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A retrospective study of orofacial clefts and associated anomalies at the Spires Cleft Centre, Oxford, United Kingdom, from 1995 to 2012.

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THIS THESIS IS SUBMITTED TO THE EXAMINATION SCHOOLS, UNIVERSITY OF OXFORD, IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF SCIENCE (BY RESEARCH) IN SURGICAL SCIENCES.

THIS THESIS IS ENTIRELY MY OWN WORK, AND EXCEPT WHERE OTHERWISE INDICATED, DESCRIBES MY OWN RESEARCH.

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A retrospective study of orofacial clefts and associated anomalies at The Spires Cleft Centre, Oxford, United Kingdom, from 1995 to 2012.

Abstract

Although it is well established that children with orofacial clefts (OCs) are often born with associated anomalies in addition to their cleft, the reported prevalence of associated anomalies varies widely between existing studies, ranging from 3% to 63%. Furthermore, details on the nature of these additional anomalies remain scant. With better antenatal diagnosis of OCs, accurate information is needed to aid parental counselling. Such data is also valuable in determining service provision, especially as few studies describe the incidence of such conditions in developed, multicultural societies such as the United Kingdom (UK). The aim of this study was to characterise children with OCs and to determine the frequency of associated anomalies in OCs within a geographically defined area within the UK.

Medical records of children with OCs born in Oxfordshire, Berkshire and Buckinghamshire between January 1995 and July 2012 were reviewed. Information was collected on socio-demographic and cleft characteristics, birth and family history, and associated anomalies. Information was cross-referenced with data from the regional birth registry.

A total of 791 infants were identified with OCs, giving a mean birth prevalence of 1.44 cases per 1,000 live births in Oxfordshire, Berkshire and Buckinghamshire over the duration of this study. There was a slight male predominance but no seasonal trends. Approximately 34% of infants with clefts had an associated anomaly, most frequently affecting the skeletal system and extremities, cardiovascular and central nervous systems. Associated anomalies were associated with non-live births, premature birth, low birth weight, cleft palate subtype, chromosomal defects and non-chromosomal syndromes.

It appears that the burden of care of multiple anomalies in children with OCs for families, carers and service providers has been widely underestimated. There is a need for a thorough investigation of infants born with a cleft, as anomalies occurred in approximately 1 in 3; and each child with a cleft should be examined with particular attention to the skeletal system and extremities, cardiovascular and central nervous systems. A significant proportion of those with additional anomalies had underlying chromosomal defects and non-chromosomal syndromes. There is also a provisionally unique group of children with OCs and additional anomalies without a unifying cause. This is a potential pool of patients with an undiscovered or undescribed genetic defect or syndrome. The findings from this study supports early genetic counselling and testing in all infants with OCs and associated anomalies.

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List of Abbreviations

The following abbreviations and acronyms are used herein:

ANOVA	Analysis of Variance
CAROBB	Congenital Anomaly Register for Oxfordshire, Berkshire and Buckinghamshire
CGH	Comparative genomic hybridisation
CL	Cleft lip
CLP	Cleft lip and palate
CL±P	Cleft lip and/or palate
CNS	Central nervous system
CP	Cleft palate
EUROCAT	European Concerted Action on Congenital Anomalies and Twins
ICBDMS	International Clearinghouse for Birth Defects Monitoring Systems
IPDTOC	International Perinatal Database of Typical Oral Clefts
M	Mean
MRI	Magnetic Resonance Imaging
N	Number
NBDP	National Birth Defects Prevention Network
NPEU	National Perinatal Epidemiology Unit
OC	Orofacial cleft
ONS	Office for National Statistics
PRS	Pierre Robin Sequence
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TOP	Terminations of pregnancy

Chapter 1: Introduction

1.0. Overview of orofacial clefts (OCs)

The World Health Organization [WHO] defines congenital anomalies – a term used interchangeably with congenital malformations, congenital disorders, or birth defects – as structural or functional anomalies, including metabolic disorders, which are present at the time of birth [1]. It is estimated that congenital anomalies affect 1 in 33 infants and result in 3.2 million related disabilities annually worldwide [1].

Craniofacial anomalies are the fourth most common congenital anomalies with orofacial clefts (OCs) being the most common [2]. The birth prevalence of OCs ranges between 1 in every 500 to 1,000 live births with ethnic and regional differences [3, 4]. They are a group of disorders affecting the lips and oral cavity [3] with cleft lip and/or palate (CL±P) resulting from failed fusion of the medial, lateral and maxillary processes, which normally fuse between the 6^{th} and 10^{th} weeks of intrauterine life [2, 5, 6]. In simplistic terms, infants with cleft lip (CL) have a fissure in the upper section of the lip, whereas infants with cleft palate (CP) have a fissure in either the soft or hard palate or both, and both CL and CP can occur separately or together [2]. The resulting defects can impact speech, hearing, appearance, and psychology with long-term adverse outcomes for health and social integration [3].

OCs can be classified as isolated, when a cleft occurs in the absence of another congenital anomaly, or associated, when other congenital anomalies are present in addition to the cleft. The term "associated anomalies" generally excludes malformations considered to be direct effects of the

cleft, such as dental anomalies [7-9]. The International Perinatal Database of Typical Oral Clefts [IPDTOC] extrapolated 7,704 cleft cases from numerous registries and found that overall, 15.9% were associated with anomalies in other systems and 7.3% occurred as part of recognised syndromes [10]. In recent years, studies have attempted to further characterise the nature of associated anomalies in OCs, but the findings have been inconsistent. The prevalence of associated anomalies in OCs has been reported to be as low as 2.8% [11] to as high as 63.4% [12], and there is a paucity of information on the nature of these anomalies.

With improvements in the antenatal diagnosis of OCs, there is increasing need for accurate information on disease burden to aid parental counselling [13]. Care for children with OCs involves several disciplines therefore such data is also valuable to determine necessary service provisions for a caseload likely to include some highly complex multiple anomalies. In addition, few studies have reported data from developed and multicultural societies such as the United Kingdom (UK). This study based at The Spires Cleft Centre is an attempt to further our understanding of associated anomalies in OCs in a UK-based cohort. Following the national reorganisation of cleft services in the early 1990s to 2000s, regional specialist centres such as The Spires Cleft Centre provide a unique opportunity to examine the birth prevalence of OCs within a reasonably well-defined population base.

First, the introduction will provide a conceptual framework of OCs including the embryology, aetiology and classification of OCs. The embryology of the face, lip and palate will be reviewed to outline the timing, complexity, and factors that may contribute to the development of OCs and associated anomalies. Second, the emerging genetic links, environmental and teratogenic influences that contribute to OCs will also be discussed. Third, the worldwide epidemiology of OCs will be summarised. Fourth, current understanding of associated anomalies in OCs in terms of frequency and types of anomalies will be evaluated. The above discussions will take place in the context of

existing studies and will synthesise information from a range of sources. Finally, the abbreviations used to describe cleft types in this study are defined in Table 1.

Table 1. Abbreviations used for describing cleft types.

Abbreviation	Definition
CL	Cleft lip with or without alveolus [excludes cleft lip and palate (CLP) and cleft palate (CP)]
CP	Cleft palate only [excludes cleft lip (CL) and cleft lip and palate (CLP)]
CLP	Cleft lip and palate [excludes cleft lip (CL) and cleft palate (CP)]
CL±P	Cleft lip with or without cleft palate [excludes cleft palate (CP)]
SMCP	Submucous cleft palate

1.1. Embryology of the lip and palate

1.1.1. Overview of facial development

The first eight weeks in human development is termed embryogenesis, and during this period, development of the lip and palate is tightly coordinated by a network of signalling molecules, transcription factors, and proteins [3, 5, 14]. During the first two weeks of human embryonic life, the embryo resembles a flat circular plate [2]. In the third week, the neural tube elongates as the cranial region expands, and with the lengthening nervous system the embryo is forced to bend on itself, bringing the cranial and caudal ends in close proximity [2]. Neural crest cells, which delaminate from neural folds, migrate through mesenchymal tissue to contribute to the formation of the facial prominences, which can be recognised around the oral cavity in the fourth week. The human face begins to form from these facial prominences which include the single frontonasal process, derived from neural-crest tissue surrounding the forebrain, and the paired mandibular and maxillary processes, derived from the first pharyngeal arch [6, 15]. Figure 1 and Table 2 show the contributions of the different facial prominences to the formation of the lip and palate.

Table 2. Embryonic structures and their contribution to face formation [6].

Prominence	Structures Formed
Frontonasal	Forehead, bridge of nose, and medial and lateral nasal prominences
Maxillary	Cheeks, lateral portion of upper lip
Medial nasal	Philtrum of upper lip, crest, and tip of nose
Lateral nasal	Alae of nose
Mandibular	Lower lip and jaw

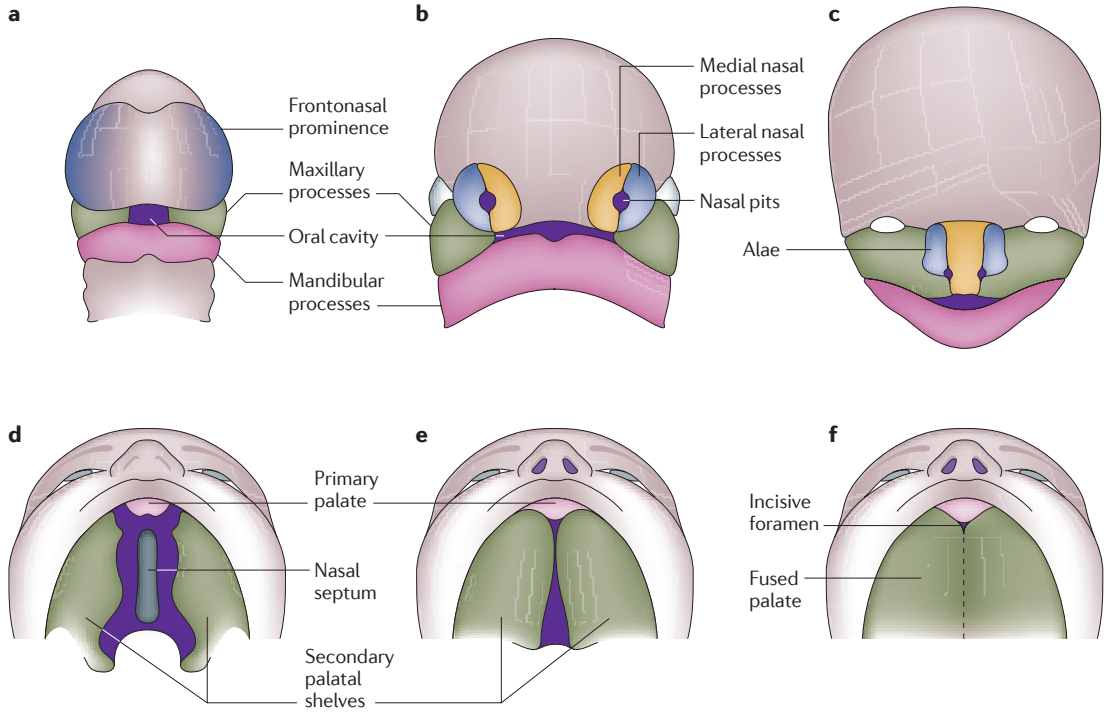


Figure 1. Development of the lip and palate. From Dixon et al., 2011. [16]. **A.** By the 4th week of embryogenesis, the frontonasal prominence, paired maxillary and mandibular processes surround the oral cavity. **B.** By the 5th week, paired medial and lateral nasal processes have formed. **C**. By the 6th week, the medial nasal processes merge with the maxillary processes to form the upper lip and primary palate, and mandibular processes fuse to form the lower jaw. **D**. During the 6th week, secondary palate develops. **E.** Palatal shelves elevate to a horizontal position above the tongue, are in contact and begin to fuse. **F.** Fusion of palatal shelves divides oronasal space into separate oral and nasal cavities.

1.1.2. Development of the primary palate

The primary palate develops at the same time as the external face, in the fifth and sixth weeks of development [2, 6]. By the fifth week, the nasal pits have formed, which divides the frontonasal process into the paired medial nasal prominence (inner edge) and lateral nasal prominence (outer edge) [6] (see Figure 1). The medial nasal prominence merges with the maxillary prominences to form the upper lip superficially, and the intermaxillary segment more deeply [6]. The intermaxillary segment is composed of a labial component, which forms the philtrum of the upper lip, an upper jaw component that carries the four incisor teeth, and a palatal component that forms the triangular primary palate [17]. Immediately before completion of these processes, they experience a peak of cell

division that renders it susceptible to teratogenic insults, therefore any disturbance at this critical time can lead to failure of the closure mechanism [18]. Similarly, merging of the mandibular prominences across the midline forms the lower lip and jaw [6, 16].

1.1.3. Development of the secondary palate

The secondary palate derives from outgrowths of the maxillary prominences and is thought to form between the sixth and twelfth weeks of development, with fusion processes generally complete by the tenth week [5, 6]. Palatine shelves appear in the sixth week of development, grow vertically down the sides of the tongue but reach a horizontal position above the tongue in the seventh week, and fuse to form the secondary palate [17]. Anteriorly, the palatine shelves fuse with the primary palate, with the incisive foramen demarcating the primary and secondary palates [6]. Mesoderm of the palate later undergoes intramembranous ossification to form the hard palate except where it remains unossified in the posterior-most region to form the soft palate [6]. The formation of the palate subdivides the oronasal space into distinct oral and nasal cavities, and subsequently enables mastication and respiration to occur simultaneously [3].

1.1.4. Cleft formation

Craniofacial anomalies are among the most common of all birth defects, with OCs being the most common [5, 15, 19]. This is unsurprising given the complexity of craniofacial development, and disturbance of the tightly controlled cascade of cell migration, growth, differentiation and apoptosis can result in OCs where the facial primordia ultimately fail to meet, fuse or form the appropriate structures [3, 5, 6]. Although CL and CP have different embryological origins, they often occur together [17]. CL results from a failed fusion of the maxillary and medial nasal prominences on one or both sides, and CP results from failure of the palatine shelves to meet and fuse [2, 6]. CL can vary in severity from a small defect in the vermillion border to a complete separation of the lip and

primary palate and similarly, CP can involve only the uvula or can involve the entire secondary palate [2]. Virtually any site where merging or fusion occurs in facial and palatal development is a potential site for cleft formation as depicted in Figure 2 [5, 6].

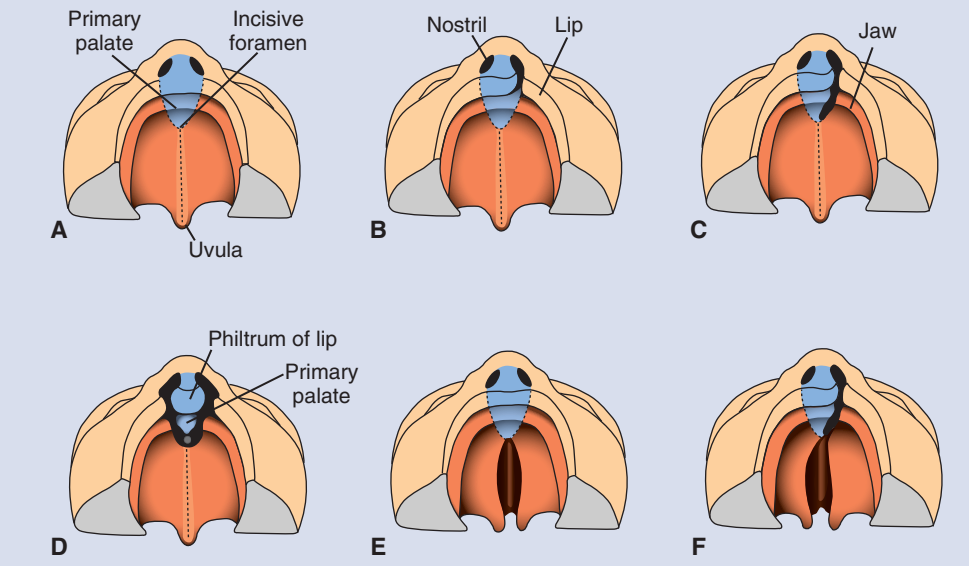


Figure 2. Types of OCs. From Sadler and Langman, 2012. [6]. **A.** Normal. **B.** Unilateral cleft lip extending into the nose. **C.** Unilateral cleft involving the lip and jaw and extending to the incisive foramen. **D.** Bilateral cleft involving the lip and jaw. **E.** Isolated cleft palate. **F.** Cleft palate combined with unilateral anterior cleft lip.

1.2. Aetiology of OCs

1.2.1. Overview of the aetiology of OCs

The complex embryology of the lip and palate render these tissues vulnerable to a number of potential disruptions in development, but the precise aetiology causing this disruption is unknown and is believed to be multifactorial [16]. Mounting evidence suggests that multiple genes and environmental factors influence the risk of OCs as described by the multifactorial threshold model [20]. That is, both exogenous and endogenous factors influence the development of OCs through their complex interactions during the critical stages of craniofacial development [15, 16, 21, 22]. One hypothesis is that the developing human can tolerate the effects of genetic variants and exogenous perturbations to a certain extent, but anomalies result once that threshold is exceeded [23, 24]. This section will outline the genes, environment, and mechanical forces thought to contribute to clefting.

1.2.2. Influence of genes

Syndromic OCs

OCs are listed as a feature of more than 500 genetic syndromes [16]. Amongst the chromosomal defects, Trisomy 13, Trisomy 18 and 22q11 deletion syndrome are most common. Of the non-chromosomal syndromes, OCs feature in Kabuki syndrome, Stickler syndrome, and Van der Woude syndrome, and are associated with other conditions, some of which are listed in Table 3 [25]. The proportion of OCs associated with specific syndromes, however, represents only 5-7% of all individuals with a cleft [26] and therefore much research has been redirected towards understanding non-syndromic clefting, including its underlying genetics.

Table 3. Common syndromes associated with OCs [25].

Genetic defect	Examples
Chromosomal	Trisomy 13
	Trisomy 18
	Velocardiofacial syndrome (22q11 deletion)
Non-Mendelian	Pierre Robin sequence
	CHARGE association
	Goldenhar syndrome
Mendelian disorders	Ectrodactyly-ectodermal dysplasia-clefting syndrome (AD)
	Gorlin syndrome (AD)
	Kabuki syndrome (unclear)
	Oto-palato-digital syndrome (XL)
	Oral-facial-digital-syndrome (XL)
	Smith-Lemli-Opitz syndrome (AR)
	Stickler syndrome (AD)
	Treacher Collins syndrome (AD)
	Van der Woude syndrome (AD)
Teratogenic	Foetal alcohol syndrome
	Foetal phenytoin syndrome
	Foetal valproate syndrome

**AD = autosomal dominant. AR = autosomal recessive. XL = X-linked inheritance.*

Non-syndromic OCs

Significant progress in understanding the genetics of non-syndromic OCs has been made possible by advances in genetic technologies [15, 21, 27]. To date, no single gene has been identified as a universal explanation for all OCs, but certain gene loci have been implicated in specific ethnic groups or cases of familial recurrence. Findings of linkage studies have suggested that several loci could have a causal role in CL±P including on chromosomes 1, 2, 4, 6, 14, 17, and 19 and at 2q32-q35 and 9q21-q33 [28-30]. Genetic polymorphisms have also been implicated, including growth factors (TGF-α, TGH-ß3), transcription factors (MSX1, IRF6, TBX22) and other metabolic factors [3, 31]. Genes linked to syndromic forms of OCs and have a Mendelian mode of inheritance can also produce phenocopies of non-syndromic clefts [32, 33]. The gene IRF6, which causes Van der Woude's syndrome, is also linked to non-syndromic OCs [32]. Other Mendelian-inherited syndromes and related genes that could modify the expression of clefting if mutated include: Kallmann's syndrome (GFGR1) [34], ectrodactyly, ectodermal dysplasia, and clefting syndrome (TBX22) [35,

36], X-linked clefting and ankyloglossia (TBX22) [37], Gorlin's syndrome (PTCH1) [38], and Margarita Island ectodermal dysplasia (PVRL1) [39].

Hereditability

Evidence from familial studies further cement the role of genes in cleft development. No predictable pattern of recurrence has been established but the risk of OCs is escalated when a positive family history exists [40, 41]. The prevalence of a positive family history for OCs is between 6% to 28% [20, 41]. When specific genetic disorders are excluded, concordance rates for CL±P are higher in monozygotic twin pairs than in dizygotic pairs, with a concordance of 40% in monozygotic twins and 4.2% in dizygotic twins. For CL±P, the risk to monozygotic co-twins of affected individuals is less than 100% but 6-19 fold higher than the risk to dizygotic co-twins [32]. For CP alone, concordance is also higher in monozygotic twins (35%) than dizygotic twins (7.8%) [42]. Meanwhile, a parent affected by CL±P is quoted as having a 3-5% risk of having an affected child [2, 31] but when parents have one affected child, parents have a 40% chance of having another affected child [43].

Parental age

Maternal age has been widely studied as a risk factor for OCs, but results remain conflicting. While earlier studies have reported increasing maternal age as a risk factor for OCs in the UK [44], Finland [45], and the USA [46], later studies have equally found no association between maternal age and OCs [47-49]. When maternal age was stratified, it was suggested that maternal age of <19 years [50] or >39 years [46] may be associated with an increased incidence of CL±P. There is also accumulating evidence that increasing paternal age causes a small but significant increase in the incidence of clefting, with evidence suggesting that increased paternal age is associated with CP [51].

1.2.3. Influence of environmental factors and teratogens

Teratogenic exposure may alter the intrauterine environment in which the embryo develops and disrupt critical processes of lip and palate formation and contribute to cleft formation [3, 16, 21]. Although both mother and baby have inborn capacities to cope with a range of insults via the action of detoxification enzymes, some individuals have deleterious variants in the detoxification genes that reduce the ability to biotransform toxic components, rendering the baby more vulnerable to teratogens [21].

Numerous maternal exposures have been reported to affect the risk of OCs, but only a few have survived scrutiny after being tested in large, well-controlled populations. The evidence is most consistent for cigarette smoking [52-54], alcohol consumption especially binge drinking [55], folic acid and vitamin B complex supplementation [56, 57], anti-folate medications [58, 59], and certain parental occupations [60]. There is some evidence for the role of maternal illnesses [61, 62], diabetes and obesity [21, 63-65] but more research is needed to clarify the role of these factors in cleft development.

Cigarette smoking

Cigarette smoking during the first trimester of pregnancy has repeatedly been associated with an increased risk of OCs [53, 66]. A meta-analysis strongly supports an odds ratio of ~1.3 for maternal smoking with OCs [54]. The precise mechanism is unknown but intermittent hypoxia induced by nicotine may affect craniofacial development [31, 67]. A genetic predisposition (altering transforming growth factor alpha) may exacerbate the risk of smoke exposure in certain individuals [68], and there is recent evidence of a particular gene-environment interaction between foetal glutathione S-transferase theta 1 [GSTT1] gene and maternal smoking [52].

Maternal alcohol consumption

Maternal alcohol use is also associated with an increased risk of clefting [21, 68]. It is suggested that embryos exposed to alcohol experience interruption in the migration and differentiation of neural crest cells [31]. As with smoking, a genetic predisposition (alteration in the gene MSX1) coupled with environmental exposure is thought to be associated with an increased risk of clefting [31, 68]. In the study by Boyles et al. (2010), heavy maternal alcohol consumption (more than five drinks per sitting) was associated with an elevated risk of OCs, only if either mother or baby carried the slow-metabolising variant of the alcohol dehydrogenase 1C [ADH1C] gene [69].

Folic acid

Women of childbearing age have been advised to take folic acid daily prior to conception and to continue throughout pregnancy [2, 70]. Folic acid not only prevents neural tube and abdominal wall defects but several studies have also shown that folic acid lowers the risk of OCs [71, 72] though it remains unclear whether this is mediated through the action of genes involved in folic acid metabolism [56, 73].

Medications

Several medications have been linked to the development of OCs when taken during the first trimester, a critical period of development for the lip and palate. Infants exposed to anticonvulsants are thought to have an increased risk of clefting, most notably with diazepam, phenytoin, and phenobarbital [3]. A positive association with maternal corticosteroid use in pregnancy has also been reported. Studies in mice have shown that cortisone affects the extracellular matrix content and the number of palatal shelf cells, delays shelf elevation and interferes with the shelves in the palatal region assuming a horizontal position [74]. The mice also showed a reduction in the size of the lateral nasal prominences [75].

Season

Studies investigating seasonal trends in the incidence of OCs demonstrate a divergence of opinion. There is an interest in the seasonal trends, as they are thought to reflect risk factors to cleft development such as deficiency of vitamins, fluctuations in mothers' diet, ultraviolet light exposure, use of fertilisers and pesticides, and infectious diseases [76, 77]. In an early study in Nigeria, Iregbulem (1982) found that children with CL±P were more often born in March and September [41]. Elliott (2008) also reported seasonal variations in the month of birth of CL±P patients born in Zambia, and agreed that more cleft births occurred in March through to August (57.2%) than in September through February (42.8%), but reported the peak of total cleft births to be in April-May [78]. Knox (1963) however found no evidence of a seasonal variation in OCs in the UK [79]. Jahanbin (2012) also failed to detect any significant seasonal influence in Iran [80]. In France, Stoll (1991) found no seasonal variation in birth incidence even when infants with OCs were compared with newborn babies in the area during the same period [81]. Studies even within the same country were inconsistent in their reports of seasonal variations and OCs. Cooper et al. (2000) reported a high birth prevalence of OCs in January through July in China [82], whereas Zhou et al. (2006) reported a peak in births in August and October in China [83]. In contrast to the previous two studies, Yin et al. (2013) added a control group, and found higher birth rates of OCs during the autumn-winter period, especially October, and lower birth rates in summer, especially June [84]. The authors hypothesised that the high birth rate of OCs in winter and early spring could be due to less sunlight during those months, but also noted that many festivities happen during January to March in China, and may reflect increased tobacco and alcohol consumption during these months [84]. It was also suggested by Coupland (1988) that the relationship between seasonal variations and OCs were modified by cleft type. CL±P had a peak occurrence in December and January, and a low frequency in May and June, whereas CP births peaked in August and September, with a low incidence in March to May [76]. Besides the possibility of a direct seasonal effect, there are numerous indirect ways in which seasonal variation in cleft incidence may arise. Comparing international studies may not be feasible when

seasonal variations also reflect the cultural idiosyncrasies of the area. It appears that no conclusion can be drawn at present about seasonal effects on OCs.

Summary

In summary, despite the well-recognised role of environment influences in OCs, only a few genetic and environmental interactions have been conclusively demonstrated such as smoking and alcohol consumption [3]. Known challenges are related to controlling exposure, sample size/statistical power, and study heterogeneity [21]. The emergence of collaborative networks such as the Gene Environment Association Studies (GENEVA) consortium [85] (http://www.genevastudy.org) may help overcome some of the limitations with sample size. Concurrently, novel study designs that increase statistical power and are not prone to confounders are needed to advance the study of gene and environmental interactions in clefting [86-88].

1.2.4. Influence of mechanical forces

Another potential mechanism of OCs may relate to the mechanical forces that interfere with normal tissue formation. A cleft will result if there is insufficient mesodermal tissue that is not able to elevate in time, or if the tongue is not positioned properly due to a mandibular hypoplasia such as in infants with Pierre Robin Sequence [89].

Pierre Robin Sequence (PRS)

Pierre Robin Sequence (PRS), or Robin Sequence, first described in 1923 by Pierre Robin, is a clinical entity of micrognathia, glossoptosis and airway obstruction [90]. In addition, CP occurs in up to 90% of children [91], but whether CP is an obligatory feature of PRS remains debated [90]. The incidence of PRS is estimated around 1 in 8,500-14,000 and the variable diagnostic criteria makes it difficult to gauge the incidence accurately [92, 93]. The primary pathogenic event is attributed to micrognathia causing displacement of the tongue, which prevents closure of the palatine shelves before the tenth week of development and results in formation of the classic inverted U-shaped CP [90, 92]. Oligohydramnios is thought to play a role as lack of amniotic fluid renders the mandible more susceptible to deformation and subsequent impaction of tongue between palatal shelves [89]. Although the morphogenesis of PRS suggests the defect in palatal closure is a result of mechanical obstruction by the tongue, transcription factors and regulatory enhancers have also been implicated in aberrant neural crest patterning and signalling [90].

1.3. Classification of cleft lip and palate

There have been many attempts to classify the variable presentations of clefts into a system of value to both clinical and research settings. A descriptive classification distinguishing between CL, CLP and CP is often used during initial assessment of cleft deformities. The CL deformity can be unilateral or bilateral, and further classified as complete, incomplete or microform. The CP deformity can be classified as unilateral, bilateral or submucous, and the CLP deformity comprises a combination of the above. There are morphological classifications that exist from the clinical point of view of surgeons in addition to classification systems which highlight the embryological aspects of lip and palate development.

1.3.1. Morphological classifications

One of the earliest classifications reported in the literature was that of Davis and Ritchie in 1922, which described three groups of clefts using the alveolar process as the line of division. This produced Group 1 (pre-alveolar clefts), Group 2 (post-alveolar clefts) and Group 3 (complete alveolar clefts), which essentially described the complete spectrum of OC deformities, but did not include continuous clefts such as those involving the lip, hard and soft palates [94]. This problem was partially addressed by Veau in 1931, who described a classification system with Group 1 (clefts of the soft palate), Group 2 (clefts of the hard palate), Group 3 (complete unilateral cleft of the lip, alveolus and palate), and Group 4 (bilateral cleft lip, alveolus, and palate) The drawback was that isolated CL was not recognised as a distinct group [95].

Various classification schemes were proposed and published in the following decades including those by Pruzansky (1953) [96], Harkins (1960) [97],) Santiago (1969) [98], amongst many others [95]. In 1976, Paul Tessier introduced a comprehensive classification system for all facial clefts including and beyond those of the lip and palate. He proposed that facial clefts occurred in well-

defined places along definite axes, and described 14 cleft types according to their location in relation to the eye and orbit, with clefts of the lip, alveolus and palate numbered as 1, 2 and 3. Tessier's classification still prevails and allows for effective communication of OCs [99].

Otto Kriens (1989) presented a novel approach to cleft classification using a palindromic acronym organisation of cleft deformities, which is currently in use by the outcomes registry of the American Cleft Palate and Craniofacial Association [100]. It is compatible with the International Classification of Diseases and Related Health Problems 10th Revision (ICD-10) and is used to code clefts on the Craniofacial Anomalies Register which attempts to record all cleft cases in Britain [25]. The acronym LAHSHAL denotes the bilateral anatomy of lip (L), alveolus (A), hard (H) and soft (S) palates, by convention from right to left, whereby uppercase letters represent complete clefts of the structure, lowercase letters represent incomplete clefts, and a period denotes no cleft of that particular structure [100]. For example, a complete right cleft lip and alveolus and incomplete left cleft lip would be noted as "LA----l". A complete right unilateral cleft lip, alveolus, hard and soft palate would be "LAHS".

1.3.2. Embryological classifications

In 1958, Kernahan and Stark were for the first time struck by the idea of the importance of classification based on embryology unlike previous classifications based on morphology [101]. Kernahan in 1971 converted this information into a Y-shaped diagrammatic classification scheme, which continued to recognise the embryological division of the primary (lip and alveolus) and secondary palates at the incisive foramen. Notable modifications enabled the cleft to be located anatomically on the right (1-3) and left (4-6), from lip anteriorly to incisive foramen (7), hard palate (8) and soft palate (9) as illustrated in Figure 3 [95, 102]. In 1991, Friedman added numbers in each diagrammatic segment to represent severity of the anatomical and functional deformity, which was

not depicted on earlier models [103]. Kernahan's classification scheme and its modifications continue to be used in many cleft centres.

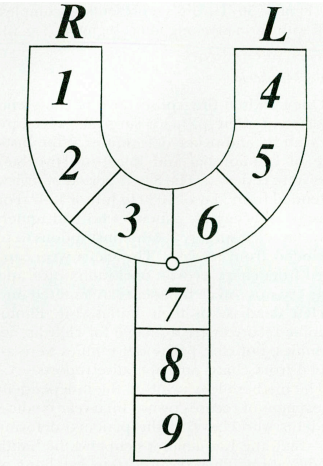


Figure 3. Kernahan striped Y classification of OCs. From Kernahan (1971) [102].

Currently, the International Confederation of Plastic and Reconstructive Surgery uses an embryonic framework to divide clefts into three groups, with further subdivisions to denote unilateral or bilateral cases as follows: Group I (defects of the lip or alveolus), Group II (clefts of the secondary palate – hard, soft or both), and Group III (any combination of clefts involving the primary and secondary palates) [104].

In this study, OCs will be grouped into CL, CP, and CLP, for ease of comparison with existing studies.

1.4. Descriptive epidemiology

1.4.1. Worldwide epidemiology of OCs

In 2002, a comprehensive investigation of cleft epidemiology was published, which reviewed data from international registries including the European Concerted Action on Congenital Anomalies and Twins (EUROCAT), International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS) and the National Birth Defects Prevention Network (NBDP) [20, 105]. The findings approximated the overall prevalence for OCs to be 1 in 700 live births with substantial ethnic and geographical variation. Data extrapolated from 57 international registries for 1993-1998 showed a variation in the birth prevalence of CL±P ranging from 3.4 to 22.9 per 10,000 births, and showed an even wider variation in the prevalence for CP, ranging from 1.3 to 25.3 per 10,000 births [106] as case ascertainment for the less externally visible CP is more susceptible to varying methodology.

1.4.2. Ethnic origin and migration

Numerous studies have shown ethnic and racial differences in the incidence of OCs. It is generally thought that Asian, Native North American and Maori populations have the highest prevalence of OCs, with Caucasian populations having intermediate prevalence, and African populations having the lowest prevalence [20, 107-110]. In the UK, the overall prevalence of OCs is commonly reported between 1.30 and 1.56 per 1,000 live births [44, 79, 111, 112], which is within the usual range reported for Caucasian populations (0.91 to 2.69 per 1,000 births) [107, 108] and consistent with the mean prevalence of 1.36 per 1,000 live births calculated for Europe [20].

The overall incidence of CL±P was thought to be lowest in African populations, only 0.67 per 1,000 live births in Malawi [113] and 0.73 per 1,000 live births reported in Uganda [114] while higher rates were reported in Ghana (1.31 per 1,000) [115] and Kenya (1.65 per 1,000) [116]. The

difference in prevalence among Europeans and Africans is likely due to underlying genetic predispositions coupled with environmental factors, however socio-cultural factors such as high infant mortality rates and unreported infanticide in children born with anomalies could also account for the low birth prevalence in certain regions of Africa [117]. In addition, there may be underreporting and lack of consistency between studies in some low-income areas, with many of the world's most deprived populations continuing to be devoid of birth defects surveillance systems [20].

The overall incidence of CL±P was much higher in Asians than in Caucasians or Africans, with a prevalence of 1.81 per 1,000 live births reported in the Republic of Korea [118] and 1.66 per 1,000 live births in China [119] according to data from the Chinese Birth Defects Monitoring Network for the period 1996-2005 which recorded births across 517 hospitals and 31 provinces, one of the most extensive epidemiological studies of OCs in China to date. Interestingly, studies of Japanese and Chinese immigrants to the USA and UK indicate that migrant groups have rates of CL±P similar to the area from which they originated than to the area into which they have migrated [120]. Despite cohabiting the same region, Lowry and Trimble (1977) found that Native American Indians (3.74 per 1,000 live births), Japanese (3.36 per 1,000 live births) and Chinese inhabitants (1.76 per 1,000 live births) of British Columbia still had higher rates of OCs compared to the overall rate for Caucasians in the region (1.97 per 1,000 live birth) [121]. Ching and Chung (1974) suggested a genetic basis to this ethnic difference in OC prevalence. Their study in Hawaii showed that Japanese immigrants continued to have high birth prevalences of OCs but that infants of interracial Caucasian-Japanese matings demonstrated only intermediate birth prevalences of OCs [120]. On the other hand, Croen et al (1998) found a difference by country in that Filipinos had the highest birth prevalence of OCs in the Philippines, an intermediate prevalence in Hawaii, and the lowest prevalence in California highlighting the impact of nongenetic risk factors such as maternal diet that may occur following migration [109]. The relationships between ethnic origin and birth prevalence of clefts is therefore complex, and remains unclear.

1.4.3. Relative proportions of different cleft types

Worldwide, CLP is thought to be the dominant cleft type with Fogh-Andersen's (1942) CL:CLP:CP ratio of 1:2:1 often quoted as the ratio for the different cleft subgroups, especially for Caucasian populations [122]. In comparing data across 5 registries in the UK, the prevalence of CL±P ranged between 7.1 (Northern) to 9.7 (Glasgow) per 10,000 births, which is higher than the range for CP, which was between 2.6 (Oxford) to 6.9 (Glasgow) per 10,000 births [123]. US and European studies suggest that unilateral CLP is the most frequent single type of cleft accounting for 30-35% of non-syndromic OC. On the other hand, CL and CP each accounted for 20-25%, BCLP for about 10%, with submucous and other clefts in the remainder [124]. Within CLP, approximately 80% are unilateral and 20% are bilateral [20]. Studies also revealed differences in the incidence of CL±P versus CP in different regions worldwide. For CL±P, rates were high in parts of Latin America, Asia (China, Japan) but low in Israel, South Africa, and Southern Europe. On the other hand, rates of CP were high in Canada and parts of Northern Europe and low in parts of Latin America and South Africa [3, 20, 105].

1.4.4. Gender

There is a general acceptance in the literature of a male predominance in CL±P and a female predominance in CP [44, 125, 126]. In white populations, the male to female ratio for CL±P is approximately 2:1. In some studies the male predominance in the CL±P group is shown to be more apparent with increasing severity of cleft, whilst in other studies the male predominance in CL±P is attenuated when the infant has associated anomalies [3, 127]. There is no generally accepted explanation for these gender differences, though it may be partially related to the sex differences in the timing of critical developmental stages in craniofacial development. For CP, gender differences in incidence may be related to the different timing of palate development. The palatal shelves move

horizontally and begin to fuse by the seventh week in a male foetus. This does not occur until 8.5 weeks in a female foetus, thus having a longer window of vulnerability to teratogens [128].

1.4.5. Terminations and stillbirths

The EUROCAT data found that 86.6% of all OCs were live births, with only 9.3% terminations of pregnancy (TOP) and 3.7% stillbirths, with the highest proportions of TOP registered in the British Isles (14.3%), South-Mediterranean Europe (13.5%) and Western Europe (11.7%) [7]. In combined data from European registries for the period 1995-1999, there were higher proportions of stillbirths (3.5%) and TOP (9.4%) for CL±P compared to the proportion of stillbirths (2.4%) and TOP (8.1%) for CP [129]. However, in isolated cases, TOP were rare for CLP (1.5%) and CL (0.9%), and it was observed in the EUROCAT data that TOP were highest for syndromes and multiple malformed cases [7, 20]. It is also probable that a significant proportion of foetal deaths were due to other associated anomalies, although precise data is lacking at present [20].

1.5. Associated anomalies in OCs

1.5.1. Overview of associated anomalies in OCs

OCs can be classified as isolated, when the child has no other anomalies, or associated, when other seemingly unrelated anomalies are present in addition to the cleft anomaly. The associated cases can be further subdivided into recognised conditions (chromosomal, monogenic, teratogens), or there may be congenital anomalies in addition to the cleft which altogether is not due to a recognised entity [7].

It has been well-established that OCs are frequently associated with other congenital anomalies, although the reported prevalence varies among studies, ranging from as low as 2.8% [11] to as high as 63.4% [12]. The wide variation in the prevalence figures may be explained by the different methodological approaches researchers have taken which restrict comparability between studies, including inconsistencies in the definition of associated anomalies, differences in sample source, method of case ascertainment, and duration of the follow-up period in which the patient is observed. Studies investigating associated anomalies are summarised in Table 4. It has not been conclusively established whether OCs are related to particular anomalies and there is a lack of consensus as to which organ systems are most affected. Given that anomalies, chromosomal defects and syndromes are associated with OCs, accurate and contemporary data is necessary to facilitate prenatal diagnosis, genetic counselling, and service provisions. Further, identification of specific associations with OCs is important to elucidate the aetiology of this heterogeneous group of conditions. The recognition of associations of birth defects is potentially useful in identifying those teratogens that do not produce isolated anomalies but affect many structures of the developing embryo simultaneously [130]. The pattern recognition can later lead to a more exhaustive evaluation of risk factor exposures and eventually allow an interpretation of pathogenic mechanisms [131].

Table 4. Summary of studies on associated anomalies in infants with OCs

Study	Country	Study period	Study type	Source	Subjects (n)	Prevalence of associated anomalies (%) in the cleft subtypes					Criteria		Most commonly affected system (% of all anomalies)
						CL	CLP	CL±P	CP	Total	Inclusion	Exclusion	
Calzolari et al. (2007) [7]	EUROCAT	1980-1999	Retrospective	Birth registers	5449	20.8	34	-	-	29.2	Live births Stillbirths SA TOP	-	Musculoskeletal CVD CNS
Milerad et al. (1997) [8]	Sweden	1975-1992	Retrospective	Registry	616	8	28	-	22	21	Live births	SMCP	Upper lower limbs/vertebral columns (33%) CVD (24%)
Rawashdeh et al. (2008) [132]	Jordan	December 2005 – September 2006	Retrospective	Medical records and interviews	196	-	-	9.9	35.3	14.3	Live births	SMCP	CVD (45.5%) Skeletal system (15.9%) CNS (13.6%)
Rittler et al. (2011) [133]	ECLAMC**	January 2003 – December 2005	Retrospective	Medical records	710	7.6	23.5	-	42.3	26.1 and 31.4*	Live births	SMCP	CVD
Sekhon et al. (2011) [127]	India	August 2001 – December 2004	Retrospective	Medical records of referral centre	1623	17	20 (U) 37 (B)	-	29 (ICP) 3 (SMCP)	14.8	Live births SMCP	-	Facial region (21%) Ocular (17%) CNS (15%)
Shaw et al. (2004) [134]	USA	1983-1997	Retrospective	Birth surveillance system	6415	-	-	59.8	71.1	-	Live births Stillbirths SA TOP	-	Respiratory "Unspecified" Eye Other musculoskeletal
Shprintzen et al. (1985) [12]	USA	1976-1982	Prospective	Patients at referral centre	1000	45	53	52	72	63.4	Live births SMCP	-	Craniofacial CNS
Stoll et al. (2000) [9]	France	1979-1996	Prospective	Registry	460	13.6	36.8	-	46.7	36.7	Live births	SMCP	CNS Skeletal CVD Urogenital
Sun et al. (2013) [135]	China	2009-2011	Prospective	Cleft centre	2180	10.6	33.6	-	47.9	30.1	Live births	Syndrome PRS	CVD CNS Skeletal system

Please note the following abbreviations: SMCP = submucous cleft palate, (U) = unilateral cleft lip and palate, (B) = bilateral cleft lip and palate, SA= Spontaneous abortions, TOP= Termination of pregnancy

***ECLAMC network includes the following countries: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador and Venezuela.*

**Rate of associated anomalies increased from 26.1% to 31.4% when infants were reassessed for associated anomalies at 1-year follow-up.*

1.5.2. Frequency of associated anomalies

The IPDTOC, which collated data from 7,704 individuals with clefts, reported that the majority of OCs were isolated (76.8%), with a proportion associated with anomalies in other systems (15.9%) and with a subset associated with recognised syndromes (7.3%) [10]. International data are robust in terms of sample size and in providing regional information but they often extrapolate data from a combination of registers, and recent studies show that data from registers often underestimate the prevalence of associated anomalies in OCs. Further, studies based on registries are limited by variations in case ascertainment, diagnostic procedures and completeness of reporting [136]. In The Netherlands, Rozendaal et al. (2012) found that data from the national register identified only 19% of associated anomalies in OCs, compared to 61% detected for the same cohort on review of existing medical records. They attributed this significant discrepancy between the data sources to delayed diagnosis and deficient recording in registers [137, 138]. Further, Tan et al (2009) detected significantly higher rates of associated anomalies for CL±P (25.9%) and CP (76.9%) in the clinical validation phase of their study which utilised interviews, examinations, and medical record reviews, than the rates for CL±P (40%) and CP (18.2%) obtained for the same cohort on perusing birth records alone [139]. Therefore value is placed on studies displaying a robust methodology, which specifically examine for associated anomalies in children with OCs. This section will provide a critical discussion of existing studies that have reported on associated anomalies.

It is noticed that except for the results of Shprintzen (1985) [12], DeRoo (2003) [50], Shaw (2004) [134] and Rozendaal (2012) [137], the common range in which OCs are found to be associated with congenital anomalies appears to be between 21-37%, and according to a recent systematic review, the prevalence of associated anomalies in infants with OCs is approximately 25% for both CLP or CL±P [13]. Shprintzen et al. (1985) reported a much higher prevalence of associated anomalies, partly due to their definition of associated anomalies, where they a considered a cleft as

associated even if only minor defects or facial features were observed, such as hypoplastic nose and ocular hypertelorism [12]. Similarly, the high prevalence of associated anomalies reported by Shaw et al. (2004) (71.1% for CP and 59.8% for CL±P) may be due to the inclusion of minor defects [134]. Further, in studies where anomalies were only selectively recorded, such as if they required follow-up or intervention, rates were comparatively lower – 14.3% in Jordan [132] and 21% in Sweden [8].

In most studies, atypical clefts and midline clefts were excluded from the study sample, but the inclusion of submucous cleft palates (SMCPs) were variable. Studies often chose to exclude infants with SMCPs given that it is typically diagnosed later in life and thus cannot be reliably determined at birth [8, 9]. However, in studies that included infants with SMCPs, it was found that this subgroup had high rates of associated anomalies [140]. In fact, Shprintzen et al. (1985) found that the highest rates of associated anomalies, reportedly 77%, occurred in the SMCP group [12]. Klockars et al. (2010) also reported a high number of chromosomal aberrations amongst infants with SMCPs [141]. It is possible that associated anomalies may have been underestimated in studies that have excluded this cleft subtype, such as in the studies by Rawashdeh (2008) [132], Milerad (1997) [8], and Rittler et al. (2011) [133], reporting rates of 14.3%, 21% and 26.1%, respectively.

Further, study samples based on all live births, stillbirths, TOP, and spontaneous abortions, tend to show higher rates of associated anomalies since those with multiple anomalies tend to be spontaneously aborted and are less likely to survive the early months of life. In the systematic review by Maarse et al. (2012), it was found that on average, 54% of foetuses with CLP had associated anomalies in prenatal studies compared to only 25% of infants with CLP in postnatal studies [13]. Calzolari et al. (2007) reported a prevalence of 29.2% for associated anomalies in OCs for all pregnancy outcomes [7], and in another study, when only prenatally diagnosed foetuses with OCs were examined by Lopoo et al. (1999), the rate of associated anomalies was as high as 75% [142].

It also appeared that samples investigating for associated anomalies in infants referred for surgical treatment showed lower prevalences of associated anomalies, as this excluded stillbirths, TOP, spontaneous abortions and included cleft infants who have survived the early months and are in reasonable health to be surgical candidates. Rajabian et al. (2000) found that only 7.7% of the 1,669 cleft patients referred for reconstructive surgery between 1976-1991 had additional anomalies [126]. In Fogh-Andersen's two survey samples, the first sample consisting of 703 surgical patients had a 6.1% rate of additional anomalies in contrast to the second sample of 164 hospital births with a rate of 14% [122]. Jensen et al. (1988) also recognised that their figure of 4.5% probably underestimated the true prevalence of associated anomalies partly because they did not include stillbirths and early deaths [143].

The reported rates of associated anomalies are likely to be lower if data are obtained from records compiled for other purposes than when patients are specifically examined for associated anomalies. In retrospective studies providing a general overview of cleft epidemiology, rates of associated anomalies were 2.8% [11], 13% [144], and 14% [145]. On the other hand, in the prospective study by Shprintzen et al. (1985) which reported a rate of 63.4% of associated anomalies, infants were carefully examined and investigated further with tests and imaging where suspicions of defects arose [12]. Stoll et al. (2000) also reported a high rate of associated anomalies (36.7%) in their prospective study which recruited a clinical geneticist to examine every cleft case; and with each suspected or confirmed case, all available records including prenatal files, maternity files, hospital records, and autopsy reports were also reviewed for additional defects [9]. A thorough investigation for associated anomalies tended to detect higher rates of associated anomalies than less thorough methods.

The ascertainment of associated anomalies is also dependent upon patient age at the time of examination for additional anomalies. It is likely that examination at the time of birth would not uncover all defects, many of which would not become evident until later in life. Studies with a follow-

up of at least one year reported a higher rate of associated anomalies [9] than when infants were only examined during the neonatal period [146]. Rittler et al. (2011) supported this idea by demonstrating that 38 infants (7.2%) initially diagnosed as having an isolated cleft were found to have associated anomalies when reviewed again at 1-year follow-up, raising the overall proportion of infants with associated anomalies from 26.1% to 31.4% [133].

1.5.3. Associated anomalies by cleft type

In the systematic review by Maarse et al. (2012), CP was found to be the cleft type most frequently associated with additional anomalies (45.9%; range 22.2-78.3%) [13]. Tsukada et al. (1993) found that up to half of patients with CP or SMCP had additional congenital anomalies [147]. It seems that in most studies except that of Milerad et al. (1997) [8] there is a much higher incidence of associated anomalies in patients with CP than in patients with CL±P or CL [9, 127, 132]. In the investigation of studies showing exceptions, the highest frequency of other defects was found in CLP patients [13], but this may be due to the exclusion of SMCP, a cleft type indicated in the previous section to be associated with higher rates of associated anomalies [140, 141].

For CLP and CL±P, most studies found a prevalence of associated anomalies of approximately 25% [13] except for the studies of Shaw et al. (2004) [134] and Rozendaal et al. (2012) [137, 138] which reported a prevalence of 60%. Rozendaal et al. (2012) specifically reviewed registers to determine whether congenital anomalies associated with CLP were underreported, whereas the significantly higher rates of anomalies reported by Shaw et al. (2004) may be accounted for by the inclusion of infants with CL±P and other defects who were stillborn or died in the newborn period [134].

1.5.4. Associated anomalies by organ system

On the basis of current published research, associated anomalies were found to occur most frequently in the cardiovascular system [7, 9, 132, 135, 148], musculoskeletal system and extremities [7-9, 132, 134, 135, 148], and central nervous system (CNS) [7, 9, 12, 132, 135, 148].

Cardiovascular anomalies

Cardiovascular anomalies were commonly identified to be associated with OCs with a five- to sixteen-fold greater incidence of congenital heart disease in children with OCs compared to the general population [8, 9]. Milerad et al. (1997) found that atrial and ventricular septal defects were most common, followed by patent ductus arteriosus and transposition of the great vessels [8]. Stoll et al. (2000) found ventricular septal defects to be the most frequent cardiac anomaly [9], which is consistent with reports by Tsukada et al. (1993) [147] and Rawashdeh et al. (2008) [132].

Central nervous system

Shaw et al. (2004) reported cerebral anomalies to be most common (noted in 16%), followed by cardiac malformations (15%), facial anomalies (14%) and anomalies in the extremities (9%) [134]. Shprintzen et al. (1985) also reported that craniofacial and CNS anomalies were most frequent, which is not unexpected given the study was conducted in a craniofacial referral centre [12]. However, Rittler et al. (2008) examined the preferential associations between OCs and other major defects and compared these with non-orofacial cleft controls, and found that of all anomalies, OCs were positively associated with anencephaly, and that there was an over-representation of brain reduction defects in infants with OCs [149].

Extremities and musculoskeletal system

Although Stoll et al. (2000) agreed that CNS defects were common, they found that extremities and skeletal system defects were most frequent in their sample, present in 15.2% of

children found with associated anomalies [9]. This was consistent with the study by Milerad et al. (1997), which reported that most associated anomalies occurred in the musculoskeletal system [8].

1.6. Conclusion

OCs are the most common craniofacial anomaly with a birth prevalence of around 1 in 700 live births, showing ethnic, gender and regional variations. It is believed that normal orofacial morphogenesis involves extensive and tightly controlled signalling between numerous embryonic tissues. Disturbances at critical stages of lip and palate development contribute to cleft formation. OCs are associated with many chromosomal and non-chromosomal syndromes, and the search continues for genes responsible for non-syndromic OCs. There is most evidence to support an association between OCs and cigarette smoking, high alcohol consumption, folic acid and certain medications, and some higher-order gene-environment interactions may contribute to this association. Although the majority of OCs are isolated, a significant proportion are associated with other congenital anomalies, as part of chromosomal defects or syndromes. The evidence for associated anomalies is strongest for CP, and additional anomalies tend to occur in the cardiovascular system, the CNS and the extremities and musculoskeletal system. Existing studies, of which many are historical, demonstrate many inconsistencies due to the various methodological differences that restrict comparability between studies. The aim of this study is therefore to investigate the birth prevalence and associated anomalies in OCs whilst addressing the methodological concerns identified above.

Chapter 2: Materials & Methods

2.0. Study aims

The aim of this thesis was to investigate the birth prevalence of OCs and associated anomalies within a geographically defined region – Oxfordshire, Berkshire, and Buckinghamshire, collectively known as the Thames Valley. The specific aims were:

1. To determine the birth prevalence of OCs in a sample of CL±P patients within a geographically defined area, that is, Oxfordshire, Buckinghamshire and Berkshire.
2. To describe the characteristics of children with OCs in terms of:
    a. Socio-demographics
    b. Prenatal history
    c. Family history
    d. Cleft characteristics
3. To determine the prevalence of associated anomalies in infants with OCs.
4. To determine the types of associated anomalies in infants with OCs.
5. To describe the distribution of associated anomalies in relation to socio-demographics, prenatal history, family history and cleft characteristics of the child.

In order to answer the research questions outlined above, we collected information on the background, cleft, and other medical characteristics of subjects, and performed appropriate statistical analyses to examine the relationships between variables. This chapter provides the rationale and

operational details of the research methods used in this study.

2.1. Ethics approval

This study was approved on 31st January 2012 by the National Health Service (NHS), Health Research Authority, NRES Committee South Central, Portsmouth. The reference number is 12/SC/0080.

2.2. Study background

2.2.1. The reorganisation of cleft services in the UK

During the early 1990s, concerns were raised by health care professionals about the standards of cleft treatment in the UK [150]. In 1996, the Royal College of Surgeons Steering Group on CL±P published recommendations for Minimum Standards of Management of Cleft Care, which were based on agreed "good practice" within Europe and the USA [151]. It concluded that (1) cleft care should be provided by multidisciplinary regional teams, (2) there should be more multicentre audit and research, and (3) cleft surgery should be limited to surgeons with extended CL±P training and it was recommended for there to be an adequate caseload for operators undertaking cleft surgery [151]. Following from this, the Clinical Standards Advisory Group (CSAG) commissioned a national investigation of cleft care to compare current practice with the recommendations made by the Royal College of Surgeons Steering Group [152-154]. The results of the CSAG audit were that cleft outcomes were generally poor, care was often fragmented, and the majority of operators of cleft surgery performed only a handful of cleft surgeries annually [155]. An earlier survey of cleft surgeons operating between 1991-1992 found that cleft surgeons were treating an average of less than 10 new cases per year [156].

Following the CSAG report and recommendations, the Department of Health reduced the number of centres treating OCs from 57 to 9 centres from 2006, each with their own specialist surgeons, clinical nurse specialists, speech and language therapists, orthodontists, paediatric dentists, psychologists, and other specialist staff [157, 158]. In April 2004, The Spires Cleft Centre was designated as one such regional specialist cleft service, and this centralisation now presents a unique opportunity in the developed world to review the birth prevalence of OCs within a well-defined population base [150, 152]. There has been a high level of patient satisfaction with the regional services' new approach to cleft management [159] with reported satisfaction rates of up to 96% [160]. Experience has shown that only a small number of patients choose care outside the NHS service and that there is limited outflow from the region in which the child was born and diagnosed. Thus the assumption behind this study is that the referral load into a given team reflects the birth prevalence of clefting in the given population covered, and reflects the specific influences related to that area.

2.2.2. Terminology in this study

In epidemiological terms, "incidence" and "prevalence" are two distinct measures of the risk of disease. The frequency of OCs is most often reported in terms of "birth prevalence", that is, the proportion of newborns found to have a cleft at birth. Ideally, incidence rates would be used instead of prevalence to measure the occurrence of birth defects in a given population within a given period of time. However, it is not possible to obtain a true incidence for OCs given that the overall number of conceptions is unknown, as is the number of cases "lost" through spontaneous abortions [161].

The term "birth prevalence" also needs to be precisely defined, as to whether spontaneous abortions and stillbirths are included. In accordance with the Congenital Anomaly Register for Oxfordshire, Berkshire and Buckinghamshire (CAROBB), the term "birth" unless otherwise stated, will be used to cover all pregnancies (from 10 weeks gestation) ending in live birth, stillbirth,

miscarriage/intrauterine death or TOP. The term "live birth" is used for babies showing signs of life at birth (and includes neonatal deaths which occur after birth), whereas a "non-live birth" covers stillbirth, miscarriage, intrauterine death and TOP [162].

2.2.3. Classification of OCs in this study

As lip and palate have distinct developmental origins, OCs are often classified and were historically investigated in two main groups – as cleft lip with or without palate (CL±P) and cleft palate (CP) in which the lip is not affected [3]. Although the phenotypic variants of CL including CL±P are thought to differ aetiologically from CP, recent evidence suggests that CL and CL±P may be aetiologically distinct and should be considered separately for research purposes [163, 164]. There has also been recent interest in CL as a separate entity, with suggestions that CL is associated with cleft-related problems (speech, hearing) more often than anticipated [165]. In this study, OCs will be subdivided into three groups: CL, CP and CLP.

2.2.4. Classification of associated anomalies in this study

An associated anomaly recorded at any point during treatment, at any age, was recorded in this study. Unlike in the studies by Milerad (1997) [8] and Rawashdeh (2008) [132], minor defects were not excluded as seemingly minor defects could later point to an important aetiological element. OCs could be classified as "isolated", when no other anomaly is present, or "associated", when other congenital anomalies are present in addition to the cleft. Tolarova et al. (1998) suggested subdividing associated cases into: monogenic, chromosomal, known environmental, associations, and defects of unknown aetiology [26]. Calzolari et al. (2007) suggested that associated anomalies should be subdivided into (1) cases due to recognised conditions (chromosomal, monogenic, sequences, teratogens) versus (2) cases of multiple congenital anomalies of unknown origin [7]. A similar

classification of associated anomalies was devised for use in this study as illustrated in Figure 4.

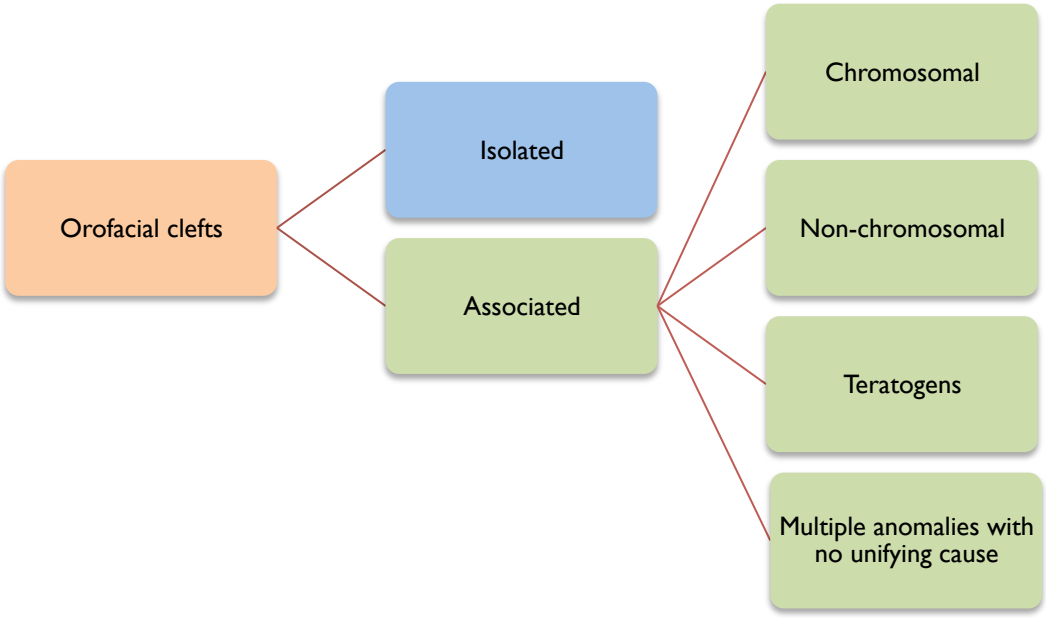


Figure 4. Classification system of associated anomalies in OCs in this present study. A cleft can be isolated or associated. Associated cases can be subdivided into those due to chromosomal syndromes, non-chromosomal syndromes, and teratogenic influences; there is a subgroup of associated anomalies with no unifying cause to explain the overall condition.

2.3. Study design

2.3.1. Type of study

This was a retrospective study, based on the review of medical records.

2.3.2. Participants

All patients attending The Spires Cleft Centre, born between January 1995 and July 2012, were included if they fulfilled the inclusion criteria and did not match any of the exclusion criteria

outlined below. A total of 791 patients with CL±P were identified and included in this study.

Inclusion criteria:

- All patients with a confirmed diagnosis of CL, CLP and CP including SMCP were included.
- Syndromic and non-syndromic clefts, including those with chromosomal anomalies, were included.
- Virtually all patients residing in Oxfordshire, Berkshire and Buckinghamshire were referred to The Spires Cleft Centre for management of their cleft. In order to provide an accurate estimate of birth prevalence within a defined region, only patients residing in these three counties were included in this study. Patients living in counties which were also serviced by other cleft teams, such as Gloucestershire, Northamptonshire, Wiltshire and Hertfordshire, were excluded.
- Live and non-live births were included, using information obtained from CAROBB.

Exclusion criteria:

- Patients with atypical clefts were excluded. In agreement with Maarse (2012), median cleft lip and atypical facial clefts should be separated from the analyses of CL±P, as they are distinct craniofacial anomalies with different pathogenesis [13].
- Patients with **occult** SMCP were excluded. In the absence of functional problems, investigations were often not performed to confirm the anomaly; therefore there is a lack of diagnostic certainty.

2.3.3. Study size

The target sample size of at least 700 patients was determined by a number of factors:

(1) **Sample sizes of similar studies:** studies specifically examining associated anomalies

in clefts had around 460 subjects [9], 616 subjects [8], and 710 subjects [133].

(2) **Timeframe allocated to conduct this Master of Science course:** this course began in January 2012 and data collection ended in April 2013. During this period, a literature review, ethics approval, data collection and analyses were to be completed.

It was decided to incorporate only patients born between January 1995 and July 2012, due to missing information for children born outside this timeframe:

(1) **Infants born before January 1995:** significant amounts of data were missing from medical records of children born before 1995, and it was not possible to obtain reliable and accurate information on associated anomalies and various cleft characteristics for this group.

(2) **Infants born after July 2012:** At the end of data collection in April 2013, many children born after July 2012 had outstanding appointments or were still awaiting the results of genetic studies. Therefore it was not feasible to include this cohort given that the clinical picture was incomplete at the time.

2.4. Data collection

2.4.1. Sources of data

Data was obtained in a systematic manner from several sources. Data files kept in several departments and by different health care teams were examined. This included: multidisciplinary team notes by staff in the cleft team and other hospital records including correspondence with other medical specialties where their services were sought. We then obtained data from the regional congenital anomaly register, CAROBB, and cross-referencing between the data sets as illustrated in Table 5 ensured completeness and identified virtually all cases of OCs in Oxfordshire, Berkshire and

Buckinghamshire. Rigorous cross-referencing minimised missing data for the variables of interest.

Table 5. Cross-referencing between data sources.

VARIABLES	SOURCES					
	The Cleft Spires Notes	Genetic records	Nursing records	ExceliCare	General Hospital Records	CAROBB
Age	✔	✔	✔	✔	✔	✔
Gender	✔	✔	✔	✔	✔	✔
Ethnicity	✔	✔	✔			✔
Residential information	✔			✔	✔	✔
Birth history	✔		✔	✔		✔
Family history	✔	✔	✔	✔		✔
Pregnancy history	✔	✔	✔	✔		✔
Genetics	✔	✔				✔
Cleft history	✔			✔		✔
Associated anomalies	✔	✔			✔	✔

*A tick indicates that information on the variable of interest was obtained from the source.

The Spires Cleft Centre

The Spires Cleft Network includes the Oxford University Hospitals NHS Trust and the Salisbury Foundation NHS Trust. The Spires Cleft Centre at the John Radcliffe Hospital, Oxford, UK, was the base for this study, and is the referral unit for Oxfordshire, Berkshire and Buckinghamshire. Referrals to the service include new cleft births, non-cleft referrals, and a number of returnees to the adult cleft service. The unit is routinely informed of new cleft births by delivery units, and infants are often examined before discharge and subsequently followed at several occasions throughout infancy and childhood by the multidisciplinary cleft team [166]. The cleft team has its own specialist cleft surgeon, otolaryngologist, clinical nurse specialists, speech and language therapists, orthodontists, paediatric dentists, psychologists, etc. There is also a genetic service, and referrals are made to other staff specialists as required. Records of these encounters are securely stored at The Spires Cleft Centre.

Information on the variables collected in this study was mostly obtained from the medical files maintained by the cleft team. A small percentage of patients are known to have left the area but many have been readily identified for follow-up. The service coordinators are well versed in exchanging datasets with agreed patient consent and details of follow-up in other services were often corresponded back to The Spires Cleft Centre, as was requested at the time of transfer of care.

ExceliCare

ExceliCare, an electronic patient record for OCs is being used on a full-time basis at The Spires Cleft Centre by the administration team and several clinical staff. Information stored on Excelicare was retrieved to complement data obtained from other sources. Excelicare did not record information on the additional anomalies found in patients with clefts, but included socio-demographic and geographic information on patients.

Clinical nurse specialists

Nursing notes were perused and clinical nurse specialists were interviewed to obtain additional information. Nursing specialists were often involved with patients from the neonatal period and recorded birth weight and gestation, from which to monitor feeding. Clinical nurse specialists have visited patients at home and have developed long-term treatment relationships, and noted personal details of patients including family history and consanguinity.

Genetic records

Genetic records of cleft patients were reviewed to obtain information on family history and any genetic diagnoses. Not all patients with clefts were reviewed by a geneticist; but where clinically indicated, patients were referred for consultation, cytogenetic and metabolic tests, and any other laboratory studies.

Congenital Anomaly Register for Oxfordshire, Berkshire and Buckinghamshire (CAROBB)

In April 2003, the Department of Health awarded funding towards the expansion of the Oxford Congenital Anomaly Register and enabled the formation of a new population-based register, CAROBB [162]. This register based at the National Perinatal Epidemiology Unit (NPEU) at the University of Oxford, receives notifications from several departments of infants born with an anomaly. Information reported in the early stages is later amended and/or confirmed by these multiple notifications. Information is stored on an electronic database and the information held by the register includes: descriptions of each anomaly, details and results of any investigations performed during pregnancy and details about mother and baby. Approval was obtained from NPEU to extract information from the CAROBB database to supplement the data collected for this study. Information on patients born in Oxfordshire was available from 1991 onwards, and for Berkshire and Buckinghamshire from 2004 onwards. From the CAROBB database, the following information was obtained: maternal age, additional anomalies, and live and non-live cleft births in the Thames Valley as per Table 5.

2.4.2. Variables of interest

Systematic compilation of data was tabulated in Microsoft Excel. Information was collected on the following variables:

(1) Age
    a. Date of birth
    b. Season of birth
(2) Gender
(3) Ethnicity
(4) Residential information
    a. Country of birth

b. Address at birth
    i. Postcode
    ii. Town
    iii. County

(5) Birth history
    a. Birth order
    b. Birth gestation (weeks)
    c. Birth weight (grams)

(6) Family history
    a. Birth order
    b. Single vs. multiple gestation
    c. Family history of clefts, noting the relation
    d. Consanguinity amongst biological parents
    e. Parental occupation

(7) Pregnancy history
    a. Maternal age
    b. Paternal age
    c. Smoking
    d. Illicit drug use

(8) Genetics
    a. Genetic diagnosis
        i. Chromosomal anomaly
        ii. Non-chromosomal syndrome
        iii. Other
    b. Pierre Robin Sequence

(9) Cleft history
    a. Cleft type
        i. CP
        ii. CL
        iii. CL±P
    b. Cleft laterality
        i. Unilateral
        ii. Bilateral
    c. Cleft side

i. Left

ii. Right

d. Prenatal diagnosis

e. Age at cleft diagnosis

(10) Associated anomalies

a. Cardiovascular

b. Central nervous system

c. Gastrointestinal

d. Respiratory

e. Urogenital

f. Skeletal and extremities

g. Neural tube defects

h. Head and neck

i. Eyes

j. Ears

k. Other

2.5. Data analysis

The data were analysed using Statistical Package for the Social Sciences (SPSS) Version 20 for Mac. Descriptive statistics (frequency, mean, median, and standard deviation) for the collected variables were calculated. For further comparisons, specific statistical tests were used.

2.5.1. Descriptive statistics

Descriptive statistics was used to calculate the following:

- The distribution and frequency of CL±P in relation to socio-demographics, gender, prenatal history, family history, genetic diagnoses, and cleft characteristics.
- The distribution and frequency of the most common associated anomalies found in CL±P
- The distribution and frequency of associated anomalies in relation to socio-demographics, gender, prenatal history, family history, genetic diagnoses, and cleft characteristics.

2.5.2. Statistical analysis between groups

- The Analysis of Variance (ANOVA), Fisher's exact test, Pearson chi-square tests and logistic regressions were used to compare groups for significant relationships. A p-value of less than 0.05 was considered to be significant. Data was often examined by stratified analysis using chi-square tests to test for statistical significance or differences in pattern.
- Denominator data was provided by the Office for National Statistics (ONS) to make comparisons between the cleft cohort and the general population. Data from the ONS included only live births and stillbirths of 24 weeks gestation or more.

Chapter 3: Results

3.0. Introduction

3.0.1. Overview

This chapter presents the findings from the study, in direct response to the aims outlined in Chapter 2 – Materials and Methods. Therefore the results will be described in terms of demographics, birth history, family history, chromosomal defects and associated anomalies, with the overall aim of the study to characterise OCs within each of these domains.

In this study, we identified a total of 791 infants born with OCs between January 1995 and July 2012 in Oxfordshire, Berkshire and Buckinghamshire. Not all of the following analyses will include the total number of subjects, as we were limited by missing data from incomplete records even after substantial cross-referencing.

3.1. Demographics

The main demographic characteristics of subjects are summarised in Table 6. Of the 791 infants with OCs, born between January 1995 and July 2012, there were 685 (86.6%) live births and 106 (13.4%) non-live births. The majority of infants were Caucasian (78.0%), there was a slight male predominance (52.8% versus 45.8%), and the main cleft type was CP (47.9%). Overall, 271 (34.3%) infants had other congenital anomalies in addition to a cleft.

Table 6. Summary of demographic characteristics of infants with OCs (N=791) in Oxfordshire, Berkshire and Buckinghamshire, born between January 1995 and July 2012.

	Live birth N (%)	Non-live birth N (%)	Total N (%)
Gender			
Male	361 (52.7)	57 (53.8)	418 (52.8)
Female	324 (47.3)	38 (35.8)	362 (45.8)
Indeterminate/ambiguous	0 (0.0)	11 (10.4)	11 (1.4)
Residence			
Oxfordshire	214 (31.2)	42 (39.6)	256 (32.4)
Berkshire	249 (63.4)	20 (18.9)	269 (34.0)
Buckinghamshire	153 (22.3)	30 (28.3)	183 (23.1)
No longer registered in the area	69 (10.1)	14 (13.2)	83 (10.5)
Ethnicity			
African	10 (1.5)	4 (3.8)	14 (1.8)
Asian	89 (13.0)	6 (5.7)	95 (12.0)
Caucasian	559 (81.6)	58 (54.7)	617 (78.0)
Mixed	13 (1.9)	0 (0.0)	13 (1.6)
Unknown	14 (2.0)	38 (35.8)	52 (6.6)
Cleft type			
CL	120 (17.5)	25 (23.6)	145 (18.3)
CLP	212 (30.9)	55 (51.9)	267 (33.8)
CP	353 (51.5)	106 (24.5)	379 (47.9)
Associated anomalies			
Yes	179 (28.8)	95 (91.3)	271 (34.2)
No	442 (71.2)	9 (8.7)	520 (65.8)

3.1.1. Residence

At the time of birth, 269 (34.0%) babies were registered with an address in Berkshire, 256 (32.4%) with an address in Oxfordshire, and 183 (23.1%) with an address in Buckinghamshire (Table 6). Only a further 83 (10.5%) were no longer registered with an address within the Thames Valley, demonstrating a low outflow from the area of birth.

3.1.2. Ethnicity

The majority of babies were Caucasian (78.0%). A further 12.0% were Asian, and 1.8% were of African descent (see Table 7).

Table 7. Ethnicity of infants born with OCs between January 1995 to July 2012 in the Thames Valley (N=739).

Ethnicity	N (%)
African	14 (1.8)
Asian	95 (12.0)
Bangladeshi	2
Chinese	7
Filipino	3
Indian	29
Indonesian	1
Middle Eastern	6
Pakistani	21
Sri-Lankan	2
Vietnamese	2
Unspecified	22
Caucasian	617 (78.0)
Mixed	13 (1.6)
Caucasian/African	4
Eurasian	8
Unknown	52 (6.6)
Total	791

3.1.3. Pregnancy outcomes

During the study period 1995 to 2012 inclusive, 106 non-live births involving OCs were identified in the Thames Valley (see Table 8). Of the 106 non-live births, 89 (84.0%) resulted in TOP, 12 (11.3%) in spontaneous abortion, and there were 5 (4.7%) stillbirths. Amongst the non-live birth cohort were 57 males, 38 females, and 11 babies of indeterminate or ambiguous sex. Males predominated with a ratio of 1.5:1. Regarding the type of cleft, 55 (52%) had CLP, 26 (24.5%) had CP, and 25 (23.5%) had CL. In those with CLP, 34 (62%) had a unilateral cleft and 21 (38%) had a bilateral cleft. The majority of non-live births had associated anomalies (89.6%).

Of the 89 cases resulting in TOP, chromosomal defects were found in 39 foetuses (41.6%), non-chromosomal syndromes in 15 (16.9%), teratogen exposure in 1 (1.1%), and there were 32 (35.9%) foetuses that had multiple structural anomalies not attributed to a chromosomal or syndromic cause as yet.

Table 8. Pregnancy outcomes for OCs in Thames Valley between January 1995 to July 2012 (N=791).

Pregnancy outcomes	N	%
Live birth*	685	86.6
Spontaneous abortion	12	1.5
Stillbirth	5	0.6
TOP	89	11.3
Total	791	100

**Includes 6 neonatal deaths*

3.1.4. Cleft type

Overall, CP was the most common cleft type (n=379, 47.9%) followed by CLP (n=267, 33.8%) and CL (n=145, 18.3%). Of the CLP cases, 205 (76.8%) were unilateral and 62 (23.2%) were bilateral. Of the unilateral CLP, the left side (62.9%) was affected more often than the right side (37.1%).

3.1.5. Sex

There were 418 (52.8%) males to 362 (45.8%) females, and there were 11 (1.4%) foetuses of ambiguous or indeterminate sex from the non-live birth database. Comparing males to females, there was a slight male predominance with a ratio of 1.15:1.

Table 9 and Figure 5 demonstrate the sex of the baby according to cleft type. Excluding the 11 foetuses of indeterminate sex, there were 376 CP cases, where 165 (43.9%) were males and 211 (56.1%) were females. Of the 260 CLP cases, 174 (66.9%) were males and 86 (33.1%) were females. Of the 144 CL cases, 79 (54.9%) were males and 65 (45.1%) were females. Chi-square tests revealed an association between gender and cleft type, where CP affected more females than males (χ^2=27.5, p=0.00) and CLP affected more males than females (χ^2=27.9, p=0.00). CL affected more males than females but the difference was not statistically significant. Furthermore, bilateral clefts were more common among males (n=48) than females (n=14) in the CLP group (χ^2=4.1, p=0.04).

Table 9. Distribution of cleft types according to sex (N=780) excluding unknowns (N=11).

Cleft types	Male (N)	Female (N)	Total (N)	Total (%)	χ^2	p-value
CL	79	65	145	18.3	0.12	0.74
CLP	174	86	267	33.8	27.9	0.00
CP	165	211	379	47.9	27.5	0.00

**Statistically significant results in red.*

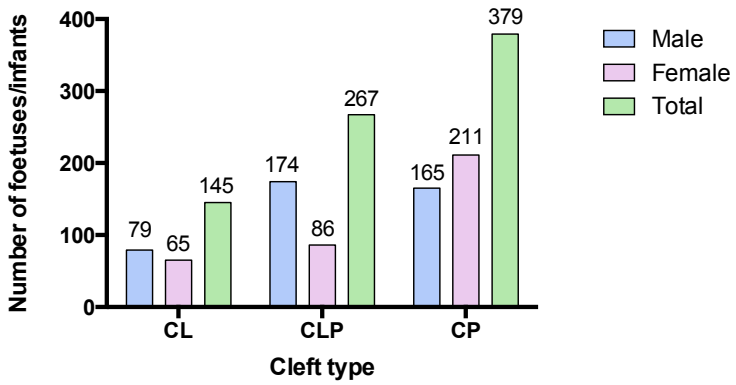


Figure 5. Distribution of cleft types according to sex (n=780) excluding unknowns (n=11).

3.2. Birth statistics

3.2.1. Calculating birth prevalence

By the time data collection was finalised in December 2012, not all children born that year had attended The Spires Cleft Centre. Therefore excluding the birth year 2012 from the analyses of birth prevalence by year, the total number of births affected by OCs varied from 33 in 1995 and 1996, to 72 in 2007 (Figure 6). For live births only, the number of children affected by OCs varied from 23 in 1997, to 63 in 2007 (Table 10, Figure 7). According to the ONS, there were 475,464 live births between and including the years 1995 and 2011 in Oxfordshire, Berkshire and Buckinghamshire [167]. Related to the number of live births within the Thames Valley, this gave a range of 0.85 to 2.1 cases of OCs per 1,000 live births and a mean birth prevalence of 1.44 cases of OCs per 1,000 live births.

Table 10. Live births affected by OCs by year of birth in the Thames Valley between January 1995 and December 2011.

Year of Birth	CL (N)	CLP (N)	CP (N)	Total clefts (N)	Total live births in Thames Valley* (N)	Birth prevalence of OCs (N per 1,000)
1995	6	9	12	27	26,657	1.01
1996	5	12	10	27	27,057	1.00
1997	4	10	9	23	27,121	0.85
1998	8	11	16	35	26,863	1.30
1999	3	9	16	28	26,969	1.04
2000	7	7	22	36	26,097	1.38
2001	8	6	14	28	25,454	1.10
2002	6	8	17	31	25,567	1.21
2003	1	5	26	32	26,910	1.19
2004	6	6	23	35	27,261	1.28
2005	7	14	25	46	27,298	1.69
2006	9	16	27	52	28,695	1.81
2007	12	27	24	63	29,716	2.12
2008	7	11	30	48	30,730	1.56
2009	14	17	25	56	30,392	1.84
2010	5	18	19	42	31,321	1.34
2011	11	21	25	57	31,356	1.82
Total	119	207	340	666	475464	1.44

**Data from Office for National Statistics [167].*

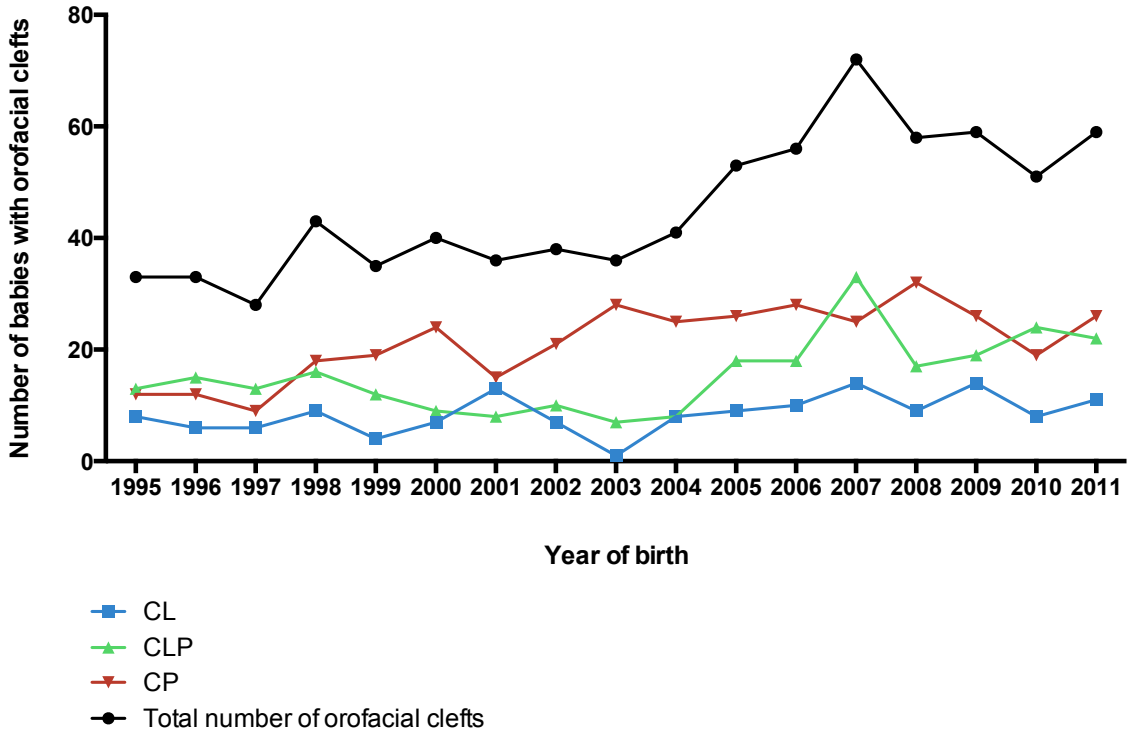


Figure 6. Total births affected by OCs by year in the Thames Valley between January 1995 and December 2011.

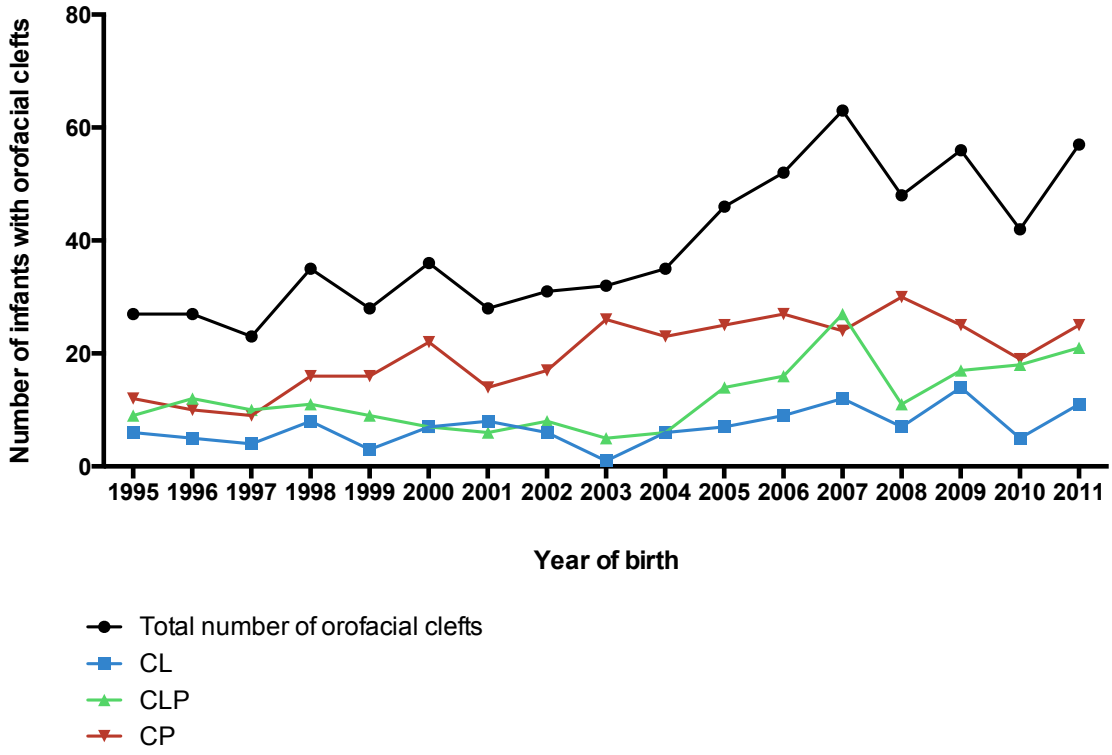


Figure 7. Live births affected by OCs by year in the Thames Valley between January 1995 and December 2011.

3.3. Birth history

3.3.1. Season of birth

For the 685 live births, the number of births peaked in July (10.1%). For CL, births peaked in May (12.5%) and July (10.8%). For CLP, births peaked in January (10.4%) and July (12.4%). For CP, births peaked in January (9.9%), March (13.0%) and May (9.9%) as outlined in Table 11.

Table 11. OCs by month of birth for live births in the Thames Valley (N=685).

Month of birth	CL N (%)	CLP N (%)	CP N (%)	Total N (%)
January	9 (7.5)	22 (10.4)	35 (9.9)	66 (9.6)
February	7 (5.8)	19 (9.0)	16 (4.5)	42 (6.1)
March	4 (3.3)	16 (7.5)	46 (13.0)	66 (9.6)
April	11 (9.2)	15 (7.1)	20 (5.7)	46 (6.7)
May	15 (12.5)	13 (6.1)	35 (9.9)	63 (9.2)
June	10 (8.3)	11 (5.2)	32 (9.1)	53 (7.7)
July	13 (10.8)	26 (12.4)	30 (8.5)	69 (10.1)
August	8 (6.7)	17 (8.0)	21 (5.9)	46 (6.7)
September	10 (8.3)	17 (8.0)	27 (7.6)	54 (7.9)
October	11 (9.2)	15 (7.1)	27 (7.6)	53 (7.7)
November	11 (9.2)	20 (9.4)	30 (8.5)	61 (8.9)
December	11 (9.2)	21 (9.9)	34 (9.6)	66 (9.6)
Total	120 (100)	212 (100)	353 (100)	685 (100)

Regarding the seasons of birth, there was a peak in the number of CP births in spring (30%), peak in the number of CLP births in winter (30%) and there was a peak in spring (27%) and summer (27%) births for CL. However, chi-square tests indicated no significant association between season of birth and cleft type (χ^2=6.29, p=0.39), and there was no significant association between season and gender, or season and the presence of associated anomalies.

Table 12. OCs by season of birth for live births in the Thames Valley (N=685).

Season of birth	CL N (%)	CLP N (%)	CP N (%)	Total N (%)
Winter	27 (22.5%)	63 (29.7%)	85 (24.1%)	175 (25.5%)
Spring	32 (26.7%)	45 (21.2%)	105 (29.7%)	182 (26.6%)
Summer	32 (26.7%)	54 (25.5%)	85 (24.1%)	171 (25.0%)
Autumn	29 (24.2%)	50 (23.6%)	78 (22.1%)	157 (22.9%)
All seasons	120 (100%)	212 (100%)	353 (100%)	685 (100%)

3.3.2. Prenatal diagnosis

Results for 675 prenatal scans were available – 669 ultrasound scans and 6 Magnetic Resonance Imaging (MRI) scans. Of the 675 infants with clefts, 249 (36.9%) were identified prenatally on ultrasound or MRI. For CP, 7 cases were identified prenatally, and nearly all (n=6) were diagnosed through MRI, with a strong clinical suspicion and/or positive family history being the indicator for the imaging study. Only 1 case of CP was diagnosed by ultrasound alone. Table 13 outlines the rates of prenatal diagnosis of OCs using ultrasound alone. Of the 352 CL±P cases (i.e. CL and CLP cases), 242 (68.8%) were diagnosed on prenatal ultrasound alone. For CL, 51.6% were identified antenatally, and for CLP, 78.1% were identified antenatally.

Table 13. Antenatal diagnosis of OCs on prenatal ultrasound only (N=669).

Prenatal diagnosis	CL N (%)	CLP N (%)	CP N (%)	Total N (%)
Yes	64 (51.6)	178 (78.1)	1* (0.3)	243 (36.3)
No	60 (48.4)	50 (21.9)	316* (99.7)	426 (63.7)
Total	124 (100)	228 (100)	317 (100)	669 (100)

**6 cases of CP were diagnosed by magnetic resonance imaging (not by ultrasound)*

3.3.3. Birth weight and gestational age

Of the infants with clefts without associated anomalies, only 5.7% had a birth weight <2500 grams in contrast to 18.4% of infants with clefts with associated anomalies. Chi-square test revealed that infants with clefts and associated anomalies were more likely to be born with a low birth weight compared to infants with clefts without associated anomalies ($\aleph^2$=24.47, p<0.001) as indicated in Table 14.

Table 14. Birth weight for live born babies and presence of associated anomalies (N=620).

Birth weight	Associated anomalies N (%)		Total N (%)
	Yes	No	
<2500 grams	33 (18.4)	25 (5.7)	58 (9.4)
≥ 2500grams	146 (81.6)	416 (94.3)	562 (90.6)
Total	179 (100)	441 (100)	620 (100)

Of the infants with clefts without associated anomalies, 7.1% were born preterm i.e. before the 37th week of gestation in contrast to 16.9% of the infants with clefts with associated anomalies (Table 15). Chi-square test indicated that infants with clefts and associated anomalies were more likely to be born premature compared to infants with clefts without associated anomalies ($\aleph^2$=10.73,

p=0.001).

Table 15. Presence of associated anomalies by gestation (n=479).

Gestation	Associated anomalies N (%)		Total N (%)
	Yes	No	
Preterm	25 (16.9)	24 (7.1)	49 (10.1)
Term	123 (83.1)	312 (92.9)	435 (89.9)
Total	148 (100)	336 (100)	484 (100)

3.4. Family history

3.4.1. Parental age

The average maternal age was 29.7 years and the average paternal age was 30.0 years (Table 16). One-way ANOVA examined the associations between cleft types and maternal ($F(2,490)=0.40$, $p=0.67$) and paternal age ($F(2,88)=0.68$, $p=0.51$), and between the presence of associated anomalies with maternal ($F(1,483)=0.55$, $p=0.46$) and paternal age ($F(1,89)=0.26$, $p=0.61$), but found no associations. Logistic regression found that advanced maternal age (≥40 years old) was associated with increased risk of isolated OCs. In fact, a U-shape was seen with maternal age and isolated OCs, with the highest risk occurring in the youngest and oldest mothers as demonstrated in Table 17.

One-way ANOVA revealed that in the cleft cohort, mothers who gave birth to infants with chromosomal anomalies were older (32.5±6.3 years, 95% CI [30.9-34.2]) than those who gave birth to infants without chromosomal anomalies (29.3±6.0 years, 95% CI [28.7-29.8]) ($F(1,482)=15.52$, $p=0.00$). Similarly, fathers of infants with chromosomal anomalies were generally older (31.6±8.0 vs. 30.0±6.3), but the association was not statistically significant ($F(1,88)=0.30$, $p=0.59$).

Table 16. Parental age by cleft type.

Cleft type	Maternal age			Paternal age		
	N	Mean (SD)	Range	N	Mean (SD)	Range
CL	96	30.1 (5.9)	15-41	10	28.6 (7.5)	17-41
CLP	209	29.4 (6.3)	17-42	28	29.3 (7.3)	18-43
CP	188	29.7 (6.1)	16-44	53	30.7 (5.7)	18-43
Total	493	29.7 (6.1)	15-44	91	30.0 (6.4)	17-43

Table 17. Logistic regressions of OCs with and without associated anomalies in the Thames Valley between January 1995 and July 2012.

	OCs and no associated anomalies		OCs and associated anomalies	
Mother age	**OR**	**95% CI**	**OR**	**95% CI**
<20	1.39	(0.59, 3.27)	0.72	(0.31, 1.70)
20-24	0.81	(0.37, 1.80)	1.23	(0.56, 2.74)
25-29	1.06	(0.48, 2.33)	0.95	(0.43, 2.08)
30-34	1.01	(0.44, 2.30)	1.00	(0.44, 2.27)
35-39	0.66	(0.23, 1.96)	1.51	(0.51, 4.47)
≥40	1.66	(0.77, 3.58)	0.60	(0.28, 1.30)
Paternal age				
<20	1.50	(0.15, 15.46)	0.67	(0.07, 6.87)
20-24	2.25	(0.23, 22.14)	0.44	(0.05, 4.37)
25-29	0.79	(0.11, 5.5)	1.27	(0.18, 8.9)
30-34	1.38	(0.21, 9.02)	0.73	(0.11, 4.77)
35-39	0.79	(0.11, 5.49)	1.27	(0.18, 8.9)
≥40	-	-	-	-

3.4.2. Consanguinity

Of the 791 babies with OCs, 18 (2.0%) were born to consanguineous parents. Of the 18 infants, 14 had a CP (77.8.0%), 4 (22.2%) had a CLP (3 = BCLP, 1 = UCLP), and there were no cases of CL. Fisher's exact test indicated that consanguineous unions were more likely to result in infants with palatal clefts than non-palatal clefts (p=0.02).

In the cleft cohort, Fisher's exact test did not show an association between consanguineous unions and chromosomal anomalies (p=0.71) or between consanguineous unions and associated anomalies in infants (p=0.15). There was also no statistically significant association between consanguineous unions and the likelihood of a pregnancy resulting in a live or non-live birth within

the cleft cohort (p=0.73).

3.4.3. Family history of orofacial clefts

One hundred and six babies (21.4%) had a positive family history of OCs. A positive family history was identified most commonly amongst infants with CLP (24.8%) followed by those with CP (21.1%) and CL (16.3%) as indicated in Table 18.

Table 18. Family history according to cleft types (N=496).

Family history	CL N (%)	CLP N (%)	CP N (%)	Total N (%)
Yes	15 (16.3)	38 (24.8)	53 (21.1)	106 (21.4)
No	77 (83.7)	115 (75.2)	198 (78.9)	390 (78.6)
Total	92 (100)	153 (100)	251 (100)	496 (100)

Of the 106 babies with a family history of clefts, 21 (19.8%) subjects had a sibling with a cleft, 46 (43.4%) had a relative with a cleft from the maternal side of the family, 32 (30.2%) had a relative with a cleft from the paternal side of the family, 7 (6.6%) had relatives with clefts in both sides of the family.

In terms of the degrees of relationship between subjects and the relative(s) with OCs, 37 (34.9%) had a 1st-degree relative (parent, sibling) affected by cleft, 12 (11.3%) had a 2nd-degree relative (grandparent, uncle, aunt) affected by cleft, 26 (24.5%) had a distant relative affected by cleft (great-grandparent, cousin), and 30 (28.3%) had more than one relative of different degrees of relationship affected by cleft.

3.5. Associated anomalies – I. Classification

3.5.1. Overview

Overall, 271 (34.3%) had an associated anomaly, and 520 (65.7%) had an isolated OC, not associated with any other congenital anomalies. In live births, only 25.5% had associated anomalies compared to 89.6% of non-live births with associated anomalies.

On closer examination of all those with associated anomalies, 25.1% had a chromosomal defect, 18.8% were identified to have a non-chromosomal syndrome, 3.0% were associated with known teratogens, and 53.1% had associated anomalies in addition to their cleft without a unifying diagnosis or recognised association to be regarded as a syndrome as depicted in Figure 8. Table 19 outlines the figures for isolated and associated anomalies by cleft type.

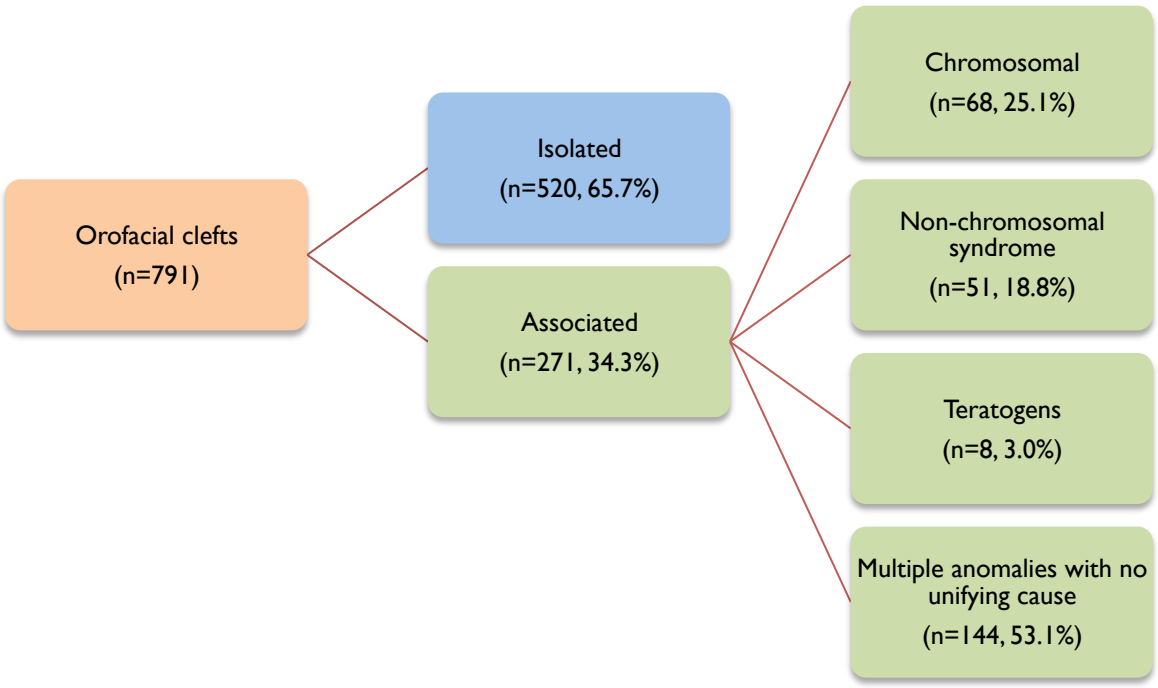


Figure 8. Total births by isolated cleft cases versus those with associated anomalies and its subdivisions by categories of chromosomal, non-chromosomal syndrome, teratogens and multiple anomalies with no unifying cause.

Table 19. Chromosomal defects, syndromes, teratogens and other anomalies associated with OCs (N=791).

	Live births N (%)			Non-live births N (%)			All births N (%)			
	CL	CLP	CP	CL	CLP	CP	CL	CLP	CP	Total
Isolated	97 (80.8)	182 (85.8)	231 (65.4)	1 (4)	8 (14.5)	2 (7.7)	98 (67.6)	190 (71.2)	233 (61.5)	520 (65.7)
Associated	23 (19.2)	30 (14.2)	123 (34.8)	24 (96)	47 (85.5)	24 (92.3)	47 (32.4)	77 (28.8)	147 (38.8)	271 (34.3)
Chromosomal	3 (13.05)	3 (10.0)	28 (22.8)	8 (33.3)	23 (48.9)	4 (16.7)	11 (23.4)	26 (33.8)	32 (21.8)	68 (25.1)
NCS	3 (13.05)	6 (20.0)	28 (22.8)	3 (12.5)	7 (14.9)	4 (16.7)	6 (12.8)	13 (16.9)	32 (21.8)	51 (18.8)
Teratogen	0 (0.0)	0 (0.0)	6 (4.9)	1 (4.2)	0 (0.0)	1 (4.2)	1 (2.1)	0 (0.0)	7 (4.8)	8 (3.0)
MCA	17 (73.9)	21 (70.0)	61 (49.5)	12 (50.0)	17 (36.2)	15 (62.5)	29 (61.7)	38 (49.3)	76 (52.7)	144 (53.1)
Total	120 (100)	212 (100)	353 (100)	25 (100)	55 (100)	26 (100)	145 (100)	267 (100)	379 (100)	791 (100)

** NCS = non-chromosomal syndrome, MCA = multiple congenital anomalies of unknown origin*

Note. The percentages for chromosomal defects, NCS, teratogens and MCAs, are calculated as a percentage of total associated anomalies.

3.5.3. Isolated clefts

In total, 65.7% had isolated OCs. While more than two-thirds of infants with CL (n=98, 67.6%) and CLP (n=190, 71.2%) had an isolated cleft anomaly, a smaller proportion of CP occurred as an isolated anomaly (n=233, 61.5%).

3.5.4. Chromosomal defects

Chromosomal defects were found in 68 infants, accounting for 25.1% of those with associated anomalies (see Figure 8). Again in Table 19, for all cleft types, chromosomal defects accounted for a significant proportion of cases of associated anomalies – for example, 33.8% of CLP cases associated with additional anomalies were also associated with a chromosomal defect. In live births, the most common chromosomal defect found in the cleft cohort was 22q11 deletion syndrome (24.2%). Other chromosomal defects found in live births are indicated in Table 20.

Table 20. Chromosomal defects in live births (N=33)

Chromosomal defect	N
5p15.2 deletion	1
12p13.1-12p13.3 deletion	1
16p11.2 and 17q25.3 deletion	1
17p13.3 duplication	2
17p13.3 deletion	1
2q13 duplication	1
4q32-4q35 deletion	1
5q23 deletion	1
8q24.11-8q24.22 deletion	1
22q11 deletion syndrome	8
Xp22.33 duplication	1
Abnormal 15q	1
Extra segment Y chromosome	1
Inversion chromosome 8 and 2p duplication	2
Translocation (unbalanced) of 22/11	1
Trisomy 13	1
Trisomy 21	3
Turner syndrome	3
Other chromosomal aberration	2
Total	33

3.5.5. Non-chromosomal syndromes

Non-chromosomal syndromes were found in 51 infants, accounting for 18.8% of those with associated anomalies (see Figure 8). In OCs with associated anomalies, non-chromosomal syndromes were found in 21.9% of non-isolated CP cases, 16.9% of non-isolated CLP cases and 12.8% of non-isolated CL cases. The most common non-chromosomal syndrome in live births was Stickler syndrome as per Table 21.

Table 21. Non-chromosomal syndromes for total births with OCs (N=51)

Syndrome	N
Branchio-oculo-facial syndrome	1
Cerebrocastomandibular syndrome	1
Cri du chat syndrome	1
Cystic fibrosis	1
Dandy-Walker syndrome	1
Diamond Black Fan syndrome	1
Dravet's syndrome	1
Ectrodactyly-ectodermal dysplasia-cleft (ECC) syndrome	1
Ehlers Danlos syndrome	1
Emery-Dreifuss	1
Fragile X syndrome	2
Goldenhar syndrome	3
Hereditary spherocytosis	1
Kabuki syndrome	4
Klippel Feil syndrome	1
Kniest syndrome	3
Marden-Walker syndrome	1
Marshall syndrome	1
Neurofibromatosis type 1	1
Noonan's syndrome	1
Peter-Plus syndrome	1
Roberts syndrome	1
Russell-Silver syndrome	1
Smith-Lemli-Opitz syndrome	1
Stickler syndrome*	10
Treacher Collins syndrome	2
Van der Woude syndrome	4
William syndrome	1
Other	2
Total	51

**10 children had Stickler syndrome, but only 6 of the 10 were diagnosed with PRS.*

3.5.6. Teratogens

Seven of the 8 (92%) cases, which were associated with teratogen exposure, occurred with CP. Seven cases were related to valproate exposure and one was related to maternal alcohol consumption.

3.5.7. Multiple congenital anomalies

In OCs with associated anomalies, multiple anomalies without a unifying cause were found in 61.7% of non-isolated CL, 52.1% of non-isolated CP and 49.3% of non-isolated CLP.

3.5.8. Pierre Robin Sequence

There were 72 cases of PRS, in which 40 cases involved isolated CP, micrognathia and glossoptosis. Amongst the infants with PRS, there were 6 cases of Stickler syndrome, 1 Marshall syndrome, 1 Neonatal abstinence syndrome, and 24 cases of multiple anomalies without a cause.

3.5. Associated anomalies – II: By cleft type, sex, birth history

3.5.1. Associated anomalies in relation to cleft type

In total, 271 (34.3%) had other anomalies in addition to their cleft. Of those with associated anomalies 47 (17.3%) had CL, 147 (54.2%) had CP, and 77 (28.4%) had CLP. Of the 77 CLP cases, 51 (66%) were unilateral and 26 (34%) were bilateral.

In examining the individual cleft types, associated anomalies were found in 38.8% of all CP cases, 28.8% of all CLP cases and 32.4% of all CL cases. A higher proportion of associated anomalies were found in children with BCLP (45.6%) than those with CLP (37.5%) (χ^2=6.80, p=0.0091). When cleft types were re-categorised into two groups, CP and CL±P, chi-square test revealed that associated anomalies occurred more frequently with CP ($\chi^2$8.70, p=0.003).

3.5.2. Associated anomalies in relation to sex

Although there were more males (n=145) than females (n=121) with associated anomalies, chi-square test demonstrated no association between the sexes in terms of likelihood of associated anomalies (χ^2=0.32, p=0.58). Females were more likely to have isolated CP (n=120 vs 76), but the sex ratio was almost even (n=75 vs 70) for CP with associated anomalies.

3.5.3. Associated anomalies by live vs. non-live births

Chi-square test demonstrated an association between birth outcome and presence of associated anomalies (χ^2=152.67, p=0.00), indicating that non-live births and neonatal deaths were more often associated with anomalies than live births.

3.5.4. Associated anomalies by maternal age

One-way ANOVA compared maternal age with presence of associated anomalies. There was no difference between maternal age and presence of associated anomalies. The mean maternal age at birth for children with associated anomalies was 29.9 years old (SD 6.3) and the mean maternal age for children without associated anomalies was 29.5 years old (SD 6.2), which was not statistically significant ($F=0.68$, $p=0.41$).

3.5.5. Associated anomalies by consanguinity

Fisher's exact test did not detect a significant association between consanguinity and associated anomalies ($p=0.14$).

3.5.6. Associated anomalies by chromosomal defects

Chi-square test revealed that those with chromosomal defects were more likely to have other anomalies in addition to their cleft ($\aleph^2=87.3$, $p=0.00$).

3.5.7. Multiple anomalies

Of the 271 babies with associated anomalies, 186 (67.9%) had *multiple* anomalies in addition to the cleft.

3.6. Associated anomalies – III: By organ system

In this section, the associated anomalies will be analysed by the organ system affected. In cases where an infant or foetus has multiple anomalies, each defect is counted as a separate case. The different organ systems affected by associated anomalies are shown in Table 22. Anomalies of the skeletal system and extremities were most common, followed by those in the cardiovascular and central nervous systems.

Table 22. Associated anomalies by organ system.

System	Males	Females	UD	Total
Cardiovascular	38	43	4	85
Central nervous system	40	44	1	85
Ears	22	20	0	42
Eyes	24	19	0	43
Gastrointestinal	28	17	1	46
Respiratory	14	7	0	21
Skeletal system and extremities	55	53	4	112
Urogenital	43	17	2	62

**UD – undetermined/ambiguous sex*

3.6.1. Anomalies in the cardiovascular system

Of the 271 babies identified with additional associated anomalies, 85 (31.4%) had a malformation in the cardiovascular system. There were 38 males, 43 females, and 4 of indeterminate sex, with no indication of a statistically significant difference between the sexes regarding the presence of cardiovascular anomalies (χ^2=1.40, p=0.24). In total, 141 cardiac defects were identified amongst the 85 babies. Only a single cardiac defect was identified in 36 cases (42%), whilst multiple cardiac defects co-existed in the majority of individuals (62%). The most common defect was ventricular

septal defect (26%) followed by atrial septal defect (19%) and patent ductus arteriosus (16%). Forty-eight (56%) infants with cardiovascular anomalies had a CP and 37 (44%) had a CL±P. There was no statistically significant difference in the distribution of cardiovascular malformations according to cleft type (χ^2=3.00, p=0.08).

Table 23. Cardiac defects (N=141) in babies with OCs and cardiovascular anomalies (N=85).

Anomaly	N
Aortic stenosis	3
Atrial septal defect	27
Coarctation of aorta	3
Dextrocardia	1
Double outlet right ventricle	2
Hypertrophic cardiomyopathy	2
Hypoplastic vessels	4
Hypoplastic left heart syndrome	3
Patent ductus arteriosus	23
Patent foramen ovale	8
Pulmonary artery stenosis	6
Transposition of great vessels	1
Tetralogy of Fallot	3
Truncus arteriosus	4
Ventricular septal defect	36
Valvular defects	6
Other	9
Total	141

3.6.2. Anomalies in the central nervous system

Of the 271 babies identified with associated anomalies, 85 (31.4%) had a malformation in the CNS. Fifty-five (65%) infants with CNS defects were non-live births, and only 30 (35%) were live births. Chi-square tests indicated that CNS defects were more often associated with non-live births due to spontaneous abortion, TOP, miscarriage and stillbirth (χ^2=188.77, p=0.00). The most common CNS defects were congenital hydrocephalus, neural tube defects and microcephaly. Other CNS defects are detailed in Table 24.

Table 24. CNS defects (N=106) in babies with OCs and CNS anomalies (N=85).

Anomaly	Number
Acrania	1
Agenesis of corpus callosum	8
Agenesis of other brain structure	5
Congenital hydrocephalus	31
Cerebellar hypoplasia	7
Dandy Walker complex	10
Encephalomalacia	1
Holoprosencephaly	11
Hydrops	2
Lissencephaly	1
Macrocephaly	2
Megalencephaly	1
Microcephaly	11
Neural tube defects	14
Univentricular brain	1
Total	106

Neural tube defects

There were 14 cases of neural tube defects, affecting near equal numbers of males and females as shown in Table 25. The majority of cases involving neural tube defects resulted in non-live births (93%). There was only one case of a neural tube defect resulting in a live birth.

Table 25. Neural tube defects (N=14).

Anomaly	Live birth (N)		Non-live birth (N)		Total (N)
	Male	Female	Male	Female	
Anencephaly	0	0	2	1	3
Encephalocoele	0	0	2	2	4
Inencephaly	0	0	1	0	1
Spina bifida	0	1	2	3	6
Total	0	1	7	6	14

3.6.3. Anomalies of the eyes

Forty-nine anomalies of the eye(s) were identified in 43 babies with cleft. The most common defect was a squint (16%) followed by coloboma (12%), nasolacrimal duct stenosis/obstruction (8%) and significant congenital visual impairment (8%).

Table 26. Anomalies of the eyes (N=43).

Anomaly	N
Amblyopia	2
Band keratopathy	1
Coats' disease	2
Coloboma	6
Congenital glaucoma (primary)	1
Dacrocystocoele	1
Iris defect	1
Lens dislocation	1
Limbal dermoid	1
Microphthalmia	3
Nasolacrimal duct stenosis/obstruction	4
Optic hypoplasia	1
Proptosis	1
Ptosis	2
Septo-optic dysplasia	1
Staphyloma	1
Strabismus	2
Structural anomaly of eye	3
Squint	8
Visual impairment	4
Other eye anomaly	3
Total	49

3.6.4. Anomalies of the ears

Forty-three anomalies of the ear(s) were identified in 42 babies with clefts. The most common anomaly was congenital hearing impairment/deafness (28%), low set ears (21%), and other external ear malformations (19%).

Table 27. Anomalies of the ears (N=42).

Anomaly	N
Absent ear	1
Accessory tragus/auricle/pinna	1
Aural polyp	1
Cholesteatoma	1
Congenital hearing impairment	12
Low set ears	9
Microtia	2
Pre-auricular ear pits	1
Pre-auricular sinus	1
Pre-auricular skin tags	4
Stahl ear deformity	2
Other external ear malformation	8
Total	43

3.6.5. Anomalies in the head and neck

Other structural anomalies in the head and neck, which are not otherwise classified under CNS, eye or ear defects, were found in 32 (12%) infants identified with associated anomalies.

Table 28. Anomalies of the head and neck (N=32).

Anomaly	N
Abnormal skull bones	14
Absent anguli oris muscle	1
Brachycephaly	2
Choanal atresia	2
Craniosynostosis	2
Dolicocephaly	1
Osteopathia striata	1
Scaphocephaly	2
Sternomastoid tumour	2
Torticollis	3
Trigonocephaly	1
Other	1
Total	32

3.6.6. Anomalies in the gastrointestinal system and abdominal wall

Of the 271 babies, 46 (17.0%) had a malformation in the gastrointestinal system. Overall, 52 gastrointestinal defects were identified amongst the 46 babies. There were 28 males and 17 females, with no difference in terms of gender or cleft type. As outlined in Table 29, the most common anomaly was inguinal hernia (which required surgical intervention) (17%), followed closely by anorectal malformations (15%). Of those with anorectal malformations (n=8), 4 had a misplaced anus, 3 had an imperforate anus and 1 had a prolapsed rectum.

Table 29. Anomalies in the GIT (N=52).

Anomaly	N
Absent diaphragm	2
Absent stomach	1
Anorectal malformation	8
Biliary atresia	1
Diaphragmatic/hiatus hernia	5
Duodenal atresia	3
Exomphalos	6
Gastroschisis	1
Inguinal hernia	9
Malrotation of bowel	1
Oesophageal atresia	1
Pyloric stenosis	2
Omphalocoele	2
Tracheo-oesophageal atresia and/or fistula	5
Umbilical hernia	1
Other	5
Total	52

3.6.7. Anomalies in the respiratory system

Of the 271 babies identified with associated anomalies in addition to their cleft, 21 (7.7%) had a malformation in the respiratory system. As per Table 30, the most common respiratory anomaly was laryngomalacia followed by bronchomalacia.

Table 30. Anomalies in the respiratory system (N=21).

Anomaly	N
Bromchomalacia	3
Hypoplastic lungs	2
Laryngomalacia	6
Laryngotracheomalacia	1
Lobar malformation	1
Pulmonary agenesis	1
Pulmonary atresia	2
Pulmonary hypoplasia	1
Tracheal stenosis	1
Tracheomalacia	1
Other	2
Total	21

3.6.8. Anomalies in the skeletal system and extremities

Of the 271 babies identified with associated anomalies, 112 (41.3%) had an anomaly in the skeletal system and extremities. Altogether, there were 67 defects identified in the upper limbs and 102 in the lower limbs, and 45 anomalies were identified in the skeletal system beyond the upper/lower limbs.

Anomalies of the skeletal system and extremities were more commonly associated with CP (59%) than CL±P (41%) (χ^2=7.27, p=0.008). Lower limb anomalies were more common in CP (66%) than CL±P (34%) (χ^2=13.10, p=0.00), but a significant association for upper limb anomalies (χ^2=2.43, p=0.13) and other skeletal anomalies (χ^2=4.12, p=0.052) was not detected.

Upper limbs

The most common congenital anomalies in the upper limbs were polydactyl (19.4%), followed by limb reduction defects (14.9%) and abnormal phalanx (10.4%) as per Table 31. Limb reduction defects typically also involved the lower limbs.

Table 31. Anomalies of the upper limbs (N=67).

Anomaly	N
Abnormal phalanx	7
Abnormal thumb	3
Absent humerus/ulna/radius	2
Absent phalanx	3
Absent thumb	2
Amputation defect of hands	2
Arthrogryposis	4
Camptodactyly	1
Camptomelic dysplasia	1
Clindodactyly	1
Congenital dislocation of shoulder	1
Cubital fossa pterygia	2
Finger webbing	2
Limb reduction defects	10
Polydactyly	13
Radial aplasia	2
Radioulnar synostosis	1
Severe restriction in range of motion of joint	3
Syndactyly	4
Other digit/hand anomaly	3
Total	67

Lower limbs

Altogether there were 102 anomalies in the lower limbs amongst 89 babies affected by lower limb malformations. The most common anomalies were talipes (33.3%), followed by syndactyly (11.8%), and limb reduction defects (9.8%) amongst others documented in Table 32.

Table 32. Anomalies of the lower limbs (N=102).

Anomaly	N
Abnormal feet	8
Abnormal hip	3
Abnormal toes	6
Absent toes	1
Absent femur	1
Acetabular dysplasia	1
Amputation defects of feet	1
Arthrogryposis	4
Camptodactyly	1
Camptomelic dysplasia	1
Clindodactyly	1
Congenital dislocation of hip	3
Deformed lower limbs	5
Femoral aplasia/hypoplasia	4
Limb reduction defects	10
Polydactyly	1
Severe restriction in range of motion of joint	2
Sirenomelia	1
Syndactylyl	12
Talipes	34
Webbing of toes	2
Total	102

Other skeletal anomalies

There were 34 babies with other skeletal anomalies. Of the 45 anomalies identified, the most common anomalies were scoliosis (17.8%) and vertebral anomalies (11.1%) as indicated in Table 33.

Table 33. Other skeletal anomalies (N=45).

Anomaly	N
Chest wall deformity	4
Congenital high scapula	1
Fusion	1
Hemivertebrae	4
Kyphosis	3
Rib deformity	4
Sacral agenesis	4
Sacral dimple	4
Sacral dysplasia	2
Scoliosis	8
Vertebral anomaly	5
Widespread skeletal deformities	3
Other	2
Total	45

3.6.9. Anomalies in the urogenital system

Of the 271 babies identified with anomalies in addition to cleft, 62 had an anomaly involving the urogenital system, and there were 70 urogenital anomalies amongst them. Males (n=43) outnumbered females (n=17), and chi-square tests revealed that males were more likely to be born with urogenital anomalies than female counterparts (χ^2=9.15, p=0.02). Urogenital anomalies were more commonly associated with CP (61%) than CL±P (39%) (χ^2=5.01, p=0.03).

The most common anomaly was hydronephrosis (23%), followed by other structural renal malformations (14%) including horseshoe-shaped kidneys or malpositioned kidneys. In males, the most common anomaly was hydronephrosis (26%) followed by hypospadia (18%) and cryptorchidism (12%). In females, the most common anomaly was other renal malformations (30%) followed equally by hydronephrosis (15%) and imperforate hymen/fused labia (15%).

Table 34. Anomalies in the urogenital system by sex (N=70).

Anomaly	Male (N)	Female (N)	Total (N)
Cryptorchidism	6	0	6
Ectopic testis	1	0	1
Hydronephrosis	13	3	16
Hypoplastic/dysplastic kidneys	3	1	4
Hypoplastic scrotum	3	0	3
Imperforate hymen/fused labia	0	3	3
Hypospadia	9	0	9
Micropenis	1	0	1
Multicystic kidneys	5	2	7
Other renal malformation	4	6	10
Rectovaginal fistula	0	1	1
Renal agenesis	1	2	3
Renal tubulopathy	1	0	1
Uterine agenesis	0	2	2
Other	3	0	3
Total	50	20	70

3.6.10. Other anomalies

Other anomalies not included in the systems above affected 31 of 271 infants (11%). The other miscellaneous anomalies are detailed in Table 35.

Table 35. Other anomalies (N=31).

Anomaly	N
Congenital muscular dystrophy	1
Arteriovenous malformation	1
Congenital facial nerve palsy	1
Conjoined twins	1
Dystonic movements	1
Extra nipples	1
Haemangioma	4
Hemifacial microsomia	3
Hypertelorism	9
Hypoplasia of thymus	1
Lymphatic malformation	5
Macrostomia	3
Total	31

Chapter 4: Discussion

4.0. Introduction

This chapter will firstly evaluate the findings of the study in the context of past studies and will relate to the literature as reviewed in Chapter 1 - Introduction. The chapter will be structured in the same format as for Chapter 3 – Results, and therefore the discussion will be structured in the order of socio-demographics, birth statistics, birth history, family history and associated anomalies. Further, methodological issues including limitations of the study will be evaluated. The final part of the chapter will draw conclusions, discuss the implications for clinical practice, and provide suggestions for future studies.

4.1. Socio-demographics

4.1.1. Sex ratio

This study demonstrated an overall male predominance in OCs (M:F = 1.15:1) (see Table 36), similar to the ratio reported for Northern Ireland (1.32:1) [111] and Northumberland and Durham in the UK (1.34:1) [79]. In comparing the sex ratios for the subgroups, this study found a male predilection for CL±P (2.02:1) and a female tendency towards CP (1.28:1). This was consistent with the male-to-female ratio reported for CL±P in other studies – 2.1:1 in Jordan [127], 2.5:1 in Korea, and 1.58:1 in Iran [126].

Table 36. Sex ratio by cleft types in the present study.

Cleft type	Males (N)	Females (N)	Sex ratio (M:F)
CL	79	65	1.22:1
UCLP + BCLP	174	86	2.02:1
CP	165	211	1.28:1
BCLP	48	14	3.43:1
UCLP	126	72	1.75:1

The differences in sex ratios within the cleft subgroups prove to be more complicated, varying with the severity of cleft and ethnic origin. In all studies of white populations, CL±P occurred more frequently in males than females, with average ratios of 2:1 [42]. In Japanese populations, there is significant male excess in the CLP group but not in the CL group [168]. In white populations, the male excess in the CL±P group becomes more apparent with increasing severity of cleft [122], a finding which was replicated in this present study where bilateral clefts were more common in males than females. In both races, there appears to be a slight excess of affected females in the CP group [42] although there remains some uncertainty and variation in the gender distribution of isolated CL.

In the present study, females were more likely to have CP alone but the sex ratio was almost even between the sexes for CP *with* additional anomalies, consistent with recent observations by Harville (2007) [169]. No generally accepted explanation for these gender differences exists, although sex differences in the timing of critical developmental stages are thought to have a role in their aetiology [128]. Overall, our results agree with previous studies, that CL±P is most frequent in males and CP is most typical in females [3, 125].

4.1.2. Cleft type

Worldwide, CLP is thought to be the dominant cleft type with Fogh-Andersen's (1942) CL:CLP:CP ratio of 1:2:1 often quoted as the typical ratio for European or Caucasian populations [122]. However, in this study, CP was the dominant cleft type (48%), in keeping with findings documented in Northern Ireland [111], Western Scotland [44], Finland [22], and Sweden [8]. The reported prevalence of CP worldwide shows greater variation than that for CL±P, and it may be that the ascertainment of cases of the less externally noticeable CP is more susceptible to methodological variations across studies [3, 13]. The distributions of cleft types also show ethnic variations. In Africa, CP is a much lower proportion of the overall prevalence of OCs, accounting for only 5.3% in a sample in Uganda [114]. In Zambia, the ratio of CL:CLP:CP was 1.54:1:0.1, the proportions being 58% CL, 38% CLP and only 4% CP [78]. In Asia, a number of Japanese studies also reported a much lower proportion of CP (12.7%) compared to comparable proportions of CL (41.3%) and CLP (46%) [170]. It has been suggested that in regions where cleft prevalence is highest, the ratio of CLP to CL is also high, and in regions of lowest cleft prevalence (e.g. Africa), the proportion of the more severe forms of clefting (palatal clefts) is also lower [20].

In certain respects, the data from this study compare well to those of previous reports but the main difference in the population distribution by cleft type is the higher proportion of CP compared to CL±P. CP, however, was also the dominant cleft type in similar studies on associated anomalies as

outlined in Table 37, accounting for 39-58% of clefts [8, 9, 12]. Another reason for the high proportion of CP may be our inclusion of SMCP, which is often not detected until later in life and is therefore potentially underestimated in studies with a limited follow-up period. When subjects with SMCP are included in studies, the distribution of cleft type resembles ours. For example, Shprintzen (1985) included SMCP and also found CP to be the dominant cleft type (58%), over CLP (36.4%) and CL (5.6%) [12].

Table 37. Comparing the cleft types in the present study to that of similar studies

	Present study	Milerad et al. (1997) [8]	Stoll et al. (2000) [9]	Shprintzen et al. (1985) [12]
Subjects (N)	791	616	460	1000
CL (%)	18.3	26	19.1	5.6
CLP (%)	33.8	35	37.2	36.4
CP (%)	47.9	39	43.7	58.0

4.2. Birth statistics

4.2.1. Birth prevalence

In this study, the overall birth prevalence of OCs in the Thames Valley varied from 0.85 to 2.12 per 1,000 live births with a mean birth prevalence of 1.44 per 1,000 live births over the period January 1995 to December 2011. This finding was consistent with the birth prevalence reported elsewhere in the UK: 1.30 per 1,000 live births and stillbirths in Birmingham (1940-1950) [112], 1.40 per 1,000 live births in the south-east of Scotland (1971-1990) [171], 1.42 per 1,000 live births in Northumberland and Durham, England (1949-1958) [79], 1.46 per 1,000 live births throughout Scotland (1989-1998), 1.47 per 1,000 of all live births in Northern Ireland (1981-2000) [111], 1.56 per 1,000 live births, stillbirths and TOP in Glasgow (1974-1985) [44], and 1.82 per 1,000 live births in the Trent Region, England (1973-1982) [76].

Our birth prevalence was also similar to that reported in studies of Caucasian infants in other European countries, ranging from 1.2 per 1,000 in Sweden (1975-1992) [8], 1.71 per 1,000 live births in Croatia (1988-1998) [172], to 1.9 per 1,000 (1979 to 1996) [9] and 2.1 per 1,000 (1995-2006) in France [173]. This result was very close to the mean 1.36 per 1,000 reported for Europe from the EUROCAT registries data (1995-2007) aggregated by country [20]. Our finding was also comparable to studies of Caucasian infants in Australia [164] and America [3], and worldwide, the birth prevalence of OC is quoted as 1.7 per 1,000 live births [3].

4.2.2. Season

The relationship between seasonality and birth prevalence of OCs remain unclear. In this study, the number of births with OCs peaked in July (10%), especially for CL±P, but similar to a recent study in the UK [111], no significant relationships between prevalence of OCs and seasonality

were found. Although the results were not significant, this study showed a peak in the number of CP births in spring and CL±P in winter.

Coupland and Coupland (1988) [76] reported a reduced incidence of children born with isolated CP in March to May, with increased incidence in August to September, which differs from the findings of Saxen and Lahti (1974) in Finland, who reported a low incidence of CP births in October to December but noted a constant incidence throughout the rest of the year [45]. However, for the CL±P group, Coupland (1988) reported a different pattern throughout the year, with a low incidence in May-June and a high incidence in Dec-Jan [76]. These results coincided with those reported by Saxen and Lahti (1974) who also found the peak incidence of CL±P to be at the end of the year, during the period October to December [45]. Sandhal (1977) in Sweden reported a similar finding to ours, and that of Saxen and Lahti, that there was a winter peak incidence for CL±P [174]. On the other hand, De La Vega (2006) found that the incidence of CL±P was highest during spring and summer and lowest during fall and winter in Puerto Rico [175]. In Zimbabwe, seasonal variations in month of birth of CL±P patients demonstrated a peak in the April-May period, and more cleft births in March through August (57.2%) than in September through February (42.8%) [78]. A similar study of seasonal variation in prevalence of CL±P in Sri Lanka also recorded the highest prevalence in April [176]. Thus far, contradictory results have been published regarding seasonality and the birth prevalence of OCs. However, there are various possible explanations to account for seasonal variations in congenital anomalies hence there are reasons why seasonality has been of interest to researchers. The presence of a teratogen at a specific time of the year e.g. viral infections, which can cause developmental anomalies, tend to show seasonal trends, and metabolic or endocrine factors could also affect seasonal incidence [76, 177]. External environmental factors related to seasonality have been examined including the use of agricultural chemicals in relation to cleft deformities with a recent meta-analysis reporting a marginally significant association between pesticides and risk of clefting [178]. Overall, studies have reported various seasonal variations in the

prevalence of birth defects with little consistency, with a possible explanation being differences in study populations, climates, environments, and methods of analysis.

4.3. Birth history

In this study, a cleft *without* associated anomalies did not appear to be associated with impaired foetal growth or preterm delivery as the majority of these infants were born at term and had a birth weight within normal limits – only 5.6% had a low birth weight and 7.1% were born preterm. In contrast to infants *with* associated anomalies, 16.9% *with* associated anomalies were born preterm, and 18.4% were born with a low birth weight. These data are in agreement with previous reports of an increased prevalence of low birth weight in infants *with* clefts and other anomalies as shown in Table 38. When compared to births in the general population, the rates of preterm delivery and low birth weight among infants with clefts *without* associated anomalies were similar to the overall rates reported in UK, but rates for clefts *with* associated anomalies were considerably higher than the 7% frequency of preterm delivery [179] and 7% low birthweight (under 2.5kg) reported for live births in the UK [167].

Table 38. Comparing preterm births and lowbirth weights in the present study to that of similar studies

Study	Live births (N)	OCs *without* associated anomalies		OCs *with* associated anomalies	
		P	LBW	P	LBW
Present study	685	7.1%	5.7%	16.9%	18.4%
Milerad (1997) [8]	616	6.2%	6.5%	21.0%	27.3%
Stoll (2000) [9]	460	5.1%	5.6%	20.6%	31.2%

**LBW – low birth weight <2500grams, P – premature <37 weeks gestation at birth*

4.4. Family history

The majority of studies before the 1970s suggest an association between increased maternal age and OCs however since 1970, a mixture of results has appeared [47]. This study found a U-shaped result with maternal age and isolated OCs, with the highest risk occurring in the youngest and oldest mothers. Similar findings were reported in a Californian study, with mothers over 39 years old being twice as likely as those between 25 to 29 years old to have an infant with a cleft [46]. Also in the US, Savitz et al. (1991) found an association between CP and mothers aged 24 years and younger [180]. Robert et al. (1996) used data from multiple registries of congenital anomalies based on a total of more than five million births, and reported a U-shaped maternal age relationship for OCs similar to the results of the present study [181]. In investigating approximately 2,000 children with OCs in Denmark, the authors concluded that both advanced maternal and paternal ages were associated with CL±P. In this study, advanced paternal age was associated with a higher risk of OCs but the result was not significant. Materna-Kiryluk et al. (2009) examined for a relationship between paternal age and a range of congenital malformations and found a positive association specifically between advanced paternal age and OCs [182]. Interestingly, it has been suggested that the influence of maternal or paternal age on the risk of CL±P increases with the advancing age of the other parent, but that the influence disappears if the other parent is young [51]. Current data on the relationship between paternal age and OCs remains unclear, and further research is needed to delineate this effect. Further, most studies of parental age in OCs have not adjusted for potential confounders such as drugs, smoking, and social status.

In this study, 21.4% of children with OCs had a positive family history, which is in agreement with existing studies reporting figures between 6% and 28% [20, 41]. In this study, only a small percentage of infants were identified to have consanguineous parents (2%) but almost all offsprings of consanguineous unions had a palatal cleft and there were no cases of CL. Existing evidence suggests

that consanguinity is a risk factor for non-syndromic OCs with implications that there is almost twice the risk of OCs in a child born to consanguineous parents [183]. In a study of 1,171 cleft patients in Riyadh of which 56.8% had consanguineous parents, CP was also the dominant subtype in their offsprings [184]. Overall, our sample size for consanguinity is too small to make conclusions and it should be noted that consanguinity was not always recorded in the patient notes that we reviewed, thus may have been underestimated.

4.5. Associated anomalies

4.5.1. Associated anomalies by cleft type

In this study, the prevalence of associated anomalies was not evenly distributed among cleft types. A higher proportion of infants with CP (43%) had associated anomalies compared to infants with CL (35%) and CLP (32%), consistent with previous reports of a tendency for associated anomalies to occur with palatal clefts rather than non-palatal clefts [9, 149]. In the systematic review by Maarse (2012), CP was the category most frequently seen in association with additional anomalies (22.2-78.3% of postnatal studies) [13]. As CP tends to be associated with syndromes, it has been postulated by Barbosa (2003) that the higher prevalence of associated anomalies in CP could be related to the presence of the syndrome rather than the cleft type [185]. However, it could also be that a more extensive cleft carries a higher risk of associated anomalies. Even for CL patients, bilateral clefts (46%) were associated with more anomalies than unilateral clefts (37%).

4.5.2. Associated anomalies by organ systems

In this study, the systems most commonly affected by additional anomalies in infants with OCs were the skeletal system and extremities, followed equally by the cardiovascular system and CNS, consistent with data elsewhere in Europe [8, 9, 148] and the Middle East [132, 186]. This is also in agreement with combined data from 23 EUROCAT registries, which revealed that anomalies in infants with OCs were most common in the musculoskeletal, cardiovascular and central nervous systems. Forrester (2006) specifically examined for associations between structural birth defects in OCs, and found that of all deformities examined, those in the extremities, limbs and CNS were most common [187]. Encephalocoele, microcephaly and syndactyly were over-represented in CP patients,

and for CL±P there were higher rates of anophthalmia/microphthalmia, single ventricle, reduction deformity of upper limbs and reduction deformity of lower limbs.

Anomalies in the skeletal system and extremities

In this study, anomalies in the skeletal system and extremities were most common. Of the 271 babies identified with associated anomalies, 112 (41.3%) had an anomaly in the skeletal system and extremities, with most defects identified in the lower limbs. The most common anomalies in the lower limbs were found to be talipes, syndactyly and limb reduction defects, and in the upper limbs were found to be polydactyl, limb reduction defects, and abnormal phalanges. Of the other skeletal anomalies, vertebral anomalies and chest wall deformities were most common. Stoll (2000) reported similar findings and identified limb deficiencies, syndactyly, polydactyly, club hand and clubfoot as the major anomalies in the skeletal system in infants with OCs [9].

Congenital talipes was the most common lower limb defect in this study, which is unsurprising as talipes is one of the most common congenital orthopaedic problems [188, 189]. It also has a complex aetiology, and there may be aberrant developmental processes implicated in the development of clefts and clubfoot. The association between OCs and skeletal anomalies is however unclear. One of the possible mechanisms for cleft development has been attributed to congenital anomalies of the cervical spine [190]. It has been postulated that a short neck with vertebral synostosis or other changes may limit the head extension of the foetus, thus interfering with the anterior and descending development of the glossomandibular complex. In turn, there may be insufficient free space above the tongue, which is required to allow for the horizontal movement of the palatal shelves and thus may induce a palatal cleft [191]. Even though some studies have observed a high rate of cervical anomalies with CP [192-194], this finding was not replicated in the present study. Further correlation of different cleft types with associated anomalies may improve our understanding on the embryogenesis and malformation processes in OCs.

Anomalies in the cardiovascular system

In this study, cardiovascular anomalies were found in 11.7% of all 791 children, and cardiovascular anomalies accounted for 31.4% of all associated anomalies in children with OCs. Cardiovascular anomalies were the commonest associated anomaly in several other studies and were found in 24% of cleft infants with associated defects in Sweden [8], and were found in nearly 25% of children with OCs in Turkey [195] and 51% of children with clefts and additional defects had a cardiovascular anomaly in Pakistan [196].

When compared to the general population, it appears that the rates of congenital heart defects are greater among cleft infants (see Table 39). According to CAROBB, the rate of cardiac defects in the Thames Valley is approximately 4.5 per 1,000 in the general population [162]. In comparison, the rate of congenital heart defects in this study was 108 per 1,000 births, which is a 24-fold increase more than that reported for the general population. Barbosa (2003) suggested that a possible explanation for the high prevalence of congenital heart disease in OC patients may be the large number of patients with a syndrome in these studies [185]. Milerad (1997) excluded infants with chromosomal and non-chromosomal syndromes as well as non-live births from this calculation, but still found congenital heart disease to be 16 times more frequent amongst cleft children compared to the general Swedish population [8]. Similarly, Stoll (2000) found congenital heart disease to be five times more common in cleft children compared to the general French population [9]. When infants with chromosomal and non-chromosomal syndromes were excluded from our calculation, 6% of infants with OCs had a cardiovascular anomaly. This is similar to the results of a large European study of 2860 patients with CL or CLP excluding those with recognised syndromes and chromosomal abnormalities, which found that at least 7% of patients had a malformation in the cardiovascular system [7].

Although studies are in agreement with the high rates of cardiovascular anomalies in infants with OCs, the nature of the cardiovascular anomaly has not been described in much detail. The most

common defect in this study was ventricular septal defect, which was also reported by Milerad (1997) [8] and Stoll (2000) [9]. In fact, the most common single associated anomaly of all systems in this study was ventricular septal defect. A recent study by Harry et al. (2013) which evaluated the nature of cardiovascular malformations in children with OCs, found that 10% of all cleft patients had a cardiovascular malformation and reported malformations of the septa, vasculature, and valves to be present in 9%, 6%, and 2% of cleft infants, respectively. In the same study, cleft repair was delayed by 2 months in patients with a cardiovascular malformation, likely delayed by the need to correct the cardiovascular anomaly prior to cleft surgery [197].

These results also suggest a potential link between palatal and cardiac development, and these structures may share genetic or environmental factors in embryogenesis, which lead to faulty development of both structures. Both the heart and palate develop between weeks five through nine of embryogenesis and relies on complex and interdependent signalling processes [198]. Several genes have been identified in syndromes that affect the heart and palate, such as IRF6 in Van der Woude syndrome, TBX1 in Velocardiofacial syndrome and DiGeorge syndrome, and SOX9 in PRS [16]. Currently, no genetic or environmental factor has been identified for the nonsyndromic malformation of both heart and palate, but future studies may reveal biomarkers for the concurrent aberrant development of these tissues [197].

Given the high rate of congenital cardiovascular malformations in OCs, and the clinical implications such as delayed surgery in those with cardiovascular anomalies, the results of this study propose routine cardiology work-up and potentially echocardiographic screening for congenital heart disease in OC patients. Further studies on perioperative complications in those with cardiovascular anomalies, and investigations into genetic and environmental aetiologies are needed.

Anomalies in the central nervous system

In this study, the CNS was the second-most affected system after the extremities and skeletal system, which is in agreement with previous studies reporting CNS defects to be common in infants

with clefts and associated anomalies [8, 9]. CNS defects were reported to account for 13.6% to 17.4% of all recorded anomalies in infants with OCs [127, 132, 148]. Stoll et al. (2000) found that even in non-syndromic OCs, anomalies of the CNS were second most common (affecting 16%) after anomalies of the extremities and skeletal system [9]. Further, Mueller (2007) reported that individuals with CLP were 13 times more likely than the general population to possess a CNS defect [199].

In this study, CNS defects were commonly due to congenital hydrocephalus (n=31) but an even greater proportion could be grouped as reduction anomalies including absence, agenesis, aplasia or hypoplasia of part of the brain (n=46), which comprised 43.4% of all CNS anomalies in this study. This finding was consistent with reports from the EUROCAT review of 23 registers, which found that even when chromosomal anomalies and recognised syndromes were excluded, reduction anomalies of the brain still accounted for 37.5% of all CNS defects in infants with OCs [7].

It is well recognised that cognitive deficits in syndromic clefting are frequent and often result in mental retardation [7]. But for a long time, cognitive deficits in individuals with nonsyndromic clefts have simply been attributed to the secondary effects of hearing impairment, mechanical speech problems, and psychosocial effects of facial disfigurement [200]. Nopoulos et al. (2002) were amongst the first to report that adult patients with non-syndromic clefts scored lower on IQ tests and had language deficits on neuropsychological testing compared to non-cleft controls [201]. Nopoulos et al. (2002) also found that adult patients with non-syndromic oral clefts had a different pattern of brain morphology on magnetic resonance imaging compared with age and sex-matched controls. This had been suggested to be due to aberrant cerebral development resulting in enlargement of anterior regions of the cerebrum and decrease in size of the posterior cerebrum and cerebellum in patients with clefts compared with controls [202]. On further studies, Nopoulos (2007) found that children with non-syndromic OC had abnormal distribution in brain tissue compared to adults with non-syndromic OC, pointing at an abnormal trajectory of brain growth in cleft patients [203]. From embryological studies, it is known that there is an intimate relationship between the development of

the face, the craniofacial skeleton and the brain [6], therefore cognitive deficits and structural brain differences in individuals with OCs is likely due to the interdependent and aberrant craniofacial development. Recently, Rosen (2011) performed a retrospective review of magnetic resonance imaging and ultrasound images of foetuses diagnosed with OCs, and found that 6.3% had brain abnormalities, and that additional infants were identified with brain anomalies on postnatal scans, suggesting for a close neuroradiology follow-up of infants with OC. Overall, the authors suggest that OCs may be manifestations of overall abnormality in craniofacial development [204]. Although many with non-syndromic OCs exhibit good cognitive function, and the findings in brain size, structure and function were generally not exaggerated; the findings support early evaluation of young patients with OCs to determine whether special developmental planning and intervention is needed.

Comparing associated anomalies in OCs with the general population

Table 39 compares the rates of associated anomalies in children with OCs in this study, to the rates of anomalies for all births in the Thames Valley. Almost all congenital anomalies occurred more frequently in children with OCs compared to children in the general population. Table 40 also shows that chromosomal anomalies were overrepresented in the cleft cohort.

Table 39. Rates of congenital anomalies in the present study compared to that in the general population, Thames Valley, UK.

Organ system	Number	Live births, stillbirths, foetal deaths and TOPFA in Thames Valley (rate per 1,000)*	Prevalence in this study population (rate per 1,000)
Cardiovascular system anomalies	85	4.5	107.5
Central nervous system anomalies	85	2.3	107.5
Neural tube defects	14	1.1	17.7
Anencephalus, encephalocoele and similar	8	0.6	10.1
Spina bifida	6	0.5	7.6
Hydrocephaly	31	0.6	39.2
Digestive system anomalies**	38	1.1	48.0
Oesophageal atresia with or without tracheo-oesophageal fistula	6	0.2	7.6
Duodenal atresia or stenosis	3	0.1	3.8
Abdominal wall defects	8	1.1	10.1
Diaphragmatic hernia	5	0.3	6.3
Gastroschisis	1	0.3	1.3
Omphalocoele	2	0.5	2.5
Genital anomalies	26	1.4	32.9
Urinary anomalies	41	2.2	51.8
Limb anomalies	112	2.4	141.6
Reduction defects	10	0.4	12.6
Talipes	34	1.0	43.0
Respiratory anomalies	21	0.5	29.0
ALL births with congenital anomalies	271	19.8	342.6

**Data from CAROBB [162]*
***Excluding abdominal wall defects*

Table 40. Rates of chromosomal anomalies in the present study compared to that in the general population, Thames Valley, UK.

Chromosomal defect	N	Live births, stillbirths, foetal deaths and TOPFA in Thames Valley (rate per 1,000)*	Prevalence in this study population (rate per 1,000)
Chromosomal anomalies	78	4.9	98.6
Trisomy 21	4	2.7	5.1
Trisomy 13	21	0.3	26.5
Trisomy 18	9	0.8	11.4
Turner syndrome	3	0.3	3.8

** Data from CAROBB [162]*

4.5.3. Chromosomal defects

In live births, the most common chromosomal defects were 22q11 deletion syndrome (20.5%), trisomy 21 (8%), and Turner's syndrome (8%). Of our non-live births, the most common chromosomal defects were trisomy 13 and trisomy 18. The latter results correspond to studies of live births in France, where the most common chromosomal defects were trisomy 13 (30.5%) and trisomy 18 (25%) [9], and in Sweden, where trisomy 21 (18%), trisomy 13 (18%) and trisomy 18 (12%) were most common [8].

Comparing chromosomal defects and genetic results of studies are difficult, given that whilst some studies provided specific information on the number of karyotyped cases and the types of chromosomal defects [133, 137], others only reported abnormal karyotypes and some did not give any specific data at all [50, 134]. As a consequence, rates of chromosomal defects could not be easily obtained for comparison. Furthermore, in studies providing specific data on chromosomal defects, chromosomal analysis was mostly performed in cases of OCs where there were associated anomalies to suspect a chromosomal or non-chromosomal syndrome. This may explain why almost all

chromosomal anomalies were accompanied by associated anomalies. Besides karyotype analysis, most studies did not report whether fluorescence in situ hybridisation (FISH) had been performed and whether microdeletions were included in the data [8, 9]. Studies by Rittler (2011) [133] and Rozendaal (2012) [137] included microdeletions and duplications, while Stoll (2000) [9] included the results of FISH 22q11 screening as from 1994. In our cohort, the 22q11 deletion syndrome is diagnosed in individuals with FISH and more recently, array comparative genomic hybridisation (CGH), performed if there is clinical suspicion for 22q11 deletion syndrome after consultation with a geneticist. In addition, this study detected higher rates of 22q11 deletion syndrome possibly because the longer follow-up period in this study allowed us to detect conditions that become evident later in life. For example, 22q11 deletion syndromes are often diagnosed at school age when speech and language difficulties become evidence, unless a cardiac anomaly manifests earlier [13, 205].

4.5.4. Pierre Robin Sequence (PRS)

The definition of PRS is variable. Most clinicians refer to PRS as a triad of micrognathia, posterior U-shaped CP and glossoptososis. However, some do not consider CP as mandatory, and some others do not include glossoptosis in the definition [90, 206]. Therefore, the reported prevalence is also variable but is estimated at around 1 in 8,500 live births [207].

It has been reported that more than half of PRS infants will have an associated syndrome, chromosomal abnormality, additional anomalies, or other medical concerns [90, 208]. In this study, there were 72 cases of PRS. Of these, 40 (55.5%) had an isolated or non-syndromic PRS, and 32 (44.5%) had PRS associated with additional congenital anomalies, non-chromosomal and chromosomal syndromes as per Table 41. In a series of 117 cases of PRS in France, Holder-Espinasse (2001) reported similar findings that 48% of PRS was non-syndromic, 35% was syndromic, and an additional 17% was associated with additional anomalies [206]. In a systematic review by Izumi (2012), pooling together a total of 1,282 patients with PRS, 653 (51%) had isolated PRS and 629

(49%) had syndromic PRS [209]. Of the 629 with syndromic features, 391 (62% of patients with syndromic PRS and 30% of total patients with PRS) had a syndromic diagnosis with Stickler syndrome being the most common (14% of total cases). In comparison, of the syndromic PRS, we had 6 cases of Stickler syndrome (19%), also consistent with the reported proportion in a US sample of 22% of syndromic PRS being Stickler syndrome [209]. Generally, reports are in agreement that between 11% and 18% of people with PRS will have Stickler Syndrome [90, 93].

Table 41. Comparing the rates of isolated and syndromic forms of PRS [209].

	Present study N	Isolated N (%)	Syndromic N (%)	Additional anomalies N (%)
Present study	72	40 (55.5)	14 (19)	18 (25)
Izumi et al. (2012) [209]	125	53 (42)	48 (39)	24 (19)
Holder-Espinasse (2001) [206]	117	53 (48)	38 (35)	19 (17)

4.6. Methodological considerations

4.6.1. Limitations of this study

This section describes the limitations of this research and illustrates the approaches taken to minimise potential criticisms.

Underestimating genetic conditions

This study could have underestimated the rates of genetic conditions as (1) a few parents declined genetic testing and (2) a clinical geneticist did not routinely review all cleft patients, but only those suspected to have a genetic defect. A genetic consult is generally offered when a child has PRS or when associated anomalies are present, however, it should be realised that the absence of PRS or associated anomalies does not exclude the possibility of an underlying genetic defect. As genetic reviews and chromosomal analysis were often not performed in such isolated cases, undetected genetic defects might have been included in our rates of isolated cases. It is thus important to realise that like most other studies, genetic analysis and results were obtained for part and not all of the cleft patients, and the inclusion of cases with undetected chromosomal defects and non-chromosomal syndromes must be considered. Furthermore, advanced genetic techniques were not available to all patients born in earlier years, and hence subtle genetic changes including microdeletions may have been missed.

Underestimating the rate of OCs

There are several factors that could have contributed to an underestimation of the prevalence of OCs, but combining data from The Spires Cleft Centre and CAROBB enabled us to identify virtually all patients in the Thames Valley (see Table 42). It is possible that there have been spontaneous abortions, miscarriages, TOP, stillbirths and neonatal deaths with undetected clefts. It is also possible that there are cleft patients not known to the NHS, although these cases may still have

been notified to CAROBB. Further, information on patients born in Oxfordshire was available from 1991 onwards, and for Berkshire and Buckinghamshire only from 2004 onwards in the CAROBB database.

Missing data

There were some missing data from patient records, hence the calculations in the 'Results' section did not always add up to the total number of subjects (n=791). However, we tried to minimise missing data as much as possible through rigorous cross-referencing as described in Chapter 2 – Materials and Methods. Another strength of this study is that patients continue to be followed by the multidisciplinary team at The Spires Cleft Centre through childhood. Therefore, data regarding anomalies found after birth has been added retrospectively, enabling us to identify those anomalies that are not immediately obvious at the time of birth.

Table 42. Participants referred to The Spires Cleft Centre versus those identified via CAROBB, and showing how utilising both databases have identified virtually all patients in the Thames Valley.

Factor	The Spires Cleft Centre	CAROBB
Non-live births	No information on non-live births	Notified of prenatally suspected OCs in spontaneous abortions, miscarriages, TOP, stillbirths and neonatal deaths. Note. The rate of OCs in non-live births is likely to be underestimated as not all foetuses with OCs survive until the morphology scan in the second trimester. Further, not all pregnancies are scanned, and even so, there is a low detection rate for CP on ultrasound.
Patients who choose care outside the NHS	Identifies patients managed in the NHS	Receives notifications of congenital anomalies from delivery suites, independent of whether the child chooses care outside the NHS thereafter.
Mothers delivering outside the Thames Valley	Even if a mother delivers a child with a cleft outside the Thames Valley, the child is likely to be referred to The Spires Cleft Centre for management and follow-up especially if the family chooses to reside in the Thames Valley.	Births to mothers resident in Thames Valley but delivering outside the CAROBB area (e.g. London) may not at present be notified to CAROBB.

4.6.2. Strengths of this study

In Chapter 1 – Introduction, several methodological concerns were identified in previous studies, which potentially accounted for the wide variation in reported prevalence of associated anomalies. This study addressed the methodological concerns as follows:

1. Diagnostic criteria: there was a lack of consensus on the definition of "associated anomaly". Some studies only recorded anomalies that required treatment or intervention, others included all anomalies detected in the child. We incorporated all anomalies as these may later provide aetiological clues.
2. Thoroughness of clinical examination: significant cross-referencing was performed to minimise missing data.
3. Age at examination: a strength of this study is that the multidisciplinary team followed patients throughout the childhood and teenage years and data regarding anomalies found after birth was added retrospectively, enabling us to identify those anomalies which are not immediately obvious at the time of birth.
4. Sample source: we incorporated live births and non-live births, and found a significant discrepancy in associated anomalies between the two cohorts. Our patient population is unique in that patients are drawn from a referral centre but also resembles a population-based study given the restructuring of cleft services in the UK.

Conclusion

The main conclusions of this study were:

- The prevalence of associated anomalies was not evenly distributed among the three main cleft types and associated anomalies occurred most frequently with CP. Even within the CL group, bilateral clefts were associated with more anomalies than unilateral clefts, suggesting that a more extensive cleft may carry a higher risk of having additional congenital defects. The proportion of different cleft types affected by associated anomalies has important implications for the clinical workload including prenatal counselling, but is also of interest in providing clues about underlying aetiology.
- The rate of associated anomalies in this cohort of OC births was 34.3%. Associated anomalies occur at a much higher rate in children with OCs compared to the general population, when we compared children born with OCs in the Thames Valley with all births identified in the Thames Valley.
- In this study cohort, associated anomalies were most frequently found in the skeletal system and extremities, cardiovascular and central nervous systems, a finding which was consistent with that of similar studies.
- There is increasing evidence for distinctive differences, both epidemiological and genetic, between CL and CLP. In this study, CL was more frequently affected by associated anomalies than CLP. The different patterns of defects associated with CL and CLP, may possibly indicate different underlying mechanisms, suggesting that CL and CLP reflect more than just variable degrees of severity, and that distinct aetiological mechanisms might be involved.
- When counselling parents regarding prognosis and risk of associated anomalies, it is essential to tailor the discussion by cleft category. As our results showed, CP is most frequently associated with additional anomalies and CLP was most frequently associated with

chromosomal defects. The frequency of associated anomalies was also higher in bilateral than in unilateral CLP. This emphasises the need for accurate prenatal subdivision of clefts. We also found that the presence of associated anomalies is often associated with chromosomal defects and non-chromosomal syndromes. The reverse was also true – chromosomal abnormalities were often associated with associated anomalies. Early genetic intervention should also be offered in cases with associated anomalies.

- A significant proportion of those with additional anomalies had underlying chromosomal defects and non-chromosomal syndromes. There is also a provisionally unique group of children with OCs and additional anomalies without a recognised syndrome or cause. This is a potential pool of patients with an undiscovered or undescribed genetic defect or syndrome, which would be of research interest.

Implications for clinical practice

The high incidence of multiple anomalies and identifiable syndromes associated with CL±P has far-reaching implications. One might think that the wide discrepancy between studies reporting on the prevalence of associated anomalies is purely of academic interest and has little bearing on clinical practice. However, the high proportion of infants with associated anomalies points to a service provision issue and a need to obtain additional genetics and paediatric support within the cleft team to cater for a medically complex caseload.

Findings also imply that individuals born with OCs are a heterogeneous group of individuals, and there is a significant group of children who have OCs and additional anomalies of an unexplained origin. This is a potential pool of patients with an undiscovered or undescribed syndrome of which the number of specific causes for the clefts could be as large as the number of patients in this unique group. Further research is needed on this group to uncover underlying aetiological mechanisms, and further identification of causal factors will provide the patient and family with correct counselling including a description of prognosis and recurrent risk.

It has become apparent that approximately 34% of patients do not have isolated clefts. Instead, these patients have multiple anomaly syndromes with clefting as one of several anomalies. Without a carefully constructed genetic and medical history, less obvious findings will not be explored, especially in cases with less severe expressions of genetic, chromosomal or disruption syndromes. A thorough evaluation of children with congenital OCs is essential and should include a medical and genetic history, a physical examination, and imaging and laboratory studies where indicated.

Directions for future research

Suggestions for future studies were integrated into the discussion above, but a summary of recommendations for future studies will also be provided below.

- Further studies are needed to examine associated anomalies in cleft patients on a larger scale by increasing the sample size to represent patients with OCs in other regional centres in the UK. Future studies should consider geographical differences between and within countries regarding the various cleft sub-phenotypes among live births, stillbirths and TOP. Multicentre collaborations would be of research value.
- Future research on OCs should investigate CL and CLP as separate entities, as there is sufficient evidence pointing towards heterogeneity in the CL±P group. A very recent study investigating cleft-type-specific associations in genes suggests that in fact, CL, CLP, CP and SMCP may represent genetically different entities, therefore should be separated in these four categories for research purposes rather than the traditional two (CL±P and CP) [210].
- More studies are needed to strengthen the literature on associated anomalies in OCs. The recognition of associations of birth defects is potentially useful in identifying those teratogens that do not produce isolated anomalies but affect many structures of the developing embryo simultaneously [130]. The pattern recognition can later lead to a more exhaustive evaluation of risk factor exposures and eventually allow an interpretation of pathogenic mechanisms [131].
- The use of array CGH in clinical practice is rising, and compared to conventional karyotyping it can detect smaller chromosome deletions and duplications. It would be of interest to perform array CGH in a large cohort of cleft cases, which would provide information about the proportion and types of chromosomal defects that are missed in cases that have not been karyotyped or investigated by array-based methods. OCs can be associated

with various microdeletions and duplications, and we suggest that CGH should be the standard technique to identify chromosomal defects in children with OCs.

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Appendix: Professional Development as a Result of this Thesis

Scientific presentations

National

Law J, Goodacre T. "Just a cleft" or more…? A population based study of associated malformations in a 17-year period to describe burden of care. Craniofacial Society of Great Britain and Ireland Annual Meeting, Birmingham, UK, 17-19 April 2013.

International

Law J, Goodacre T. Associated malformations in children with orofacial clefting: 17-year data from a large UK centre with implications for required care. 12th International Congress on Cleft Lip/Palate and Related Craniofacial Anomalies, FL, USA, 5-10 May 2013.

Law J, Goodacre T. "Just a cleft lip" or more…? A UK-based study of related problems and associated malformations in children with isolated cleft lip. 12th International Congress on Cleft Lip/Palate and Related Craniofacial Anomalies, FL, USA, 5-10 May 2013.

Service to professional associations

Session Co-chair, Health Care and Epidemiology #7, 12th International Congress on Cleft Lip/Palate and Related Craniofacial Anomalies, FL, USA, 5-10 May 2013.

Other conference attendance

British Isles Network of Congenital Anomaly Registers (BINOCAR) Annual Meeting, St Anne's College, Oxford, 25 September 2012.

Craniofacial Society of Great Britain and Ireland Annual Conference, Bristol Marriott Royal Hotel, Bristol, United Kingdom, 25-27 April 2012.

Publications

Articles currently in preparation for submission to the Cleft-Palate Craniofacial Journal.

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This thesis is submitted to the Examination Schools, University of Oxford, in partial fulfilment of the degree of Master of Science (by Research) in Surgical Sciences.