Heterogeneity of Oral Clefts in Relation to Associated Congenital Anomalies

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Summary. *Background and Objective. The first step in the search for the genetic basis of oral clefts should be the well-accepted classification and clinical data protocols, which are important in distinguishing separate phenotypic groups. The aim of this study was to compare the frequency of congenital malformations associated with oral clefts between the different groups of oral clefts.*

Material and Methods. The study population comprised 238 patients with oral clefts and one or more major congenital anomalies. All cases of oral clefts were subdivided into 2 groups: patients with the recognized conditions (n=97, 40.8%) and patients with the multiple congenital anomalies of unknown origin (n=141, 59.2%). The frequency of associated congenital anomalies was compared between the cleft palate (CP) and cleft lip and/or palate (CL/P) groups as well as between the cleft lip only (CL) and cleft lip with cleft palate (CLP) subgroups.

Results. A total of 420 anomalies associated with oral clefts were diagnosed in 141 patients with multiple congenital anomalies (2.98 anomalies per proband) with the highest incidence being in the CP group (3.5 anomalies per proband). Comparison of the CP and CL/P groups showed that some of associated congenital anomalies such as atresia and stenosis of the small intestine and micrognathia occurred significantly more often in the CP than CL/P group (2.1% vs. 0% and 3.5% vs. 1.1%; P<0.05). Meanwhile, comparison of the CL and CLP subgroups revealed accessory auricle, other specified anomalies of the ear, congenital anomalies of the circulatory system, and certain congenital musculoskeletal deformities of the spine to be more common in the CL than CLP group (5.1% and 0.5%, 11.9% and 5.1%, 3.4% and 0%, 3.4% and 0%, respectively; P<0.05).

Conclusions. The highest incidence of associated congenital anomalies was in the CP group followed by the CL, CL/P, and CLP groups. Generally, the anomalies of the musculoskeletal system, cardiovascular system, and face including eye, ear, and neck were most common. The careful analysis of associated anomalies and cases of oral cleft subgroups with multiple congenital anomalies is helpful in identifying the etiologic entities and underscores the need for thorough evaluation and competent distinction of various types of oral clefts.

Introduction

It has been known for more than 80 years that cleft lip (CL), cleft lip and/or palate (CL/P), and isolated cleft palate (CP), collectively termed oral clefts (OCs), are frequently associated with congenital anomalies (1). The earliest reports published in the literature demonstrated that the incidence rate for cleft lip and/or palate was 18.4 per 10 000 (1:544 live births) in Lithuania during 1993–1997. The cases of isolated CL/P accounted for 74.1% of all clefts, while the remaining part was associated with other congenital defects (2). In general, OCs are the most common congenital anomalies of the head and the neck. However, the mechanisms of this pathology and the genetic causes of susceptibility have not been completely understood yet. The nature of OCs is multifactorial, with a genetic component playing a significant role in the etiology of OCs. The investigations of this complex pathology have to be multistage and require well-organized work of the team of a variety of specialists.

The first step in the search of the genetic basis of OC pathology should be thoroughly defined classifying and clinically protocolling the cases of OCs. According to the recent publications, it is important to distinguish the separate phenotypic groups of OCs (1, 3–7). Although CP is usually regarded as a defect distinct from CL/P, there is still a question to discuss if the latter should be considered as a variant of the same defect or should be divided into separate groups of cleft lip only (CL) and cleft lip with cleft palate (CLP) (8–10). Moreover, it has long been known that OCs are frequently associated with other congenital anomalies, but the reported

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incidence and type of anomalies associated with OCs vary considerably across different studies. The aim of this study was to compare the frequency of congenital malformations associated with oral clefts between the different groups of oral clefts. We expect that this report on associated anomalies in cases of different OC phenotypes will be significant in the identification of the etiologic basis of OCs.

Material and Methods

The data for this retrospective study were obtained from the Centre for Medical Genetics of Vilnius University Hospital Santariškių Klinikos, Clinic of Maxillofacial and Oral Surgery, the Institute of Odontology of Vilnius University, the Lithuanian Registry of Congenital Anomalies (LIRECA), and the National Pathology Centre at the Ministry of Health of the Republic of Lithuania between 1993 and 2006.

All patients were examined by several team members (clinical geneticists, orthodontists, maxillofacial and oral surgeons). If it was required, the laboratory tests, such as cytogenetic (standard chromosome analysis performed from GTG banded metaphases with the resolution level of 400–500 bands), metabolic, and molecular testing; x-ray pictures (e.g., cranial, chest, spine, extremities, and panoramic dental x-ray), and other necessary instrumental investigations (brain, heart, and visceral organ ultrasound, MRI, etc.), were performed.

The cases without OCs or with submucous CP, bifid uvulae, and isolated OCs were excluded from the study. The study population comprised patients with OCs and one or more major congenital anomalies. Major anomalies were determined to be of functional or cosmetic significance and required certain medical interventions. Minor anomalies and sequences were not included as associated anomalies, but they were important for the identification of the syndromes. Based on the available clinical data, each case was assigned to one of the two following categories:

- Recognized conditions (chromosomal aberrations, monogenic diseases, sequences) such as trisomy 13, Treacher Collins syndrome, amnion rupture sequence, etc.,
- Multiple congenital anomalies (MCAs) of unknown origin.

The patients with OCs and having MCAs were examined. Malformations other than OCs were grouped according to the codes of the British Pediatric Association (BPA) (codes 740.0–759.9) (11).

OCs were subdivided into the CP and CL/P groups. The latter CL/P group was divided into two subgroups: CL and CLP due to the assumption to different etiologic mechanisms. This distinction was made based on the different embryological timings of primary and secondary palate closure (4).

The distribution of congenital anomalies coexisting with OCs was compared between 2 groups: CP versus CL/P and CL versus CLP. The chi-square and exact Fisher tests were used to establish statistically significant differences between the groups of OCs. A P value of <0.05 was considered statistically significant.

Results

The study population comprised 238 individuals with OCs and associated anomalies, who were examined between 1993 and 2006. All patients were subdivided into 2 groups: those with the recognized conditions (n=97, 40.8%) and those with the MCAs of unknown origin (n=141, 59.2%).

Of the 97 patients with the recognized conditions, 27 had chromosomal abnormalities with more than half of them being autosomal trisomies such as trisomy 13 (n=16, 59.3%), trisomy 18 (n=1, 3.7%), and trisomy 21 (n=1, 3.7%). The remaining 70 patients had syndromes and sequences (19 different nosological entities) such as Pierre Robin sequence (n=31, 44.3%), holoprosencephaly (n=6, 8.6%), and amnion rupture sequence (n=4, 5.7%).

The type of OCs was defined in 141 patients with MCAs. There were 420 associated anomalies in patients with MCAs, with a mean number of 2.98 anomalies per proband. The highest frequency of associated congenital anomalies was in the CP group (3.5 anomalies per proband) followed by the CL, CL/P, and CLP groups (3.1, 2.8, and 2.7 anomalies per proband, respectively). However, no significant differences in the number of associated congenital anomalies per proband between the groups were found ($P=0.19$ in CP vs. CL/P; $P=0.37$ CL vs. CLP; $\alpha=0.05$; $P>\alpha$). Among these 141 patients, 41 patients (9.8%) had 1 associated anomaly; 33 patients (7.9%), 2 associated anomalies; 33 patients (7.9%), 3; and 34 patients (8.1%), 4 or more. The organ systems affected by the associated anomalies are shown in Fig. Generally, the anomalies of the musculoskeletal system (n=129, 30.7%), cardiovascular system (n=90, 21.4%), and face including eye, ear, and neck (n=64, 15.2%) were most common.

Associated Congenital Anomalies in CP Versus CL/P Groups. Of the 141 OC patients with MCAs of unknown origin, 41 (29.1%) had CP and 100 (70.9%) had CL/P. There were no significant differences in the distribution of patients with MCAs of unknown origin by the organ system affected comparing the CP and CL/P groups except for 2 organ systems: urinary ($P<0.05$) and ear, face, and neck ($P<0.05$) (Table 1).

There were 144 and 276 associated anomalies in the CP and CL/P groups, respectively. Some of

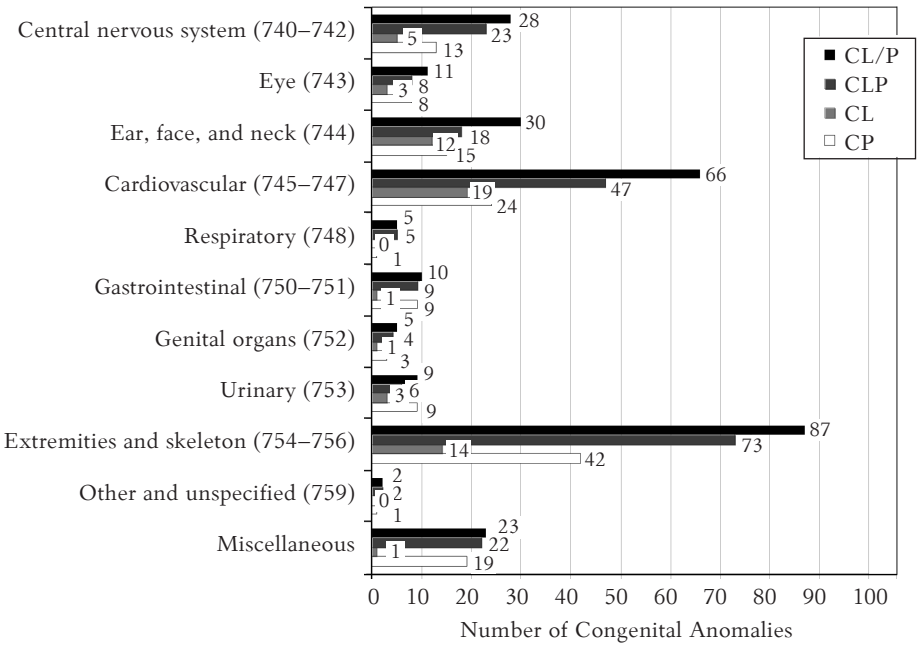

Fig. Distribution of congenital anomalies by the type of cleft
CL/P, cleft lip and/or cleft palate; CLP, cleft lip and cleft palate; CL, cleft lip; CP, cleft palate.

Table 1. Affected Organ Systems in the Cleft Palate (CP) and Cleft Lip and/or Cleft Palate (CL/P) Groups

Organ System (BPA Code)	CP Group n=41	CL/P Group n=100	*P**
Central nervous system (740–742)	13 (31.7)	28 (28.0)	0.847
Eye (743)	8 (19.5)	11 (11.0)	0.161
Urinary (753)	9 (22.0)	9 (9.0)	0.044
Ear, face, and neck (744)	15 (36.6)	30 (30.0)	0.019
Cardiovascular (745–747)	24 (58.5)	66 (60.0)	0.182
Respiratory (748)	1 (2.4)	5 (5.0)	0.662
Gastrointestinal (750–751)	9 (22.0)	10 (10.0)	0.443
Genital organs (752)	3 (7.3)	5 (5.0)	0.686
Other and unspecified (759)	1 (2.4)	2 (2.0)	0.992
Miscellaneous	19 (46.34)	23 (23.0)	0.867

Values are number (percentage). *Exact Fisher test.

associated congenital anomalies such as atresia and stenosis of the small intestine and micrognathia occurred significantly more often in the CP than CL/P group (2.1% vs. 0% and 3.5% vs. 1.1%; $P<0.05$) (Table 2).

Associated Congenital Anomalies in CL Versus CLP Groups. The further analysis of associated congenital anomalies with OCs was performed within the CL/P group. Of the 100 CL/P cases investigated in this study, 19 (19.0%) had CL and 81 (81.0%) had CLP. Of the 420 anomalies, there were 59 associated anomalies (14.0%) in the CL group and 217 associated anomalies (51.7%) in the CLP group. Table 3 shows the frequency of congenital anomalies in the CL and CLP groups. Accessory auricle, other specified anomalies of the ear, congenital anomalies of the circulatory system, and certain congenital musculoskeletal deformities of the spine were significantly more common in the CL group than CLP group (5.1% and 0.5%, 11.9% and 5.1%, 3.4% and 0%, 3.4% and 0%, respectively; $P<0.05$).

Discussion

This study compared the frequency of associated congenital anomalies in cases of MCAs in the different phenotypic groups of OCs of unknown origin. Based on the embryological and epidemiological data, CP is commonly regarded as etiologically distinct from CL/P, in which CL is only a variant of the same defect differing mainly in the severity (9). Nevertheless, recent discussions in literature suggest that CLP and CL may have etiologic differences as well (8–10). The methodological aspects of this study involve both of these assumptions and thus are acceptable for more detailed studies on the causes of OCs regarding associated anomalies. The considerable part of OC cases of unknown origin demonstrates the complexity and etiologic heterogeneity

Table 2. Congenital Anomalies in the Cleft Palate (CP) and Cleft Lip and/or Cleft Palate (CL/P) Groups

Congenital Anomaly (BPA Code)	CP Group n=144	CL/P Group n=276	*P**
Microphthalmia (743.1)	3 (2.1)	1 (0.4)	0.074
Congenital cataract and lens anomalies (743.3)	2 (1.4)	0 (0.0)	0.083
Other unspecified anomalies of face and neck (744.9)	2 (1.4)	4 (1.4)	0.817
Atresia and stenosis of small intestine (751.1)	3 (2.1)	0 (0.0)	0.023
Obstructive defects of renal pelvis and ureter (753.2)	3 (2.1)	1 (0.4)	0.074
The main anomalies of the maxilla size	5 (3.5)	3 (1.1)	0.046
Umbilical hernia	2 (1.4)	0 (0.0)	0.083

Values are number (percentage). *Exact Fisher test.

Table 3. Congenital Anomalies in the Cleft Lip (CL) and Cleft Lip and Cleft Palate (CLP) Groups

Congenital Anomaly (BPA Code)	CL Group n=59	CLP Group n=217	*P**
Accessory auricle (744.1)	3 (5.1)	1 (0.5)	0.021
Other specified anomalies of ear (744.2)	7 (11.9)	11 (5.1)	0.040
Other congenital anomalies of circulatory system (747)	2 (3.4)	0 (0.0)	0.035
Certain congenital musculoskeletal deformities of spine (754.2)	2 (3.4)	0 (0.0)	0.035
Syndactyly (755.1)	3 (5.1)	3 (1.4)	0.081

Values are number (percentage). *Exact Fisher test.

of this pathology. According to the 2001 version of the London Dysmorphology database, there are 487 identified monogenic syndromes with OCs.

In this study, the most common syndromes were Pierre Robin sequence (n=31, 44.3%), holoprosencephaly (n=6, 8.6%), and amnion rupture sequence (n=4, 5.7%). Lilius reported the following syndromes: Robin sequence (29 cases; 1.8%), Van der Woude's syndrome (n=22, 1.4%), diastrophic dysplasia (n=6, 0.4%), velocardiofacial syndrome (n=6, 0.4%), and fetal alcohol syndrome (n=6, 0.4%) (12). In a Latvian study performed from 1980 to 2005, the following genetic syndromes were identified in 55 patients with OCs: Pierre Robin sequence (n=32, 58.2%), Van der Woude's syndrome (n=12, 21.8%), fetal alcohol syndrome (n=4, 7.3%), Holzgreve syndrome (n=1, 1.8%), Marfan syndrome (n=1, 1.8%), myotonic dystrophy (n=1, 1.8%), Klippel-Feil anomaly (n=1, 1.8%), and Potter's sequence (n=1, 1.8%) (13).

In our study, among the 27 cases with chromosomal abnormalities, trisomy 13 was most common (n=16, 59.26%), and trisomy 18 accounted for 3.7% (n=1) of all chromosomal abnormalities. Our findings are in line with the data in the study by Stoll et al. where trisomy 13 (n=11, 30.56%) and trisomy 18 (n=9, 25%) made up 55.56% of all chromosomal syndromes (14). However, Sárközi et al. reported higher percentages in their study: trisomy 13 and trisomy 18 accounted even for 96.77% (n=30) of all chromosomal syndromes with trisomy 13 being most prevalent (93.55%) (1). Such findings on the distribution of trisomies within the groups of chromosomal abnormalities suggest that with the improvement of diagnostic possibilities, more changes of chromosome number will be determined. Moreover, more detailed variances of chromosomal structure will be identified, which will have an impact on the distribution of chromosomal changes in this group.

However, many patients with OCs have syndromes and sequences that have not been discovered yet. According to Shprintzen et al., the number of specific causes resulting in clefts could be as large as the number of patients in this group (15). The identification of specific anomalies associated with OCs is significant in order to improve the definition of the etiology of this group of malformations (10). There are many different opinions regarding which organ system is the most commonly affected by associated malformations. It is not known whether clefts are really related to the specific types of other congenital malformations. In the present study, the diagnosis was confirmed in 40.8% of OC patients with associated malformations; the remaining patients formed the group of unidentified syndromic OCs with one or more associated major anomalies, which was further analyzed. There were 420 anomalies in 141 patients with OCs of unidentified origin, giving a mean number of 2.9 anomalies per proband. In 1992, Lilius reported a total of 560 anomalies in 345 patients with OCs, with a mean number of 1.6 anomalies per proband (12). In our study, the highest rate of associated malformations among three types of cleft (CL, CP, and CLP) was documented in the CP group: there were 144 anomalies in 41 patients with a mean number of 3.5 anomalies per proband. Our findings did not corroborate the data in the study by Shafi et al. (16).

There is still no agreement on which other congenital anomalies are the most common in patients with OCs. In our study, the anomalies of the mus-

culoskeletal system were most common among all associated anomalies. This group of anomalies included 3 smaller groups: other congenital musculoskeletal anomalies (BPA codes 756.0-756.7); certain congenital musculoskeletal anomalies (BPA codes 754.0-754.8); and other congenital anomalies of limbs (BPA codes 755.0-755.8). It is worth noting that the number of associated anomalies, such as cardiac defects and structural anomalies of the brain, the kidney, and other internal organs, may not be diagnosed during the neonatal period. It is difficult to compare the results with those of other similar studies because the definition and classification of cases and anomalies vary across studies, and this complicates the identification of the proportion of cases diagnosed by objective techniques. Moreover, age- and race-related differences might be associated with different incidences of clefts (12, 14).

Stark reported clubfoot to be the most common malformation in infants with clefts (17). Thereafter, other investigators reported an increased number of the central nervous system malformations and anomalies in the head region in cases with OCs (15, 18). A Finnish study reported that anomalies affecting the extremities accounted the largest proportion of all anomalies, followed by cardiovascular and other facial anomalies (12). In the study by Stoll et al., the anomalies of the extremities and the skeletal system were most commonly associated with OCs (14). Shafi et al. reported that congenital heart disease was more common in comparison with the anomalies of other systems (16), but in the same year, Shaw et al. based on epidemiological data gathered from the California population demonstrated different results (19). An epidemiologic study of nearly 6 million births in 23 EUROCAT registries confirmed that musculoskeletal, cardiovascular, and central nervous system defects were frequently associated with CLP and CL (10). In general, these anomalies dominate in all mentioned studies.

As expected, there were some differences comparing the CP and CL/P groups. This might be due to the following: first, the frequency of associated congenital anomalies was higher in the CP than CL/P group. These results are in line with those reported by Shaw et al. (19), FitzPatrick et al. (4), and Shafi (16). Second, in agreement with Shaw et al. (19) and Kallen et al. (3), the affected organ systems were similar both in the CL and CL/P groups, but still there are a few differences suggesting that genetically they are 2 different entities. Our results were not consistent with the data reported by Kallen et al. (3) regarding the structure of congenital anomalies most commonly associated with CP as well as CL/P. The small sample size in our study could be responsible for this disagreement. Kallen et al. (3) observed alimentary atresia to appear significantly more frequently in patients with CL/P than CP, but these data are controversial to the findings reported in our study.

In some recent publications, the CL/P group was analyzed as two subgroups of OCs: CL and CLP (8–10). The associated affected organ systems in cases of MCAs could also be useful for the explanation of etiologic heterogeneity of these 2 entities. Harville et al. (9) indicated a few reasons why CL might be distinct from CLP. The origin of CL is a malformation of the primary palate only, while CLP involves both the primary and secondary palates. Besides, CL can be found with the separated cleft of the soft but not the hard palate, which suggests 2 different defects. Forrester et al. also listed some reasons for an etiologic distinction between 2 types of CL (8). Our study revealed some differences in affected organ systems (such as congenital anomalies of the circulatory system, BPA code 747) and specific associated anomalies (one of them accessory auricle, BPA code 744.1) with CL, but not CLP, and vice versa. Further investigations involving a larger number of cases are necessary to confirm the findings of this study.

Conclusions

The highest incidence of associated congenital anomalies was in the cleft palate group (3.5 anomalies per proband) followed by the cleft lip (3.1 anomalies per proband), cleft lip and/or palate (2.8 anomalies per proband), and cleft lip with cleft palate (2.7 anomalies per proband) groups. Generally, the anomalies of the musculoskeletal system, cardiovascular system, and face including eye, ear, and neck were most common.

Genetic counseling to carefully evaluate the patients with congenital oral clefts is essential. The careful analysis of associated anomalies and cases of oral cleft subgroups with multiple congenital anomalies is helpful in identifying the etiologic entities and underscores the need for the thorough evaluation and competent distinction of various types of oral clefts.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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