Role of environmental factors in onset of non-syndromic orofacial cleft in Italian population

A Avantaggiato 1, F Cura 2, A Girardi 3, D Lauritano 4*

Abstract

Introduction

Non-syndromic cleft lip with or without cleft palate is the most common craniofacial anomaly affecting around 1 in 700 live births worldwide. Clefts of the human face can be classified anatomically as cleft palate only (CPO), cleft lip only, cleft lip and palate (CLP) or a combined group of cleft lip with or without cleft palate, based on differences in embryologic development.

These malformations have a genetic origin, in fact several association studies have been performed to obtain important information about the candidate genes; but more important are gene–environment interactions that play an increasing role in its aetiology. In this review we analyse the role of environmental and genetic factors related to onset of cleft.

Conclusion

Epidemiological studies have shown how environmental factors (alcohol, smoking and drugs), as well as possible gene–environment interactions, play an important role in the onset of the malformation. On the contrary, folic acid intake seems to have a protective effect.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committee related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Environmental factors

Alcohol

Materno–foetal intoxication with ethanol during pregnancy causes a well-known syndrome—the so-called foetal alcohol syndrome—characterised by pre/postnatal growth retardation and facial dysmorphism; but the association between alcohol and non-syndromic (NS) CL/P is inconsistent. In fact, during the past two decades, a series of epidemiologic studies have also revealed the role of alcohol in determining NS OFC.

One study showed that alcohol increases the risk of NS CL ± P, whereas no significant association was found between alcohol and CPO, or in syndromic clefts. In 1999, other authors observed that the risk of delivering infants with OFC phenotypes for mothers who take alcohol during pregnancy is dose related. In the same year, other authors examined the allelic variants of three genes—transforming growth factor alpha (TGF-A), TGF-B3 and MSX1—and their interaction with two exposures during pregnancy (cigarette smoking and alcohol consumption). They demonstrated that the development of CL ± P and CPO may be influenced by these risk factors, especially when they interact with specific allelic variants.

Drugs

There are drugs whose effects on chronic pain syndrome (CPS) development are demonstrated. Diazepam assumption has an important effect in developing CPS during...
embryogenesis\textsuperscript{11,12}. Diphenylhydantoin, a teratogen known to induce cleft palate in human newborns might be related to anomalous palate development\textsuperscript{13,14}. In fact, the morphogenetic processes during palate development are related to extracellular matrix composition, which is important both in cell activities and in gene expression. This drug can modify cytoskeletal components and extracellular matrix-cell adhesion influencing the expression of genes involved in the development of the palate.

In another study, it has been shown that other factors, such as the TGF-β, retinoic acid and γ-aminobutyric acid ergic are potentially involved in the malformation\textsuperscript{15}.

**Cigarette smoking**
The role of cigarette smoking has been analysed in epidemiologic studies by several authors, sometimes with conflicting results; although its clefting effect is universally accepted, the type of cleft induced is less clear. One study\textsuperscript{16} investigated whether parental periconceptional cigarette smoking was associated with an increased risk of offspring with CL \( \pm \) P. They also investigated in which way the genetic variation of the TGF-A locus could interact with smoking in causing cleft. The authors found that risks associated with maternal smoking were most elevated for isolated CL \( \pm \) P and for CPO when mothers smoked 20 cigarettes or more per day. Cloting risks were even greater for infants with the uncommon TGF-β type. Smoking was associated with a moderately increased risk of oral clefts and TGF-A genotype nor its interaction with maternal smoking.

Some authors\textsuperscript{19} performed a meta-analysis whereby they found a small, but statistically significant association between maternal cigarette smoking consumption in the first trimester of gestation and the risk of CL \( \pm \) P or CPO. However, in a further study\textsuperscript{20}, the same author employed large samples from the 1996 and 1997 U.S. Nationality database indicating that smoking is only a minor risk factor. In a large case-control study\textsuperscript{21}, the authors found a positive dose-response association between smoking and infants with syndromic CL \( \pm \) P.

**Folic acid**
The debate about folate interference began when it was demonstrated that women periconceptionally taking multivitamins containing folic acid lowered their risk of having children with CL \( \pm \) P\textsuperscript{22}. Although some authors\textsuperscript{23} did not find any evidence to confirm a protective association between the periconceptional use of folic acid supplements and the risk of oral clefts, further evidence to support the role of folic acid was produced by other investigators\textsuperscript{24}.

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folic acid metabolism. The C677T mutation of the MTHFR gene produces a form of MTHFR thermolabile with reduced activity. This characteristic has been related to elevated plasma homocysteine levels and lowered plasma folate on account of reduced MTHFR activity\textsuperscript{25}. Some authors\textsuperscript{26}, in an investigation on Irish population, highlighted that the homozygosity for the common folate-related polymorphism associated with the thermolabile form of MTHFR is more frequent both in CL \( \pm \) P patients and sporadic CPO. Linkage disequilibrium was not found by some authors\textsuperscript{27} in their evaluation of parental allele transmissions. However, this study reported that the MTHFR polymorphic system was not in the Hardy–Weinberg equilibrium among mothers of CL \( \pm \) P patients. The authors suggested that homozygosity of either the T or C allele of C677T polymorphism in females constitutes an important susceptibility factor for CL \( \pm \) P onset; they postulated that the CT heterozygotes would have an advantage over the homozygotes in relation to this trait.

Other authors\textsuperscript{28} observed that maternal hyperhomocysteinaemia may be a risk factor for having CL \( \pm \) P offspring, which is interesting if we consider that one effect of reduced MTHFR activity is hyperhomocysteinaemia.

In 2001, it was demonstrated that there was a significantly higher mutation frequency for C677T polymorphism at MTHFR in the mothers of CL \( \pm \) P patients as compared with controls. The results support the involvement of the folate pathway in the aetiology of CL \( \pm \) P, and sustain the hypothesis of an effect due to the maternal genotype, rather than an influence of the embryo genotype. These findings have been borne out by a subsequent-independent study\textsuperscript{29}.

In a subsequent research some authors have investigated c.665C > T (commonly known as 677C > T; p.Ala222Val) and c.1286A > C (known as 1298A > C; p.Glu429Ala) polymorphisms in the MTHFR gene in 110 non-familial patient/parent triads and 289 unrelated controls. The results of the study highlight that mutations in the MTHFR gene in pregnant women are responsible for a higher risk of having CL \( \pm \) P affected children\textsuperscript{30}.

Folate receptors (FOLRs) mediate the delivery of 5-methyltetrahydrofolate to the interior of cells or between cells in a process known as potocytosis.
In order to verify whether FOLRs could be responsible for the onset of NS CL + P, using linkage and linkage disequilibrium, a study sample consisted of patients and their mothers from 71 families CL/P pedigrees and 75 sporadic cases of the Italian population. The results of this study have shown only a silent mutation in FOXL1 present in a mother and her child. But this does not allow supporting FOXL1 and FOXL2 genes in the onset of CL/P.

In addition, the involvement in CL/P aetiology of four genes belonging to the folate pathway was verified: transcobalamins (TCN1 and TCN2), methionine synthase (MTR) and MTR reductase (MTRR). The results suggest that TCN2 is involved in causing CL + P, through a reduction of homocysteine remethylation efficiency. These data are highly interesting and require further investigation of different sample collection.

In an Italian study, genetic variants of folate and homocysteine metabolism in influencing the risk of OFCs were evaluated; but did not find significant level of association between betaine-homocysteine methyltransferase (BHMT and BHMT2) and cystathionine beta-synthase variants with CL+/-P. The common MTHFR 677T variant leading to reduced folate availability in the mother and then for the embryo, who uses maternal reserves. In fact, this genetic variant is an important factor associated with increased risk of CL/P.

Several studies have tested the interaction between MTHFR and folate ABCB1 genotypes. ABCB1 gene codes for P-glycoprotein, a drug-transport pump in charge to protect the cell by harmful exposures by actively exporting various substrates across the cell membrane.

A family-based association study was performed to verify the involvement of ABCB1 polymorphisms in CL/P aetiology, including a possible foetal-maternal genetic interaction between ABCB1 and MTHFR, but no evidence of association was detected. Lack of association could mean that the sample size was not sufficient to detect a very low effect. A sample selection criteria including periconceptional drugs or medication assumption may help to increase the power of the study; thus identifying the possible foetal-maternal interaction between ABCB1 and MTHFR genotypes.

Genetic factors

CL/P are among the most common birth defects, with an incidence of 1/700–1/1000 of born alive children. Both genetics and environmental factors contribute to its onset, thus CL/P is defined as a multifactorial disease. Although the question about the nature of the genetic contribution is still under profiling, some chromosome regions have been successfully investigated and some genes have been suggested. The presence of multiple candidate genes makes oral cleft a complex disorder. Some of these candidate genes have been identified with the employment of linkage analysis and mouse-model knockout studies.

Chromosome 6 has been largely investigated and evidence of linkage was highlighted between the 6p23 chromosome region and the malformation. The role of TGFA-A was taken into account in the oral cleft onset, in light of its contribution in cell proliferation, differentiation and development. Some studies reported an association between TGFA and oral cleft, but some others did not reply this result.

Positive association was found out between markers on chromosome 19q13.2 and OFC malformation. Some authors classified the phenotypes found in a series of patients with NS CL/P and isolated cleft palate, concluding that NS CL/P can be classified according to laterality that can be under genetic control.

Mutations in interferon regulatory factor 6 (IRF6) can lead to Van der Woude syndrome, a dominant disorder that has CL/P as a common feature. Recently, it has been proposed and confirmed a strong association between genetic polymorphisms at the IRF6 locus and NS CL/P particularly in Asian and South American populations.

In addition, MYH9, a gene encoding for the heavy chain of non-muscle myosin IIa, is also considered a potential candidate. The reason for its possible involvement must be sought in its abundant and characteristic expression in epithelial cells of palatal shelves before their fusion. During palatal fusion, the expression of MYH9 appears to gradually decrease until it disappears completely, once fusion is ended. Thus, MYH9 might be a predisposing factor for CL/P, although more investigation is needed to better define its pathogenetic role.

Besides MYH9, the expression profile of another gene JARID2 follows the approach of palatal shelves up to their fusion. Seen that expression data suggesting clearly a role for JARID2 in palate development, some authors investigated its involvement in CLP in a family-based linkage disequilibrium study. Their results confirmed the role of JARID2 in this clinical manifestation, although its functional roles are still unknown.

TFAP2A is a transcription factor with peculiar characteristics that prompted another research group to verify its involvement in the onset of the NS CLP. In fact, the gene that encodes this protein is located in the 6p24 region, widely recognised as the NS CLP candidate region. Moreover it carries out its function as a regulator, modulating the expression of IRF6, in turn already associated with increased risk of cleft lip. In addition, TFAP2A is involved in the branchio-oculo-facial syndrome, a congenital disease that includes CL/P. Both single marker
and haplotype analysis, confirmed the existence of association between TFAP2A and NS CLP.

Among various mechanisms involved in embryonic development, the epithelial-mesenchymal transition has always captured the attention of researchers, urging them to investigate several molecules that appear to be cardinal players in orofacial development. LEF1, specifically, is a transcription factor with a key role for the correct flow of events. In fact, data show how LEF1 maternal genotype is associated with the occurrence of CLP.

**Conclusion**

It is well established that NS OFC is a congenital disease that may affect the lip with or without the involvement of the palate (CL +/− P) or just the palate. Both have a genetic origin, but also environmental factors play an important role in the onset of these malformations.

Epidemiologic studies have demonstrated a relationship between certain environmental factors (alcohol, drugs and cigarette smoking) during pregnancy and a higher risk of having a child with OFC. On the contrary, folic acid intake has a protective effect.

However, despite all the advances made to identify the genetic and environmental causes of NS CLP, published data are still conflicting and much work is still needed to clearly define CLP aetiology.

**Abbreviations list**

BHMT, betaine-homocysteine methyltransferase; CLP, cleft lip and palate; CL+/−P, cleft lip with or without cleft palate; CPO, cleft palate only; CPS, chronic pain syndrome; FOLL, folate receptors; IRF, interferon regulatory factor; MTHFR, methylenetetrahydrofolate reductase; NS, non-syndromic; OFC, orofacial clefting; TCN, transcobalamins; TGF, transforming growth factor.

**References**

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