REVIEW ARTICLE

Cleft lip and palate: Associated genetic and environmental factors

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Abstract

Cleft lip and palate are among the most common craniofacial anomalies of the human species causing considerable morbidity to affected individuals. The etiology of isolated or non-syndromic cleft lip and palate is complex and heterogeneous, and its occurrence is associated with the interaction of different genetic and environmental factors. To explore the genetic and environmental factors involved in the etiology of non-syndromic cleft lip and palate, an extensive literature review was conducted on PubMed and SciELO databases. A number of environmental factors such as smoking, alcohol consumption, diet quality, supplementation with folic acid, exposure to chemicals and contaminants, parental age and medication use may be associated with mutations in several genes increasing the risk of development of cleft lip and cleft palate. Advances in the study of gene-environment interactions and the expansion of knowledge about its etiology should lead to improvements in the prevention, diagnosis and treatment of non-syndromic cleft lip and palate.

Introduction

The cleft lip and palate are among the most frequent craniofacial anomalies of the human species. It is estimated that cleft lip and palate affect 1 in 700 live births, with large geographic and racial variation.[1] While not a major cause of infant mortality in developed countries cleft lip and palate cause considerable morbidity to the affected children and imposed a substantial financial impact on families and society. Individuals with clefts require complex treatments involving surgery, dentistry, speech therapy, and psychosocial interventions.[2]

The etiology of isolated or non-syndromic cleft lip and palate is complex and multifactorial. Often result from the interaction between genetic and environmental factors.[1-3] Thus, since its high incidence in the population, it is important the presentation of current knowledge about the complex etiology of cleft lip and palate.

Literature Review

The craniofacial development comprises one of the most complex events during embryogenesis, coordinated by a chain of transcription factors, signaling molecules and proteins that together confer cell polarity and cell and ectomesenchymal interactions. Disturbances in this precisely synchronized cascade can lead to failures in the fusion of the primary facial structures between the 6th and 8th week of intrauterine life resulting in clefts.[4]

Maternal exposure to environmental factors during the embryonic development period may increase the likelihood of an embryo to develop structural anomalies that include cleft lip and palate.[5]

In a recent systematic review, 14 different classifications were found for cleft lip and palate, and the most widely used classification divides clefts into two groups, cleft lip with or without cleft palate (CL/P) and cleft palate (CP),[6] as they are etiologically distinct, both embryologically as epidemiologically.[6,7]

Clefts are also classified in isolated or non-syndromic and syndromic, with approximately 70% of CL/P and almost 50% of FP occurring singly.[4] Non-syndromic clefts occur without other concomitant anomalies and seem to result from a complex interaction between genetic variations and environmental factors. When one or more additional anomalies occur, the clefts are considered syndromic, and can be subdivided into different categories: Chromosomal abnormalities, Mendelian disorders, teratogenic effects and unknown syndromes.[4] Over 400 Mendelian disorders associated with cleft lip and palate were listed.

The cleft lip and palate occur at a frequency of approximately 1 case per 700 births, but with wide variation in relation to race and the studied geographic region.[1]

Among the Asian countries, in northern China, a prevalence of 1/515 births was found in 2000 and later in 2007 a prevalence of 1/568.[8] It is believed that China presents the largest number of babies with cleft lip and palate in world.[3]

In Europe, the prevalence observed in the Nordic countries was 1 case per 454 live births in Norway,[9] 1/555 in Denmark,[10] 1/588 in Sweden[11] and 1/574 births in Finland.[12] Considering other European countries, the prevalence in Spain was 1/694 born between 1990 and 2004.[13] Analyzing regional variation in the prevalence of cleft lip and palate in the Netherlands, Rozendaal *et al.*[14] reported that the northern Netherlands had a significantly higher prevalence of cases (1/467-662) than the rest of the country (1/621-757).

In the United States approximately 1 in every 870 live births present CL/P 1 while in 1500 are affected by CP.[15] In Brazil, Martelli-Júnior *et al.*[16] found a rate of 1 case per 684 live births.

The etiology of isolated or non-syndromic cleft lip and palate is complex and multifactorial. Often result from the interaction between genetic and environmental factors.[1-3]

Several environmental factors have been associated with the occurrence of lefts as smoking,[3,5,17-19] alcohol consumption,[19,20] diet,[21,22] medicines[23,24] and chemical products.[23,25] Currently, great attention has been paid to the identification of genes related to increased susceptibility to the mentioned environmental factors. In the study of genetic susceptibility to cleft lip and palate, correlations between specific genetic variations with specific environmental risk factors are sought.[26] Therefore, it will be explored separately the environmental and genetic factors involved in the etiology of non-syndromic cleft lip and palate.

Among environmental factors, maternal smoking is one of the most studied,[2,5,18] with some disparities in results that can be explained by population differences, variations in samples and variations in pharmacogenetics susceptibility to the smoking effects.[18]

A meta-analysis of 24 case-control and cohort studies on the association between maternal smoking during pregnancy and cleft lip and palate identified statistically significant but modest associations between maternal smoking and CL/P and between maternal smoking and CP.[27]

Analyzing two large population groups affected by cleft lip and palate (Iowa [USA] and Denmark), Shi *et al.*[18] observed increased risk to CL/P in the offspring of both Danish and American smoking.

A case-control study involving 933 cases of children with non-syndromic CL/P, 528 cases of non-syndromic CP and 3390 controls in the United States, aimed to analyze the association between maternal smoking, maternal exposure to tobacco smoke and the occurrence of cleft lip and palate. Maternal smoking in the periconceptional period was associated with CL/P, but weakly associated with FP. Mothers who smoked more than 25 cigarettes a day had twice the risk of generating child with cleft lip and palate, however, only 2.2% of mothers of cases and 1.3% of controls mothers reported that level of cigarette consumption. Among nonsmoking mothers, exposure to tobacco smoke in the workplace or home in the periconceptional period was not associated with the occurrence of CL/P or CP. The study confirmed then a modest association between maternal smoking and cleft lip and palate.[17]

In another study conducted in the United States, it was also evaluated the relationship between maternal smoking and the occurrence of cleft lip and palate (1654 cases of non-syndromic or isolated clefts in a national cohort of more than 4 million births). It was concluded that pregnant smokers had significantly higher risk of generating children with clefts when compared to nonsmokers, regardless of an ethnic group.[5]

A case-control study in Brazil, involving 274 cases of cleft lip and palate and 548 controls, in order to assess the association between maternal and paternal smoking and alcohol consumption and the risk of non-syndromic clefts, found that the prevalence of maternal smoking in the first trimester of pregnancy was higher in the case group, however, not statistically significant. Passive maternal smoking, non-smoking mothers exposed to tobacco smoke during pregnancy (at home or in the workplace) was associated with CL/P but not with FP. The paternal smoking did not increase the risk for the occurrence of cleft lip and palate. The results of this study suggest that maternal smoking increases the risk to non-syndromic cleft lip and palate.[19]

Aiming to investigate whether maternal and paternal smoking early in pregnancy increased the risk to the occurrence of cleft lip and palate in China, Zhang *et al.*,[3] conducted a case-control study (304 cases of non-syndromic clefts and 454 controls). It was observed that paternal and maternal (active and passive) smoking in the peri-conceptional period, were associated with significantly increased risk of generating child with cleft lip and palate. The risk associated with maternal smoking (1-10 cigarettes/day) in the first trimester was more critical than the pre-pregnancy maternal smoking. A statistically significant dose-response trend was also demonstrated since the risk to clefts increased with increasing paternal smoking during pregnancy. The association between passive maternal smoking and cleft lip and palate in this study was consistent, modest and statistically significant.

The consulted scientific evidence suggests that maternal smoking increases the risk to the occurrence of non-syndromic cleft lip and palate, although this risk is modest. The association between passive maternal smoking, parental smoking and cleft lip and palate is inconclusive.

Leite and Koifman[19] examined whether alcohol consumption increases the risk to non-syndromic cleft lip and

palate. The risk factors evaluated were the maternal consumption of alcohol during the year prior to pregnancy and during the first trimester of pregnancy, the amount of alcohol ingested per day (≤96 g or >96 g) and the consumption pattern (daily or occasional). The results indicated that the risk to CL/P and CP was increased with daily doses of ethanol and that the risk to CL/P tended to increase with frequent consumption of alcoholic beverages. The maternal consumption of alcohol during the first trimester of pregnancy was also associated with CL/P and FP.

Lebby *et al.*[5] found no association between cleft lip and palate and maternal consumption of alcohol in the United States in a sample with 1654 cases of non-syndromic cleft patients.

A case-control study conducted in Norway (483 cases of cleft lip and palate and 503 controls) aimed to examine the association between maternal alcohol consumption and risk to clefts. It investigated alcohol consumption by mothers in the first trimester, which is the relevant period for facial development. The research included the average number of days per week or month in which the mother drank alcohol and the average doses consumed on each occasion. It was observed that women who consumed alcohol at high levels (≥5 doses/occasion) in the first trimester of pregnancy, had a high risk for the development of children with cleft lip and palate.[20]

The association between maternal alcohol consumption and cleft lip and palate has been demonstrated in epidemiologic studies,[19,20] although not in all.[5] This inconsistency may occur, in part, by the need for ingesting large quantities of alcohol to the occurrence of the anomaly. As the excessive consumption of alcohol during pregnancy is unusual, the small number of women exposed in many studies could hamper the evaluation of this association.[20]

Several studies have sought the association between maternal and paternal age with the occurrence of cleft lip and palate. A meta-analysis was conducted in order to verify the association between parental age and the occurrence of non-syndromic cleft lip and palate. There was no statistical association between paternal or maternal age <20 years and the occurrence of clefts. However, parents aged over 40 were more likely to generate a child with CP compared to parents aged 20-29 years. Mothers aged 40 or more had 1.56 times the risk of having a child with CL/P and 1.28 times more likely to have a child with CP compared to mothers between 20 and 29 years old. Mothers between 35 and 39 were also at statistically higher risk of generating a child with CP than those between 20 and 29 years.[6]

Bille *et al.*[28] also observed an association between advanced maternal and paternal age and increased the risk of birth with CL/P, although only advanced paternal age has been associated with an increased risk of CP.

A case-control study conducted in China with 713 non-syndromic cleft lip and palate cases and 221 controls, trying to analyze the relationship between environmental factors and clefts, found that although the average maternal age in both groups was similar (26.53 years for cases and 27,14 group for the control group), when subgroups were analyzed divided into five age groups, mothers with more than 35 years had more children with cleft lip and palate and the difference between the case and the control group was statistically significant.[7]

Opposing previous works, a case-control study conducted by Zhang *et al.*[3] in China showed that mothers with more than 35 years had a lower risk of generating children with clefts, whereas paternal age below 25 years was significantly associated with all types of clefts. A similar situation was observed by Lebby *et al.*[5] in the United States. The authors also found that increasing age of mothers was consistently associated with significantly lower risk of conceiving a child with a cleft in all ethnic groups.

Factors that may explain the association between the occurrence of clefts and the advanced age of parents refer to cumulative changes in the gametes throughout life as a result of environmental exposures or chromosomal changes. In women, with the aging process, the uterus becomes less selective for defective embryos and the placenta becomes more permeable teratogenic agents.[6]

There is limited evidence that periconceptional diet can influence the risk of occurrence of clefts. Nutrients such as folate, niacin, thiamin, vitamins B6 and B12, riboflavin, zinc, amino acids and carbohydrates have been associated with neural crest cell defects, including cleft lip and palate.[21]

There is currently a consensus that folic acid supplements ingested in the periconceptional period substantially reduce the risk of neural tube defects.[22] To test the hypothesis that the intake of supplements containing folic acid during pregnancy reduces the prevalence of non-syndromic cleft lip and palate, Badovinac *et al.*[29] performed a meta-analysis and found a consistent trend of the protective effect of folic acid. Using the results of case-control studies it was found that mothers who used supplements containing folic acid during pregnancy had 33% less risk of generating a child with any type of cleft, 29% less risk of generating child with CLP and 20% less risk of generating child with CP. Nevertheless, the authors stated that evidence that supplementation with folic acid during pregnancy reduces the prevalence of non-syndromic cleft lip and palate in humans is still inconclusive.

A case-control study investigated the relationship between environmental factors and the occurrence of clefts in the Chinese population and observed that supplementation with folic acid in early pregnancy had a protective effect, associated with a reduced risk of non-syndromic cleft lip and palate.[7]

Shaw *et al.*,[30] in a large proportion case-control study in the United States (1108 CL/P cases and 2594 controls), investigated whether the consumption of certain nutrients in the peri-conceptional period, increased or decreased the risk to the occurrence of clefts. The frequency of consumption of 58 foods during the year before pregnancy was evaluated. There was some evidence of decreased risk for CL/P (30% reduction) with increasing intake of total protein, choline, and methionine. Similarly, the decrease in risk of clefts appear to be associated with the increased consumption of carbohydrates and cysteine. However, the results did not show decreased the risk of CL/P or FP with maternal consumption of vitamin supplements containing

folic acid, contradicting findings of several studies. The reason for this lack of association is unknown; the authors speculated that the protection afforded by folic acid through supplements might no longer be working due to high levels of folic acid in fortification of foods in the United States during the study period.

Using two diet quality indices, Carmichael *et al.*,[31] in a later case-control study also in the USA, sought to examine whether better quality maternal diet was associated with a decreased risk of birth defects, including CL/P. The authors found that a better diet quality in the year before pregnancy was associated with lower risk of neural tube defects and clefts, even after adjusting for potential confounding factors such as consumption of vitamin supplements. The results suggested that the overall quality of the diet is more predictive of the risk of birth defects than the consumption of individual nutrients such as folic acid.

Although the evidence that the use of folic acid or vitamin B6 is not yet confirmed, its use should be recommended to all women in the peri-conceptional period.[1]

Several studies have attempted to identify the teratogenic effects of various drugs. Regarding cleft lip and palate, an association has been investigated between the use of anticonvulsants and CL/P. Werler *et al.*[32] evaluated the use of specific antiepileptic drugs during pregnancy and its relation to birth defects. Increased risk to the occurrence of clefts was observed with the use of valproic acid.

The use of benzodiazepines during pregnancy has also been considered a risk factor for the occurrence of clefts in some studies. Safra and Oakley[33] found a positive association, particularly between the use of diazepam in pregnancy and the occurrence of cleft lip and palate. These findings were not corroborated by the case-control study of Rosenberg *et al.*[34] Dolovich *et al.*[35] conducted a meta-analysis in order to determine whether exposure to benzodiazepines in the first trimester of pregnancy increase the risk to malformations and cleft lip and palate. They concluded that the data from cohort studies showed no association between the use of benzodiazepines and clefts, however, a small but significant increased risk to cleft lip and palate was found in the case-control studies.

The association between the use of corticosteroids during pregnancy and the risk of cleft lip and palate also remain inconclusive. Although epidemiological studies have suggested that association,[36,37] many of these studies showed a limited statistical power.[24] A large national cohort study was conducted in Denmark in order to determine whether the use of corticosteroids during pregnancy would be associated with the occurrence of clefts. The authors found that the association was not significant for the general use of corticosteroids during pregnancy. However, the use of dermatological corticosteroid was associated with an increased risk of CL/P, unlike corticosteroids administered by oral, nasal sprays, inhalants and other means of topical use.[24] It has been recommended caution in the use of corticosteroids during pregnancy.

The potential impact of exposure to contaminants such as waste solvents, pesticides, and metals in human reproduction has been investigated.[23]

As for solvents, case-control studies have demonstrated a relationship between maternal occupational exposure to these products and congenital malformations, especially CL/P.[38,39] Garlantézec *et al.*[25] conducted in France a cohort study of 3421 pregnant women in order to test the hypothesis that maternal exposure to solvents has an impact on the risk of congenital malformations. The working contact frequency was evaluated with 11 classes of products containing solvents (paints, varnishes, dyes, gasoline, glues, degreasers, detergents and cleaning agents, agents for textiles and cosmetics). It was concluded that there was an association between maternal exposure to solvents and the occurrence of cleft lip and palate whereas women who worked as laboratory technicians, beauticians, hairdressers, and cleaners, had significant exposure to them.

Considering pesticides, since the 1940s, there was a worldwide dramatic increase in the use of agricultural supplies, including herbicides, insecticides, and fungicides. Some of these chemicals are suspected or known to be teratogenic, mutagenic or carcinogenic in animals. Still, relatively little attention has been given to hundreds of chemical formulations and their effects on the health of populations.[23]

In the 1980s, Gordon and Shy[40] raised the hypothesis that intrauterine exposure to agricultural chemicals during the peak period of use of herbicides and pesticides, especially in the first trimester of pregnancy could be associated with an increased risk of birth defects. The study, despite its limitations, showed independent effects of exposure to these products in the occurrence of cleft lip and palate.

Sources of maternal exposure to pesticides include agricultural work, living in agricultural areas and gardening.[23] Shaw *et al.*[41] explored the relationship between various sources of maternal and paternal exposure to pesticides in peri-conceptional period and congenital anomalies in offspring (cleft lip and palate, neural tube defects, conotruncal defects and limbs anomalies). Regarding cleft lip and palate, it was observed that the paternal occupational exposure to pesticides has been associated with an increased risk of CL/P and that pesticide use by pregnant women in gardening activities was associated with ≥OR 1.5 for most of the anomalies studied. However, as there was not in this study specific information about the chemical agents and the level of exposure, it was not possible to adequately assess the validity of the effects observed, which could have been influenced by bias.

Thulstrup and Bonde[42] conducted a literature review on the association between maternal occupation during pregnancy and the occurrence of some birth defects (neural tube defects, cleft lip and palate, congenital heart defects, urinary tract defects and limb defects). Regarding cleft lip and palate, they suggested the association with maternal exposure to pesticides as well as organic solvents and glycol ethers, however, the evidence is still limited. They concluded that there was growing concern about the possible teratogenic effects of these chemicals, but there is no convincing evidence linking maternal exposure during pregnancy with congenital anomalies in offspring.

Shirangi *et al.*,[43] in a systematic review of the literature, assessed the current epidemiological evidence of the association

between residents of nearby areas with application of pesticides in agriculture and adverse reproductive outcomes, including congenital malformations. They concluded that the scientific evidence suggests an association with congenital malformations; however, due to methodological limitations of the various studies, a definitive conclusion was not reached.

It is noted, thus, that the epidemiological research has not convincingly demonstrated the teratogenic effects of many chemicals, although suggested them. Methodological shortcomings with respect to establish an exposure-response relationship, to control confounding factors and assess large populations represent the challenges to be overcome by future researches.[43]

Fogh-Andersen[44] first defined genetic factors as etiological factors of cleft lip and palate, which was later confirmed by segregation analysis. Since then, a variety of genetic approaches has been used to identify genes and loci involved in the etiology of clefts. The last few years witnessed many advances in mapping of genes involved. These include IRF6, MSX1, PVRL1, TBX22, FGFR1, TGFA, TGFb3, RARE, NAT2.[26,45]

The use of animal models has been of great importance in identifying the genes involved in the etiology of clefts.[1,4,45]

Twin studies have also contributed to the understanding of the genetic component of the clefts etiology. The simultaneous occurrence in monozygotic twins varies between 40% and 60%, and 5% in dizygotic. The lack of 100% concordance in monozygotic twins suggests that genetic events alone are not responsible for cleft lip and palate phenotype. However, the simultaneous occurrence in monozygotic strongly supports the importance of the genetic component.[1]

Many studies have provided strong evidence that syndromic forms of clefts may favor the knowledge of the genetic etiology of non-syndromic or isolated clefts.[1,45] There is evidence that these genes contribute to the etiology of syndromic and non-syndromic clefts, perhaps by variable penetrance or action of different modifiers.[4]

The autosomal dominant Van der Woude syndrome is an important model studied. Mutations in IRF6 gene are related to the syndrome and variations in this gene were significantly associated with non-syndromic clefts.[46]

Another recessive autosomal syndrome, the Margarita Island ectodermal dysplasia, is characterized by the occurrence of cleft and mutations in the PVRL1 gene. These same changes were considered significant risk factors for the occurrence of clefts in non-syndromic Venezuelans.[47]

The Msx proteins have a known role in epithelial-mesenchymal interactions during craniofacial development.[48] MSX1 gene mutations caused dental agenesis and various combinations of cleft lip and palate in a Dutch family.[49] In patients with non-syndromic cleft lip and palate of different ethnic groups, it was observed that up to 2% of patients had mutations in the gene MSX1.[50]

Another example of a gene involved in the etiology of syndromic fissure which has been examined as a candidate gene in the etiology of non-syndromic clefts is FGFR1, which mutations result in the autosomal dominant Kallmann syndrome, characterized by hypogonadism, anosmia and cleft lip and palate for 5-10% of cases. Mutations in FGFR1 gene were also found in some individuals with isolated or non-syndromic cleft lip and palate.[45]

A variety of mutations in other genes (TP63, TTF-2, TBX22, FOXE1, FLNA) associated with syndromic clefts has been investigated, and may facilitate the mapping of genes involved in isolated or non-syndromic forms.[4,45]

The number and diversity of genes involved in the etiology of cleft lip and palate probably reflects the reason for these deficiencies to figure among the most frequent in humans. Despite advances in genetic studies, the challenge is still to relate this genetic heterogeneity to the environmental and socioeconomic factors involved in the etiology of clefts.[4]

Several studies have investigated interactions between a number of environmental factors such as smoking, alcohol consumption, multivitamin supplementation and medications with mutations in several genes.[18,45] Some studied correlations refer to variations in TGFA (smoking, vitamin deficit), TGFb3 (smoking, drinking), MSX1 (smoking, drinking), ADH1C (drinking), EPHX1, GSTT1, NAT1, NAT2, CYP1A1 (smoking, use of drugs, exposure to chemical agents) and RARA (smoking, consumption of vitamin A).[1,26] Importantly, these genetic variations that confer susceptibility to environmental factors can in many cases be detected only in the mothers, not the affected children. Thus, based on genetic variations detected in pregnant women, one could identify environmental risk factors, thereby avoiding the exposure of pregnant women to them.[26] In other cases, the genetic variation is found only in the fetus and combined with an environmental maternal risk factor could lead to the phenotype expression.[18] Or the genetic variation can be detected in both the mother and the child.[20]

With the recent advances in genetics and a deeper study of genetic-environmental interactions, researchers are beginning to unscramble the causes of cleft lip and palate, which should provide new opportunities for improvement of diagnosis, prevention and treatment of this complex congenital anomaly.

References

1. Murray JC. Gene/environment causes of cleft lip and/or palate. Clin Genet 2002;61:248-56.
2. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: Understanding genetic and environmental influences. Nat Rev Genet 2011;12:167-78.
3. Zhang B, Jiao X, Mao L, Xue J. Maternal cigarette smoking and the associated risk of having a child with orofacial clefts in China: A case-control study. J Craniomaxillofac Surg 2011;39:313-8.
4. Stanier P, Moore GE. Genetics of cleft lip and palate: Syndromic genes contribute to the incidence of non-syndromic clefts. Hum Mol Genet 2004;13:R73-81.
5. Lebby KD, Tan F, Brown CP. Maternal factors and disparities associated with oral clefts. Ethn Dis 2010;20:S1-146.
6. Herkrath AP, Herkrath FJ, Rebelo MA, Vettore MV. Parental age as a risk factor for non-syndromic oral clefts: A meta-analysis.

J Dent 2012;40:3-14.
7. Jia ZL, Shi B, Chen CH, Shi JY, Wu J, Xu X. Maternal malnutrition, environmental exposure during pregnancy and the risk of non-syndromic orofacial clefts. Oral Dis 2011;17:584-9.
8. Wang W, Guan P, Xu W, Zhou B. Risk factors for oral clefts: A population-based case-control study in Shenyang, China. Paediatr Perinat Epidemiol 2009;23:310-20.
9. Sivertsen A, Wilcox A, Johnson GE, Abyholm F, Vindenes HA, Lie RT. Prevalence of major anatomic variations in oral clefts. Plast Reconstr Surg 2008;121:587-95.
10. Grosen D, Bille C, Pedersen JK, Skytthe A, Murray JC, Christensen K. Recurrence risk for offspring of twins discordant for oral cleft: A population-based cohort study of the Danish 1936-2004 cleft twin cohort. Am J Med Genet A 2010;152A:2468-74.
11. Cedergren M, Källén B. Maternal obesity and the risk for orofacial clefts in the offspring. Cleft Palate Craniofac J 2005;42:367-71.
12. Derijcke A, Eerens A, Carels C. The incidence of oral clefts: A review. Br J Oral Maxillofac Surg 1996;34:488-94.
13. Dehli CR, Tenreiro CM, López EG, Toral JF, Fernández AR, Galán IR, *et al.* Epidemiología de las fisuras labiales y palatinas durante los años 1990-2004 en Asturias. Ann Paediatr 2010;73:132-7.
14. Rozendaal AM, Mohangoo AD, Ongkosuwito EM, Buitendijk SE, Bakker MK, Vermeij-Keers C. Regional variation in prevalence of oral cleft live births in the Netherlands 1997-2007: Time-trend analysis of data from three Dutch registries. Am J Med Genet A 2012;158A:66-74.
15. Shaw GM, Carmichael SL, Yang W, Harris JA, Lammer EJ. Congenital malformations in births with orofacial clefts among 3.6 million California births, 1983-1997. Am J Med Genet A 2004;125A:250-6.
16. Martelli-Júnior H, Bonan PR, Santos RC, Barbosa DR, Swerts MS, Coletta RD. An epidemiologic study of lip and palate clefts from a Brazilian reference hospital. Quintessence Int 2008;39:749-52.
17. Honein MA, Rasmussen SA, Reefhuis J, Romitti PA, Lammer EJ, Sun L, *et al.* Maternal smoking and environmental tobacco smoke exposure and the risk of orofacial clefts. Epidemiology 2007;18:226-33.
18. Shi M, Christensen K, Weinberg CR, Romitti P, Bathum L, Lozada A, *et al.* Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. Am J Hum Genet 2007;80:76-90.
19. Leite IC, Koifman S. Oral clefts, consanguinity, parental tobacco and alcohol use: A case-control study in Rio de Janeiro, Brazil. Braz Oral Res 2009;23:31-7.
20. Boyles AL, DeRoo LA, Lie RT, Taylor JA, Jugessur A, Murray JC, *et al.* Maternal alcohol consumption, alcohol metabolism genes, and the risk of oral clefts: A population-based case-control study in Norway, 1996-2001. Am J Epidemiol 2010;172:924-31.
21. Krapels IP, van Rooij IA, Ocké MC, West CE, van der Horst CM, Steegers-Theunissen RP. Maternal nutritional status and the risk for orofacial cleft offspring in humans. J Nutr 2004;134:3106-13.
22. van Beynum IM, Kapusta L, Bakker MK, den Heijer M, Blom HJ, de Walle HE. Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: A registry-based case-control study in the northern Netherlands. Eur Heart J 2010;31:464-71.
23. Leite IC, Paumgartten FJ, Koifman S. Chemical exposure during pregnancy and oral clefts in newborns. Cad Saude Publica 2002;18:17-31.
24. Hviid A, Mølgaard-Nielsen D. Corticosteroid use during pregnancy and risk of orofacial clefts. CMAJ 2011;183:796-804.
25. Garlantézec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: A prospective study in the general population. Occup Environ Med 2009;66:456-63.
26. Stuppia L, Capogreco M, Marzo G, La Rovere D, Antonucci I, Gatta V, *et al.* Genetics of syndromic and nonsyndromic cleft lip and palate. J Craniofac Surg 2011;22:1722-6.
27. Little J, Cardy A, Munger RG. Tobacco smoking and oral clefts: A meta-analysis. Bull World Health Organ 2004;82:213-8.
28. Bille C, Winther JF, Bautz A, Murray JC, Olsen J, Christensen K. Cancer risk in persons with oral cleft – A population-based study of 8,093 cases. Am J Epidemiol 2005;161:1047-55.
29. Badovinac RL, Werler MM, Williams PL, Kelsey KT, Hayes C. Folic acid-containing supplement consumption during pregnancy and risk for oral clefts: A meta-analysis. Birth Defects Res A Clin Mol Teratol 2007;79:8-15.
30. Shaw GM, Carmichael SL, Laurent C, Rasmussen SA. Maternal nutrient intakes and risk of orofacial clefts. Epidemiology 2006;17:285-91.
31. Carmichael SL, Yang W, Feldkamp ML, Munger RG, Siega-Riz AM, Botto LD, *et al.* Reduced risks of neural tube defects and orofacial clefts with higher diet quality. Arch Pediatr Adolesc Med 2012;166:121-6.
32. Werler MM, Ahrens KA, Bosco JL, Mitchell AA, Anderka MT, Gilboa SM, *et al.* Use of antiepileptic medications in pregnancy in relation to risks of birth defects. Ann Epidemiol 2011;21:842-50.
33. Safra MJ, Oakley GP Jr. Association between cleft lip with or without cleft palate and prenatal exposure to diazepam. Lancet 1975;2:478-80.
34. Rosenberg L, Mitchell AA, Parsells JL, Pashayan H, Louik C, Shapiro S. Lack of relation of oral clefts to diazepam use during pregnancy. N Engl J Med 1983;309:1282-5.
35. Dolovich LR, Addis A, Vaillancourt JM, Power JD, Koren G, Einarson TR. Benzodiazepine use in pregnancy and major malformations or oral cleft: Meta-analysis of cohort and case-control studies. BMJ 1998;317:839-43.
36. Pradat P, Robert-Gnansia E, Di Tanna GL, Rosano A, Lisi A, Mastroiacovo P, *et al.* First trimester exposure to corticosteroids and oral clefts. Birth Defects Res A Clin Mol Teratol 2003;67:968-70.
37. Carmichael SL, Shaw GM, Ma C, Werler MM, Rasmussen SA, Lammer EJ, *et al.* Maternal corticosteroid use and orofacial clefts. Am J Obstet Gynecol 2007;197:585.e1-7.
38. Lorente C, Cordier S, Bergeret A, De Walle HE, Goujard J, Aymé S, *et al.* Maternal occupational risk factors for oral clefts. Occupational Exposure and Congenital Malformation Working Group. Scand J Work Environ Health 2000;26:137-45.
39. Chevrier C, Dananché B, Bahuau M, Nelva A, Herman C, Francannet C, *et al.* Occupational exposure to organic solvent mixtures during pregnancy and the risk of non-syndromic oral clefts. Occup Environ Med 2006;63:617-23.
40. Gordon JE, Shy CM. Agricultural chemical use and congenital cleft lip and/or palate. Arch Environ Health 1981;36:213-21.
41. Shaw GM, Wasserman CR, O'Malley CD, Nelson V, Jackson RJ.

Maternal pesticide exposure from multiple sources and selected congenital anomalies. Epidemiology 1999;10:60-6.
42. Thulstrup AM, Bonde JP. Maternal occupational exposure and risk of specific birth defects. Occup Med (Lond) 2006;56:532-43.
43. Shirangi A, Nieuwenhuijsen M, Vienneau D, Holman CD. Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: A review of the literature. Paediatr Perinat Epidemiol 2011;25:172-91.
44. Fogh-Andersen P. Inheritance of harelip and cleft palate. Copenhagen: Munksgaard; 1942.
45. Jugessur A, Murray JC. Orofacial clefting: Recent insights into a complex trait. Curr Opin Genet Dev 2005;15:270-8.
46. Scapoli L, Palmieri A, Martinelli M, Pezzetti F, Carinci P, Tognon M, *et al.* Strong evidence of linkage disequilibrium between polymorphisms at the IRF6 locus and nonsyndromic cleft lip with or without cleft palate, in an Italian population. Am J Hum Genet 2005;76:180-3.
47. Sözen MA, Suzuki K, Tolarova MM, Bustos T, Fernández Iglesias JE, Spritz RA. Mutation of PVRL1 is associated with sporadic, non-syndromic cleft lip/palate in northern Venezuela. Nat Genet 2001;29:141-2.
48. Alappat S, Zhang ZY, Chen YP. Msx homeobox gene family and craniofacial development. Cell Res 2003;13:429-42.
49. van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. Nat Genet 2000;24:342-3.
50. Jezewski PA, Vieira AR, Nishimura C, Ludwig B, Johnson M, O'Brien SE, *et al.* Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. J Med Genet 2003;40:399-407.