major anatomic variations in oral clefts

Abstract

Background: We describe morphological variations of oral clefts in a large population-based sample, especially variations in severity and laterality. We present 3616 cleft cases treated in Norway for oral clefts between 1967 and 1998.

Methods: Classification of cleft morphology was based on clefting in nine anatomic focal areas anterior and posterior to the incisive foramen. A three-digit coding system provides a total of 63 possible cleft combinations. Their distribution in the population is presented as a whole and stratified by baby's sex and the presence of accompanying malformations. The relative proportion of cleft types is illustrated in modified striped-Y Kernahan diagrams.

Results: Clefts of the lip or palate are more severe when both types of clefts are present. Among babies with cleft lip, 18% lips were severe (i.e. complete cleft of the primary palate) in the absence of cleft palate, compared with 81% severe when cleft palate was also present. Similarly, among babies with cleft palate, 40% were severe (complete cleft of the secondary palate) in the absence of cleft lip, compared with 93% when cleft lip was also present. The more severe the cleft lip, the more likely that the baby had an accompanying cleft palate. Girls were more likely to have severe clefts, as were cases who had other types of birth defects. Although cleft lip was more frequent on the left side, clefts were not more severe on the left side. In bilateral cleft lip, severity was similar on both sides.

Conclusion: Our data provide a population-based reference for common and rare variants of oral clefts.

Paper II

ORIGINAL ARTICLE

Completeness of registration of oral clefts in a medical birth registry: a population-based study

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Abstract

Background. Epidemiological surveillance and research on birth defects require accurate diagnosis and adequate registration. In this regard, the performance of national birth registries is not well described. **Methods.** We linked clinical data from all 3,616 cleft cases treated in Norway between 1967 and 1998 with data from the Medical Birth Registry of Norway, and calculated the proportion of clinically verified cases reported to the Registry, stratified by severity. **Results.** The cleft type most completely ascertained was cleft lip and palate (CLP), of which 94% were reported. Ascertainment was less complete for cleft lip alone (83% recorded), and cleft palate only (CPO) (57% recorded). For each of the three types of clefts, completeness of reporting depended on severity of the cleft. For example, 71% of cases with severe CPO were reported, while only 11% of cases with mild CPO were reported. **Conclusions.** Ascertainment was strongly related to cleft type and severity. To the degree that severity of birth defects may be related to their cause, these patterns of registration have implications for surveillance of birth defects as well as the conduct of etiologic studies. The large proportion of cleft palate cases unrecorded at birth suggests that clinical examination of the newborn palate is often inadequate.

Key words: Oral cleft, ascertainment, birth registry

Background

Since its establishment in 1967, all births in Norway have been reported to the Medical Birth Registry of Norway (MBRN). Birth defects detected during the initial stay of up to 7 days at the delivery unit are recorded as part of the report. Diagnoses are made by physicians, while midwives are responsible for the reporting (1). Similar medical birth registries operate in all Nordic countries and several other countries. Together with Norway, many of these registries collaborate in international scientific networks (www.eurocat.ulster.ac.uk; www.icbd.org). A shared objective for these registries is to provide epidemiological surveillance of new epidemics of birth defects (caused for example by new medical treatments), and to provide a basis for etiological research on birth defects. To serve this purpose, it is

crucial to ensure that the data provided by the birth registries is of high quality. Sound diagnosis and relatively complete reporting of birth defects are necessary to maintain high standards of data quality.

Medical birth registries presumably have more complete registration of major than minor birth defects, although information on this is limited. Oral clefts are a category of birth defects with a particularly large range of anatomic variations. There are minor forms of both cleft lip and cleft palate that may easily be overlooked, while the most severe forms of cleft lip and palate (CLP) are unmistakable, and require extensive treatment.

We established a population-based database of 3,616 cleft cases that received surgical treatment in Norway in the period 1967–1998. These cases are clinically verified and carefully described. In

particular, they have been graded by anatomic severity of the defect (2). By linking these clinical data with the MBRN (1.9 million births), we were able to estimate variations in registration according to the severity of the defect.

Materials and methods

Surgical treatment of oral clefts in Norway has been centralised in two major hospitals, Haukeland University Hospital in Bergen and Rikshospitalet University Hospital in Oslo. Virtually all cleft cases in the country have been sent to one of these two hospitals for treatment. The two surgical centres co-operate in the treatment of clefts, and shared routines have been established for documentation of clefts. For example, all patients have preoperative photos in their medical records, supplemented since 1970 with study models.

The MBRN includes all live births and stillbirths with a gestational age of 16 weeks or more. Birth defects identified at the maternity unit are recorded on a standard form and then reported to the registry. The MBRN includes unique personal identification numbers for all births in Norway. This person's specific number is also the identification used in the medical records. These identifiers permit linkage of clinical and birth registry records with virtually 100% match. Stillbirths and terminated pregnancies (for which no clinical data are available) were not included in the study.

Cleft cases were classified in the MBRN according to the International Classification of Diseases, Eight Revision (ICD-8). The 3-digit ICD-8 code indicates the oral cleft birth defect, while a fourth digit lists cleft lip, cleft palate and CLP separately. Based on the clinical data obtained from medical records (including photos and study models), the 3,616 cleft cases have been classified in a separate 3-digit numerical coding system (2,3). In this coding system, the first number describes right-sided cleft lip, the middle number describes cleft palate, and the third number describes left-sided cleft lip. In this right-palate-left (RPL) system, the digit at a given position represents severity (0 = none to 3 = severe). For example, a severe cleft palate without cleft lip would be '030'.

More specifically, the RPL system codes normal embryonic development of the primary palate as '0' (position R or L). '1' (mild) in the R or L position includes everything from minor scarring to a well-defined cleft lip, while '2' (moderate) represents a cleft in the lip and alveolar ridge. A complete cleft of the lip and primary palate, as far as to the incisive foramen, is coded '3' (severe).

In the middle position (P) of the three-digit code, '0' represents normal embryonic development of the secondary palate and '1' (mild) represents submucous cleft in the soft palate. A visible cleft in the soft palate is coded '2' (moderate), and a cleft that involves the hard palate is coded '3' (severe).

Based on the RPL code, we grouped the 3,616 cleft cases in the clinical material into three main categories. Cleft lip only (CLO) are clefts anterior to the incisive foramen without posterior involvement ($n=992$). CLP includes clefts involving structures both anterior and posterior to the incisive foramen ($n=1,400$). Cleft palate only (CPO) are clefts posterior to the incisive foramen without anterior involvement ($n=1,224$). Our analyses were carried out separately for these main categories.

The RPL coding is a numerical version of the 'striped Y of Kernahan' (4,5), a visual representation of the cleft defect. In this Y-diagram, R corresponds to the first upper limb of the Y, P to the stem of the Y, and L to the second upper limb of the Y (Figure 1). The percentage of cases reported at birth is represented by the white area within the box (Figures 2–4). These percentages are also presented as numbers in the figures. These figures correspond to the three main categories of clefts, and the segments within

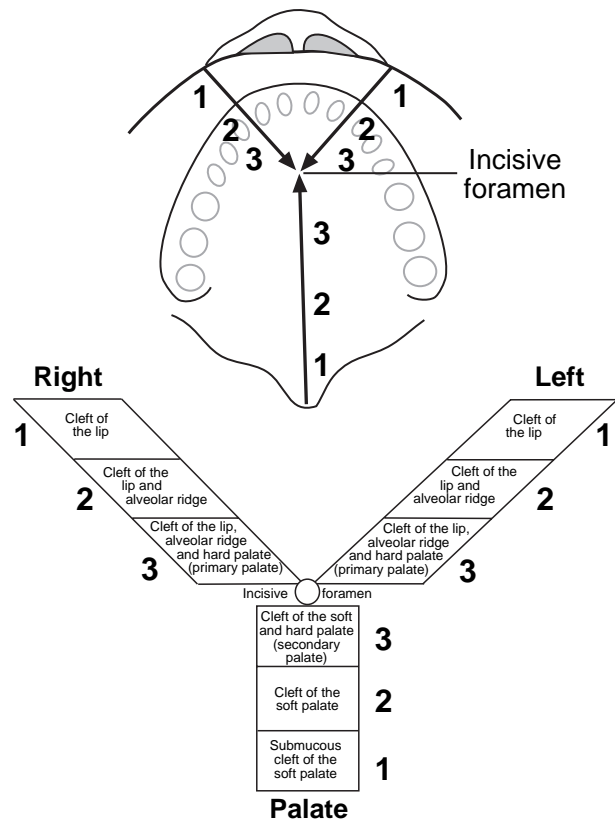


Figure 1. Morphological structures corresponding to a grading of 1, 2 or 3 of cleft severity.

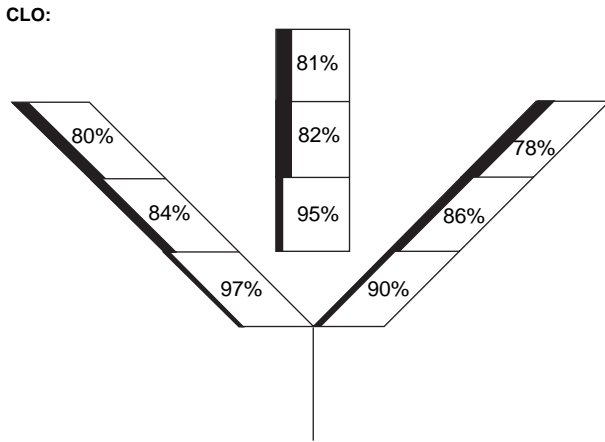


Figure 2. The proportion of cleft cases reported to the Medical Birth Registry of Norway in relation to severity of the cleft defect in the category cleft lip only. The percentage of cases reported within each subgroup of severity of the cleft defect is represented by the size of the white area within the box. Bilateral cleft lips are displayed as a separate branch between the upper limbs of the Y.

each figure correspond to the subgroups of severity. The proportions of cleft cases not reported to the registry are shaded in figures.

For cases in the CLP category (Figure 3), both the cleft lip and the cleft palate are marked in the Y-diagram. Bilateral cleft lips are displayed as a separate branch between the upper limbs of the Y-diagram. The more severe of the two cleft lip

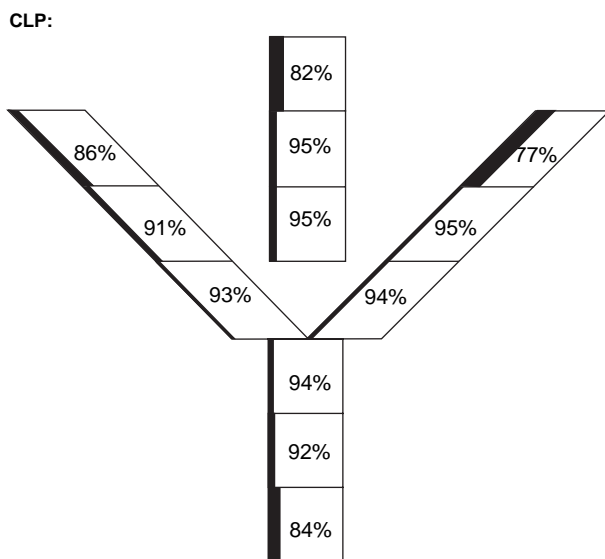


Figure 3. The proportion of cleft cases reported to the Medical Birth Registry of Norway in relation to severity of the cleft defect in the category cleft lip and palate. The percentage of cases reported within each subgroup of severity of the cleft defect is represented by the size of the white area within the box. Bilateral cleft lips are displayed as a separate branch between the upper limbs of the Y. Note that the grade of clefting in the primary and secondary palate are presented separately. Every cleft lip and palate case is, therefore, represented twice.

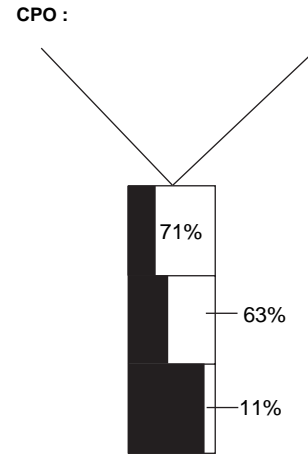


Figure 4. The proportion of cleft cases reported to the Medical Birth Registry of Norway in relation to the severity of the cleft defect in the category cleft palate only. The percentage of cases reported within each subgroup of severity of the cleft defect is represented by the size of the white area within the box.

defects defines the severity of a bilateral cleft in the Y-diagram. For example, the RPL code '201' refers to a bilateral cleft lip without cleft palate, in which the right-sided cleft lip is moderate, and the left-sided cleft lip is mild. This case would be in the branch for bilateral clefts between the upper limbs in the middle box ('moderate').

When clinical data were linked with data from the Registry, we found a total of 639 cleft cases in the MBRN that were not present in the clinical data. 151 of these were stillborn, while 195 died during the first year of life; the remaining 293 could not be accounted for. Some in the last group may have received surgical treatment in other hospitals in or outside the country. They may also have had other birth defects severe enough to postpone surgical treatment of the cleft or they may have been erroneous diagnoses.

We estimated trends in ascertainment by the registry by grade of severity, and calculated test-for-trend *p*-values by log-binomial regression using STATA version 9.2. We also tested for a change in ascertainment by dividing the time period into two parts: 1967–1990 and 1991–1998.

Results

The proportion of clefts registered in the MBRN was highest for CLP (94%). For CLO, 83% were registered, while 57% were registered for CPO. The completeness of registration at birth was strongly related to severity of the defect. The proportion of left-sided CLO cases registered at birth was 78% for mild cases, 86% for moderate cases, and 90% for severe cases (Figure 2). These percentages were

similar for right-sided cleft lip, and the trend was also present for bilateral cleft lip. Overall, the trend in registration by severity of CLO was highly significant ($p < 0.001$).

The registration of cleft lip was more complete when accompanied by a cleft palate ($p = 0.01$) (Figure 3). In the presence of cleft palate, the proportion of registered cases of left-sided cleft lip was 77% for mild cases, 95% for moderate, and 94% for severe cases. This was very similar for right-sided and bilateral cases (Figure 3). The overall trend in registration by severity was again significant ($p = 0.008$).

Severity was most strongly associated with registration for CPO (Figure 4). Among the mild, submucous clefts of the soft palate (still serious enough to require surgery), 11% of cases were registered at birth. 62% of newborns with a moderate cleft palate were registered. For patients with a severe cleft involving the hard palate, the proportion of cases registered was 71%. This trend with severity was once again highly significant ($p < 0.001$).

The registration of cleft palate was better when accompanied by a cleft lip ($p < 0.001$) (Figure 3). Among CLP cases, 84% of the mild cleft palates, 92% of the moderate, and 94% of the severe were registered.

The proportion of registered cases with cleft lip decreased in the birth registry over time, from 90% in 1967–1990 to 87% in 1990–1998 ($p = 0.04$). In contrast, there was little evidence for a change in the proportion of cleft palate cases reported to the registry (from 56% in 1967–1990 to 59% in 1990–1998 ($p = 0.33$)).

Discussion

This study provides estimates of the completeness of registration for oral clefts in a national birth registry, linking comprehensive clinically verified data from the two national centres of cleft treatment with data from the MBRN.

The registration of cleft lip (CLO or CLP) was more complete than the registration of CPO. This suggests better registration of externally visible clefts (such as cleft lip) than clefts that require a diagnostic procedure (such as opening the child's mouth for inspection and palpation of the palate).

The registration of an oral cleft depended heavily on the anatomical severity. This was the case for all three main categories of clefts, but was most evident for CPO. 11% of all mild cleft palate cases were reported at birth, compared with 71% of the severe cleft palate cases.

Virtually all cases operated for an oral cleft in Norway over a period of 31 years (1967–1998) are included in our data. The 2 treatment centres have routinely carried out high quality documentation needed for modern multidisciplinary treatment that favours our retrospective classification. The classification was provided by two experienced clinicians with special interest in cleft treatment (2). The clinical diagnosis of submucous cleft palate was most often given in early school age because the diagnosis assumes velopharyngeal insufficiency with hypernasality and articulation failure in the patient (6,7).

The MBRN operated with the same routines for registration through the whole period and collected data from the newborns first week of life. Based on comparison with a paediatric database in the 1980s, Lie *et al.* estimated the proportions of cleft lip or cleft palate cases reported to the MBRN (8). Stillbirths were included in their study. They found that the overall estimated proportion of cases reported was 78%. In another analysis of Norwegian data restricted to live births (9), the proportion of cleft cases ascertained by the MBRN was 93% for CLP and 83% for CLO.

In our study, only one in ten with submucous cleft palate had the cleft diagnosed in the clinical newborn examination, presumably because the detection of submucous cleft palate in the newborn is difficult. In contrast, an open cleft in the hard or soft palate should be quite easy to detect in the newborn. The fact that one in three newborn babies with open cleft palate was not recorded in the Registry is a matter of concern. It suggests that many babies with cleft palate were not diagnosed until after their first week of life. Early diagnosis of the cleft palate is important also because up to 50% of the babies with cleft palate have other congenital birth defects (10). Thus, a diagnosis of cleft palate should trigger an even more careful evaluation of the newborn. The incomplete registration of open cleft palate in Norway suggests a lack of care in the clinical examination of the newborn palate. This is consistent with a recent paper suggesting the need for more vigilance in detection of cleft palate at the neonatal clinical examination (11).

Perhaps more surprising, there was also a tendency to miss cases of mild and moderate cleft lip in the birth registry, even though they are clearly visible and require surgery. This under-representation of mild clefts may have consequences for research based on registry data. For example, studies examining teratogens that are associated mostly with mild cleft defects may underestimate the importance of such teratogens.

The ascertainment of clefts did not change markedly over time. After a period with very high ascertainment (90%), the ascertainment of cleft lip was slightly poorer after 1990. Ascertainment depends both on the availability of a diagnosis and on the reporting. A revision of the registration in 1998 has extended the responsibility for reporting from midwives at obstetric units to pediatric departments and others, in order to capture birth defects with a more complicated diagnosis. The revision also introduced a 12-month follow-up period of all newborns and reporting of terminated pregnancies after the 12th gestational week. For cleft lip, however, diagnosis may be a smaller problem than the problem of responsibility for the reporting.

Some surveillance systems are based entirely on birth records, while others work with more specific notification of malformed infants. The efficiency of record systems depends highly on local tradition and organisation. Few data are available on the quality of registration of oral clefts in various registries. No previous study has been able to examine registration in relation to severity of clefts. In a Danish study from 1992, based on multiple sources of ascertainment, the completeness of registration of oral clefts in the Register of Congenital Malformations was more than 90% (12). The under-reporting of cleft palate in this study was, to some extent, because cleft in the soft palate was wrongly diagnosed as 'bifid uvula'.

Our analysis included only patients referred for treatment of their oral cleft. Cleft cases among stillborns terminated pregnancies and among children who died before referral are therefore not included. We know little about whether the trends we observe may be generalised to those cases. It is, however, unlikely that ascertainment of clefts in a registry is better among these groups.

The completeness of clefts ascertainment in the Norwegian birth registry was clearly related to the severity of the cleft. One in three patients with open cleft palate was not recorded at birth, which suggests incomplete examination of the palate in the

newborns. The identification of severe cleft lip is fairly complete, but much reduced for less severe clefts. To the degree that severity is related to the etiologic factors producing the cleft, this systematic under-representation of mild cases may have practical importance both for surveillance and for the discovery of preventable causes.

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Paper III

Familial risk of oral clefts by morphological type and severity

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Abstract

Background The tendency for clefts to recur in families is very strong and point to a genetic contribution to the etiology. Clefts vary substantially in morphology, but little is known about recurrence risks for subcategories of clefts.

Material and methods Data on patients treated in the two national treatment centres in the period 1967 to 2001 were linked to the population-based Medical Birth Registry of Norway (4138 cases and 2.1 million births). Detailed clinical information on cleft morphology was combined with virtually complete ascertainment of biological family members for the whole country over a 35-year period. We estimated the recurrence risk of an isolated oral cleft from parent to child and between full siblings for anatomic subgroups of clefts.

Results The risk of clefts among children of affected mothers and affected fathers was similar, and also similar for full siblings of affected children. The recurrence risks of cleft lip (with or without cleft palate) and cleft palate alone were 31.5-fold (95% CI: 24.6-40.3) and 56.2-fold (95% CI:37.2- 84.8), respectively, and significantly different (p diff=0.02). The cross-over risk between CL and CPO was increased (RR=3.0, 95% CI, 1.3-6.7). We found no effect of cleft lip or cleft palate severity on recurrence risk, and the mean severity of cleft in the recurrent cases was similar after mild, moderate or severe clefts.

Conclusion Cleft lip and cleft palate alone have almost distinct genetic causes which seem to work mainly through the fetal genes. The anatomic severity of a cleft does not affect the recurrence risk of clefts in first degree relatives, which weakens the multifactorial threshold model as a model of the effect of genes and environmental factors on risk.

Oral clefts are among the most common birth defects. The birth prevalence in Norway is 2.2 per 1000 live births, (Sivertsen, Wilcox et al. In Press) among the highest rates of clefts in the western world. The defects range from mild forms to complete clefts affecting both the lip and the palate. Although the Online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov/Omim/) lists more than 400 Mendelian disorders associated with oral clefts, the majority of oral clefts appear as isolated defects without signs of malformation syndromes. The genetic and environmental causes of non-syndromic oral clefts are largely unknown.

Given the uncertainty about its causes, the tendency for clefts to recur in families is striking. Fogh-Andersen (1942) used familial recurrence to show that cleft palate is etiologically different from cleft lip with and without cleft palate, demonstrating that families at high risk for one are not at increased risk for the other (Fogh-Andersen 1942). Recurrence risks as estimated from epidemiological studies are regularly used in genetic counselling (Harper 1998; Firth and Hurst 2005).

We used a population-based study of clinically-verified cases to estimate familial recurrence risks in first degree relatives, and to describe recurrence risk by severity of the cleft. We also considered whether having a cleft of a certain type (or having a child with a cleft) affects subsequent reproduction.

Material and Methods

The population-based Medical Birth Registry of Norway includes all children born in Norway since 1967 (about 2.1 million). All live births and stillbirths at a gestational age of at least 16 weeks are included in the registry. Babies born with oral clefts in Norway are treated in one of two national centers, Haukeland University Hospital and Rikshospitalet University Hospital. From 1967 to 2001, there were 4138 patients with oral clefts treated in these hospitals. The morphological classification of the clefts was carried out by two experienced clinicians for consistent application of the definitions of severity. We linked these clinical data to the population registry using Norway's unique national person identification numbers. This allowed us to combine high-quality clinical information on cleft morphology with virtually-complete ascertainment of biological family members for the whole country over a 35-year period.

Permission for this study was obtained from the Regional Committee on Research Ethics for Western Norway and the Data Inspectorate of Norway.

There were 944 908 babies born in Norway between 1967 and 1983. We excluded plural births from these analyses, since twinning has a heritable component and is also associated with the risk of oral clefts (Robert, Kallen et al. 1996). We were able to follow this cohort of children to 2001 when the cohort was between 18 and 34 years of age. During this time, 367 301 became parents of babies reported in the registry (Figure 1).

We calculated rates of reproduction in persons with and without oral clefts. Recurrence risk of facial clefts from parent to child was expressed as relative risk (RR) with 95% confidence intervals (CI). Those relative risks were estimated by odds ratios in logistic regression models in STATA 9.2. Correlation between repeated observations within nuclear families was accounted for using robust estimation of variances. When risks of two different outcomes were compared, for example risk of CPO after CL with CL after CPO, the reference group was split randomly in two halves that were used as reference for each of the two risks.

We also linked full siblings in order to estimate the recurrence risk of clefts in sibships. By 2001, there were 572 772 babies who had at least one subsequent full sibling (with the same mother and father) (Figure 2). Again, recurrence risk was estimated as relative risk of recurrence.

We estimated the recurrence risk of the same or dissimilar cleft types among these first-degree relatives using three categories of clefts: cleft lip only (CLO), cleft lip and palate (CLP) and cleft palate only (CPO) (Table 1). CLO includes cases of cleft lip confined to the lip or primary palate. CLP includes cases in which the cleft extends through the primary palate into the secondary palate, while CPO includes cases with clefting in the secondary palate only. We also combined CLO and CLP into the more general category of “cleft lip” (CL) (Table 2, Table 3).

We graded the severity of the cleft based on the morphological details of the cleft in the clinical records (initial surgical report, photos and study casts) (Sivertsen, Wilcox et al. In Press). Cleft lip was classified by laterality (right or left), and graded “1” (mild) when restricted to the lip, “2” (moderate) with cleft in the lip and alveolar ridge and “3” (severe) with complete cleft of the primary palate. Cleft palate was graded “1” (mild) in cases with submucous cleft of the soft palate, “2” (moderate) with overt cleft of the soft palate, and “3” (severe) with complete cleft of the secondary palate.

Cleft cases with any other birth defect were excluded because of the possibility that syndromic cases might have a different underlying genetic predisposition with different recurrence risk. The index cleft cases were also restricted to cases who had been referred to surgical treatment, since these cases had a morphological description of their cleft. When

estimating recurrence risks we considered the total risk of oral clefts, including stillbirths, cases with other birth defects, and cases in the registry who never received surgery (usually because of death). In the estimations of severity and side-location of the cleft in the recurrent cases, we could only use the clinically verified cases since these had a morphological description of their clefts. If the cleft description in the clinical record did not match the diagnosis in the Medical Birth Registry, we used the diagnosis in the clinical record.

Results

Follow-up from parent to child

We followed children born 1967 through 1983 and in 2001 they were at least 18 years of age. Of the females born with a cleft, 45% had become mothers by 2001 compared with 48% of females without a recorded birth defect ($p=0.16$) (Figure 1). Among males, 24% of those with a cleft and 30% of those without birth defects had become fathers ($p<0.001$). Among those who had become parents, the total number of children was the same (mean 1.9) whether or not the parent had oral clefts. Overall, the prevalence of clefts in offspring was 3.6% for mothers with oral clefts and 4.7% for fathers with oral clefts, while the prevalence was 0.2% for parents without clefts.

Follow-up in siblings

There were 1554 women in the registry whose first registered child had an oral cleft (Figure 2). Of these women, 879 (57%) had a subsequent child with the same father, similar to the proportion of mothers whose first registered child had no defect. Among the subsequent siblings of the clefts cases, 4.6 % had an oral cleft, compared with 0.2% of the siblings of unaffected babies.

Specificity in recurrence of cleft types

The estimates of recurrence were not different for parents-offspring recurrence and sibling recurrence neither for CLO ($p \text{ diff}=0.32$), for CLP ($p \text{ diff}=0.21$), nor for CPO ($p \text{ diff}=0.86$) (estimates shown in Table 1). We therefore pooled the generational data with the full-sibling data to estimate joint recurrence risks of subgroups of clefts for first-degree relatives.

When we compared the relative risk of recurrence of CLO (RR=30.0, 95% CI: 16.6-54.2) with that of CLP (RR=41.1, 95% CI: 27.8-60.7), we found no evidence of difference ($p=0.38$). The recurrence risk of CLO was also similar after CLO and CLP ($p \text{ diff} = 0.50$) and

the recurrence risk of CLP was similar after CLO and CLP (p diff=0.36). Since these numbers support the assumption that CLO and CLP are just different expressions of the same underlying condition, we pooled those categories into a category denoted CL of all isolated cleft lip cases with or without cleft palate.

Recurrence for first-degree relatives combined

The overall recurrence risk of CL was estimated at 31.5-fold (95% CI: 24.6-40.3) (Table 2). There was no difference in recurrence of CL from mother to offspring and from father to offspring (relative risks of 27.1 and 26.6, respectively; p diff=0.97). Sibling recurrence of cleft lip was slightly higher than recurrence from parents to offspring (relative risks of 35.1 and 26.7, respectively), but these estimates were not significantly different.

The overall recurrence risk of CPO was 56.2-fold (95% CI:37.2- 84.8) (Table 2), and significantly different from recurrence of CL (p diff=0.02). Although CL and CPO has been considered distinct defects, the combined cross-over risk of CL after an index case had CPO and risk of CPO after an index case had CL was significantly increased (RR=3.0, 95% CI, 1.3-6.7, p =0.007). There were no differences between mother-offspring and father-offspring recurrence or between parent-offspring and sibling recurrence for CPO (Table 2).

Recurrence by severity

Relative risk of recurrence of CL was 25.7 after a mild CL (severity 1), 42.8 after a moderate CL (severity 2), and 31.2 after severe CL (severity 3) (p diff=0.53) (Table 3). The mean severity of CL in the recurrent case was 2.4 after a mild CL, 2.3 after a moderate CL, and 2.3 after a severe CL. Recurrence relative risk was 29.6 with unilateral CL in the index case and 39.2 with bilateral CL (p diff=0.35).

We repeated similar analysis for cleft palate. Relative risk of recurrence was 43.9 after mild (submucous) CPO, 41.4 after moderate (soft palate) CPO, and 81.9 after severe (hard palate) CPO (p diff=0.34). The mean severity of cleft palate was 1.5 after mild CPO, 2.1 after moderate CPO, and 2.5 after severe CPO.

The risk of cleft lip recurrence in first degree relatives was not different with right- or left-sided unilateral CL (data not shown) (p diff=0.59). There was no apparent tendency for the CL in recurrent cases to occur on the same side as the index case. The well-known left predominance of cleft lip was found in cases born into unaffected families as well as in the recurrent cases.

Discussion

Oral clefts have one of the highest rates of familial recurrence of any class of birth defects (Lie, Wilcox et al. 1994; Skjaerven, Wilcox et al. 1999; Lie, Wilcox et al. 2001). Confining our analysis to isolated or assumed non-syndromic cases, we found very similar rates of recurrence among both types of first-degree relatives (siblings and parent-child). This has also been observed in previous studies (Mitchell and Risch 1992; Christensen and Mitchell 1996; Mitchell and Christensen 1996; Lidral and Murray 2004). Accordingly, we pooled all first-degree relatives in our estimation of recurrence risks.

As in the classic study of Fogh-Anderson (Fogh-Andersen 1942), recurrence risk in our data was quite specific for CL-to-CL and for CPO-to-CPO. There was, however, a significant three-fold elevation of the cross-over risks which may be caused by genes like *MSX1* (van den Boogaard, Dorland et al. 2000) or rare syndromes (Gorlin, Cohen et al. 2001) that can produce both CL and CPO.

Within CL cases, we found no evidence for specificity of risk between CLO and CLP; risks for both were high following either. This reinforces the assumption that these two types of cleft lip comprise a single genetic risk group.

More surprising was the absence of any effect of cleft severity on recurrence risk. The mildest CL produced a recurrence risk indistinguishable from the risk seen with the most severe CL. Furthermore, there was no difference in the severity of the recurring defect. This contradicts widely-used textbooks in medical genetics (Mueller and Young 2001; Nussbaum, McInnes et al. 2001), which state that the familial risk of cleft recurrence increases with cleft severity in the proband, and claim that this pattern of recurrence is an example of the multifactorial threshold model of inheritance. A general principle of this model is that the familial risk is greatest among relatives of the most severely-affected patients, because more severe disease presumably indicates a greater load in the family of the alleles assumed to predispose to disease (Carter 1969; Carter 1970; Fraser 1976).

Our data are consistent with some previous studies suggesting that cleft lip and cleft palate do not fit the multifactorial threshold model (Melnick, Bixler et al. 1980; Melnick 1992; Mitchell and Risch 1992; Wyszynski, Beaty et al. 1996; Gorlin, Cohen et al. 2001). Previous estimates of absolute risk in first-degree relatives have ranged from 2.5% to 5.7% using coarser definitions of severity of clefts (Harper 1998; Firth and Hurst 2005). With much more statistical power and careful clinical criteria for severity we still did not find evidence of an effect of severity on recurrence risk.

Another prediction of the threshold model is that if a condition is more common in one sex (say, females), then relatives of an affected male will be at a higher risk than relatives of an affected female. Our data had a predominance of females in the CPO category and a predominance of males in CL (Sivertsen, Wilcox et al. In Press), but no difference in risk by sex of the index case. Other genetic models that explain why severity is nearly independent of heritability are therefore needed.

The lack of difference in mother-offspring and father-offspring recurrence for CL and CPO has implications for the genetic model (Lie 2007). Fetal genes are likely to explain entirely the genetic component of risk in oral clefts. If maternal genes operating during pregnancy (or mitochondrial mechanisms) had a major impact, mother-offspring recurrence should have been higher than father-offspring recurrence. Lack of difference also indicates that genes subject to genomic imprinting are not major contributors to the risk of oral clefts. Furthermore, since we did not find sibling recurrence to be higher than parent-offspring recurrence, persistent environmental factors carried by the mother should have much weaker effects than genes. The estimated sibling recurrence of CL was higher (although not significantly different), indicating that environmental effects could be more important for CL than for CPO.

Our findings also have implications for the genetic counselling of families with oral clefts. A severe cleft in one child does not increase the risk of a subsequent affected child; similarly, the occurrence of a mild defect does not insulate the family from the generally high recurrence of clefts, or from a severe version of the defect in other family members, should it occur.

Regarding the social impact of facial clefts, we observed a slightly decreased rate of reproduction among women with clefts, and a stronger decrease for men with clefts within our follow-up period. These differences have been suggested in earlier data from Norway (Skjaerven, Wilcox et al. 1999; Lie, Wilcox et al. 2001). We did not see any tendency for couples with one affected child to change their subsequent reproductive patterns.

Given the low population risk of around 2 per 1000 of clefts, relative risks were estimated as odds ratios in logistic regression models. Log-binomial regression of relative risks was used to confirm that this approximation was good, but since log-binomial regression did not always converge and produce estimates, we preferred to present the results from logistic regression analyses.

This study had its strengths by combining large sample size and population coverage with a higher level of clinical detail and verification from surgical examinations (Sivertsen,

Wilcox et al. In Press). The use of the compulsory national registration of births in the Medical Birth Registry, together with an insignificant emigration made the data virtually complete.

In summary, we found almost complete specificity of recurrence risk by the two major types of clefts, supporting that they have almost distinct etiologies. We found similar risk of clefts to the children of affected fathers and affected mothers, and these were again similar to recurrence between siblings. This indicates that autosomal fetal genes have the major contribution to the recurrence risks. There was no evidence in our data that severity of the defect affects recurrence risk for either type. This questions the validity of the widely-accepted theory of a multifactorial threshold model of inheritance for oral clefts, and opens new possibilities for a genetic model in which severity of disease is independent of genes predisposing for oral clefting.

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Figur legends

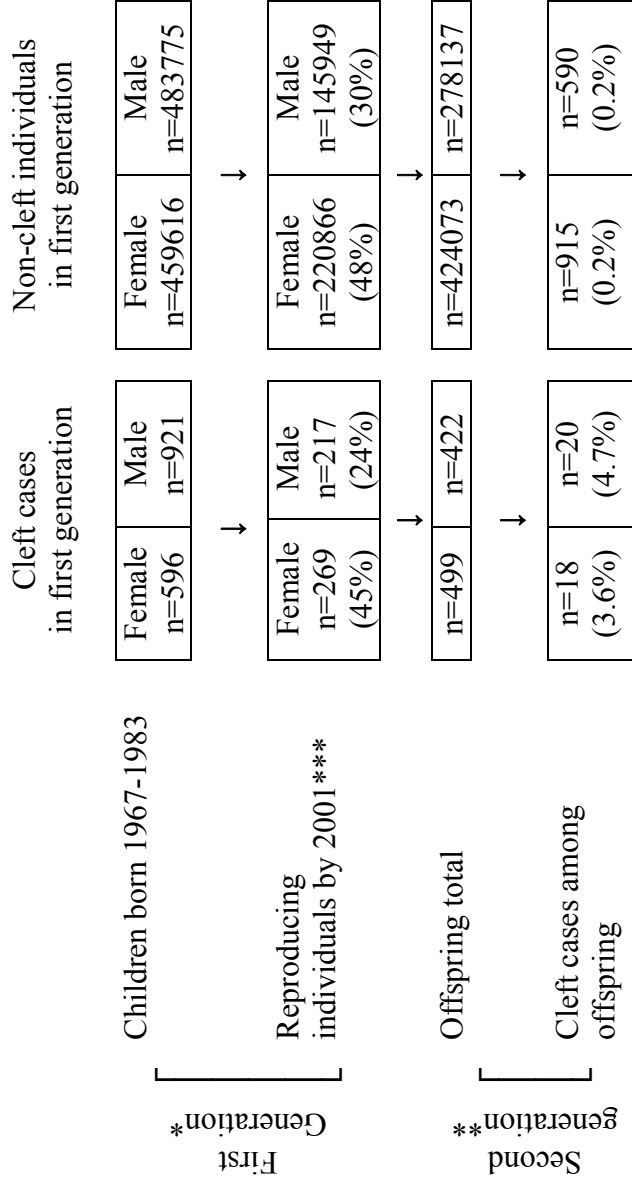
Figure 1.

Follow-up through generations.

Figure 2.

Follow-up of babies who had at least one subsequent full sibling.

Figure 1.
Follow-up through generations



* Individuals without non-cleft birth defects. The cleft diagnosis was clinically verified.

** The cleft diagnosis was verified in the Medical Birth Registry and / or in the clinical data. Stillborns and individuals with non-cleft birth defects are included.

*** The overall marked lower proportion of males who have reproduced is because women tend to have children with men older than themselves (men born before 1967 and not included in the study-cohort).

Figure 2.

Follow-up of babies who had at least one subsequent full sibling

	Index baby with cleft	Index baby without cleft
Index children*	n= 1554	n=1007156
	↓	↓
Children who had one or more later born full- siblings **	n=879 (57%)	n=571893 (57%)
	↓	↓
Subsequent full- siblings***	n=1221	n=803065
	↓	↓
Cleft cases among siblings***	n=56 (4.6%)	n=1678 (0.2%)

* A woman's first recorded baby. Stillborns and babies with non-cleft birth defects are not included. The cleft diagnosis was verified in the clinical data.

** Number of mothers who had more than one baby (not plural births) with the same partner.

*** Stillborns and babies with non-cleft birth defects are included. The cleft diagnosis was verified in the Medical Birth Registry and / or in the clinical data.

Table 1.

The risk of cleft types to recur across generations and between full-siblings in the three main categories of clefts: Cleft lip only (CLO), Cleft lip and palate (CLP) and Cleft palate only (CPO). Risks relate to the prevalence of CLO, CLP and CPO in offspring or siblings of index cases without oral cleft **.

Follow-up	Index cases			Recurrent cases			Relative risk* (95% CI)		
	Main categories of cleft	At risk	CLO	CLP	CPO	CLO	CLP	CPO	
Through generations	CLO	293	3	7	2	18.7 (6.1 - 57.5)	28.6 (13.6 - 59.8)	9.3 (2.3 - 37.4)	
	CLP	340	5	8	1	27.0 (11.2 - 64.9)	28.1 (14.0 - 56.7)	4.0 (0.6 - 28.5)	
	CPO	288	0	1	11	-	4.1 (0.6 - 29.1)	54.0 (29.7 - 98.0)	
From first registered child to subsequent born full-siblings	No cleft (reference)	702210	388	601	516	1	1	1	
	CLO	388	8	9	0	37.0 (18.3 - 74.7)	30.2 (15.5 - 58.8)	-	
	CLP	491	5	18	2	18.1 (7.4 - 43.9)	48.4 (30.2 - 77.4)	5.6 (1.4 - 22.3)	
CPO	342	0	0	14	-	-	58.1 (32.8 - 102.8)		
	No cleft (reference)	803065	457	631	590	1	1	1	

* Relative risk estimated as odds ratio in logistic regression models

** Index cases are without non-cleft birth defects and have the cleft diagnosis clinically verified. Recurrent cases also include stillborns, cases with non-cleft birth defects and cases who have the cleft registered in the Medical Birth Registry alone.

Table 2.

Estimated relative risks of recurrence for cleft lip (with or without cleft palate) for first degree relatives when the index had an isolated cleft lip and for cleft palate only when the index had an isolated cleft palate**.

	At risk	Cases	RR*	95% CI	P difference
Cleft lip	Mother-offspring	295	11	27.1 (14.9-49.2)	0.97
	Father-offspring	338	12	26.6 (15.0-47.2)	
	Parent-offspring total	633	23	26.7 (17.7-40.3)	0.31
	Subsequent full sibling	879	40	35.1 (25.5-48.4)	
	First degree relative total	1 512	63	31.5 (24.6-40.3)	
Cleft palate only	Mother-offspring	204	7	48.4 (22.7-103.1)	0.60
	Father-offspring	84	4	67.8 (25.3-181.7)	
	Parent-offspring total	288	11	54.0 (29.7-98.0)	0.98
	Subsequent full sibling	342	14	58.1 (32.8-102.8)	
	First degree relative total	630	25	56.2 (37.2-84.8)	

* Relative risk estimated as odds ratio in logistic regression models

** Index cases have the cleft diagnosis clinically verified. Recurrent cases also include stillborns, cases with non-cleft birth defects and cases who have the cleft registered in the Medical Birth Registry alone.

Table 3.

Estimated relative risks of recurrence and severity of cleft in the recurrent cases for first degree relatives by severity of cleft lip (with or without cleft palate) and severity of cleft palate only in the index cases***.

Index cases		Recurrence of similar cleft type			
Cleft type	Cleft severity	At risk	Number recurrent cases	Mean severity of the cleft ***	Relative risk* (95% CI)
Cleft lip	Mild	438	15	2.4	25.7 (15.4 - 42.8)
	Moderate	251	14	2.3	42.8 (25.2 - 72.6)
	Severe	823	34	2.3	31.2 (22.3 - 43.7)
Cleft palate only	Mild	96	3	1.5	43.9 (13.8 - 139.2)
	Moderate	299	9	2.1	41.4 (19.9 - 85.9)
	Severe	229	13	2.5	81.9 (47.6 - 140.9)

* Relative risk estimated as odds ratio in logistic regression models

** The mean severity was estimated in the clinically verified recurrent cases. 57 of 63 recurrent CL cases and 20 of 25 recurrent CPO cases were clinically verified. In bilateral cleft lip the more severe of the two clefts defined the severity.

*** Index cases are without non-cleft birth defects and have the cleft diagnosis clinically verified. Recurrent cases also include stillborns, cases with non-cleft birth defects and cases who have the cleft registered in the Medical Birth Registry alone.

Paper IV

American Journal of Medical Genetics Part A 143A, (2007), Sivertsen, Åse; Lie, Rolv Terje; Wilcox, Allen J. Åbyholm, Frank; Vindenes, Hallvard; Haukanes, Bjørn Ivar and Gunnar Houge, Prevalence of Duplications and Deletions of the 22q11 DiGeorge Syndrome Region in a Population-Based Sample of Infants With Cleft Palate, pp. 129–134. Copyright 2006 Wiley-Liss, Inc. Abstract only. Full-text not available due to publisher restrictions.

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Prevalence of Duplications and Deletions of the 22q11 DiGeorge Syndrome Region in a Population-Based Sample of Infants With Cleft Palate

Abstract

The prevalence of duplications and deletions of the 22q11.2 (DiGeorge syndrome) region was studied among babies born in Norway with open cleft palate without cleft lip (cleft palate only, CPO). During a 5-year period (1996–2001), there were 245 live births with CPO that were referred for surgery. DNA was available from 174 cases with overt cleft palate. DNA copy number was analyzed with the multiplex ligation-dependent probe amplification (MLPA) technique, and an unambiguous result was obtained in 169 (97%) of the samples. We found no 22q11.2 duplications, and one known, and two previously undiagnosed cases with 22q11.2 deletions. All three del22q11-syndrome cases also had heart malformations, which represent one-third of the 10 babies with heart malformations in our study population. The prevalence of del22q11-syndrome among babies with cleft palate with or without additional malformations was 1 of 57 (1.8%). Because the prevalence of CPO in the 35 22q11.2 duplication cases published was 20%, we also investigated if dup22q11-testing was warranted in this group. However, no 22q11.2 duplications were found, indicating that the duplication cases ascertained so far might not be representative of the dup22q11-group as a whole. We conclude that neither del22q11 nor dup22q11 testing is warranted in babies with overt cleft palate as the only finding.

Key words: cleft palate; DiGeorge syndrome; del22q11 syndrome; 22q11.2 deletions; 2q11.2 duplications