NEW INSIGHTS IN OROFACIAL CLEFT: EPIDEMIOLOGICAL AND GENETIC STUDIES ON ITALIAN SAMPLES

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SUMMARY
Cleft of the lip and/or palate (CL±P) is the most common congenital craniofacial anomaly affecting around 1 in 700 live births worldwide.
Clefts of the human face can be classified anatomically as cleft lip only (CL), cleft palate only (CP), cleft lip and palate (CLP) or a combined group of cleft lip with or without cleft palate (CL±P), based on differences in embryologic development.
CL±P has a genetic base and several linkage and association analyses have been performed in order to obtain important information about the role of candidate genes in its onset; not less important are gene-environment interactions that play an increasing role in its aetiology. In CL±P, several loci have been seen associated with the malformation, and, in some cases, a specific gene mapping in a locus has also been identified as susceptibility factor. In CP, one gene has been found, but many more are probably involved. In this short review the genetic studies carried out on CL±P, and the interaction with environmental factors (alcohol, smoking, drugs) are discussed.

Key words: alcohol, cleft, folic acid, gene, linkage, methylenetetrahydrofolate reductase, smoke.

Introduction

Cleft of the lip and/or palate (CL±P) is a disembriogenetic disease characterized by failure of the nasal process and/or palatal shelves fusion. This birth defect is one of the most common; in fact, the incidence is in the range of 1 in 700 to 1 in 1,000 among populations (1, 2). Genetic factors seem to play a relevant role in the aetiology of this congenital malformation. Many studies provided population-based evidence that CL±P has a strong genetic component (3-8).
Although the CL±P genetic has been investigated for many years, the results are controversial. The discrepancy is probably a result of both the sample and the models used. Indeed, sample studies were sometimes collected including subjects from different geographical areas having different racial and ethnic origins. Conversely, studies based on a limited number of families could be biased. Indeed, CL±P investigations are limited by the small pedigrees usually available, the reduced number of affected individuals in the pedigree (plausibly as a result of the low penetrance), and the genetic heterogeneity exhibited by this malformation (9-13). In CL±P, several loci have been identified, and, in many cases, specific genes have also been found. Thus, no definitive results can be reached without a large sample size (13-18).
Furthermore, environmental factors such as smoke, steroids, or anticonvulsants seem to have an important role in the development of CL±P.

These environmental factors may have a different influence even in homogeneous populations. The genetic studies of CL±P, and the interaction with environmental factors are discussed with different approaches (19-23).

Human genetic factors

CL±P is the most common malformation of born children, with an incidence of 1/700 alive born. Both genetics and environmental factors contribute to its onset, thus CL±P is considered a multifactorial disease. Although the question about the nature of the genetic contribution in CL±P is still under profiling, some chromosome regions have been successfully investigated and some candidate genes have been suggested. The presence of multiple candidate genes makes CL±P a complex disorder (23, 24). Some of those 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 6p23 chromosome region and CL±P. The role of transforming growth factor alpha (TGF-A), mapping on 2p13, was taken into account in the CL±P onset, in light of its contribution in cell proliferation, differentiation and development. Some studies reported an association between TGF alpha and CL±P, but some others did not reply this result (25, 26).

Another positive association was found out between markers on chromosome region 19q13.2 and orofacial cleft malformation (27).

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, on 1p32.2 and CL±P, particularly in Asian and South American populations (26, 28-31).

Moreover, MYH9, a gene encoding for the heavy chain of non-muscle myosin IIA, is also considered a potential candidate in the CL±P onset. In fact, this gene expression is frequent before fusion of palatal shelves and gradually is reduced at the end of this process. Thus, MYH9 might be a predisposing factor for CL±P, although more investigations are needed to better define its pathogenetic role.

The expression profile of another gene, JARID2 induced some Authors to investigate its involvement in CL±P onset in a family-based linkage disequilibrium study. Their results confirmed the role of JARID2 in CL±P malformation, although its functional role is still unknown (12).

TFAP2A is a transcription factor with peculiar characteristics that prompted another research group to verify its involvement in the onset of the CL±P. In fact, the gene that encodes this protein is located in the 6p24 region, widely recognized as CL±P candidate region. Moreover it carries out its function as regulator, by modulating the expression of IRF6, in turn already associated with an increased risk of CL±P (31, 32). In addition, TFAP2A is involved in the branchio-oculofacial syndrome, a congenital disease that includes CL±P. Both single marker and haplotype analysis confirmed the existence of association between TFAP2A and CL±P (12).

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 CL±P. 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 CL±P (6).

Human genetic factors and environment

Alcohol abuse during pregnancy causes a well-known syndrome – the so-called fetal alcohol syndrome – characterized by pre/postnatal growth retardation and facial dysmorphism; but the association between alcohol and L±PC is not

consistent. Some epidemiologic studies have also stated the role of alcohol in developing CP and L±PC. Some studies showed that alcohol increases the risk of L±PC, whereas no significant association was found between alcohol and CP, or in syndromic clefts. Other Authors observed that the risk of delivering infants with CP and L±PC for mothers who take alcohol during pregnancy is dose-related. Other Authors studied the allelic variants of three genes – TGFA, TGFB3, and MSX1 – and their relation with tobacco smoking and alcohol abuse during pregnancy, reporting that the development of CP and CL±P may be influenced by these risk factors, especially when they interact with specific allelic variants. The risk estimated for maternal smoking was significantly elevated in the case of CP, and was higher among infants with allelic variants at the TGFB3 or MSX1 sites. By contrast, the risk estimated for maternal alcohol abuse was significantly more elevated in the case of CL±P, and was higher among infants with allelic variants at the MSX1 site (33).

The role of cigarette smoking has been analysed in epidemiologic studies by several Authors (34, 35), sometimes with conflicting results; although the effects of smoking are universally accepted, the type of CP and CL±P malformation induced is less clear. Some studies investigated whether parental periconceptional cigarette smoking was associated with an increased risk of offspring with CP and CL±P malformation. They also investigated in which way the genetic variation of the TGFA locus could interact with smoking in causing CP and CL±P malformation. The Authors found that risks associated with maternal smoking were most elevated for CP and CL±P malformation when mothers smoked 20 cigarettes or more per day. Clefting risks were even greater for infants with the uncommon TGFA allele (26).

Subsequently, investigators studied the effects of smoking and TGFA alleles in an ethnically homogeneous setting. Unlike previous findings, Authors have shown that smoking was associated with a moderately increased risk of CL±P, but not of CP. TGFA genotype was not associated with either CP or CL±P, and no synergistic effect with smoking was observed. Instead, a case-control study of non-syndromic oral clefts couldn't confirm neither the association between oral clefts and TGFA genotype nor its interaction with maternal smoking (26).

Some Authors (36) 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 CP and CL±P. However, in a further study, the same Author employed large samples. Natality database indicating that smoking is only a minor risk factor. In a large case-control study the Authors found a positive dose-response association between smoking and infants with syndromic CL±P (26).

Supplements and vitamins during pregnancy

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 CP and CL±P malformation. Although some Authors did not find any evidence to confirm a positive association between the use of folic acid supplements during pregnancy and the risk of CP or CL±P (37), further evidence to support the role of folic acid was produced by other investigators (38, 39).

Methylenetetrahydrofolate reductase coded by MTHFR gene is a key enzyme in folic acid metabolism. The C677T mutation on MTHFR sequence produces a form of methylenetetrahydrofolate reductase thermolabile with reduced activity. This reduced enzymatic activity has been related to elevated plasma homocysteine levels and lowered plasma folate. Some Authors highlighted that the homozygosity for the common variant of MTHFR C677T polymorphism is more frequent both in CL±P patients and sporadic CP (40).

Other Authors (39), in their evaluation of

parental allele transmissions (TDT analysis), did not find linkage disequilibrium. However, they reported that the MTHFR polymorphic system was not in equilibrium amongst mothers of CL±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±P onset; they postulated that the CT heterozygotes would have an advantage over the homozygotes in relation to this trait.

Other Authors observed that maternal hyperhomocysteinemia might be a risk factor for having CL±P offspring: this is interesting if we consider that one effect of reduced MTHFR activity is hyperhomocysteinemia.

In one study was demonstrated a significantly higher frequency for 677T variant at MTHFR rs1801133 SNP in the mothers of CL±P patients as compared to controls. The results support the involvement of the folate pathway in the aetiology of CL±P, and sustain the hypothesis of an effect due to the maternal genotype, rather than an influence of the embryo genotype (40). These findings have been borne out by a subsequent independent study (16, 40, 41).

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/parents triads and 289 unrelated controls. The results of these study highlight that polymorphic variants at the MTHFR gene are responsible for a higher risk of having L±PC affected children.

Folate receptors (FOLRs) mediate the delivery of 5-methyltetrahydrofolate to the interior of cells or between cells in a process known as potocytosis (20, 28, 29).

In order to verify whether FOLRs could be involved in the onset of non-syndromic CL±P, a sample study consisted of patients and their mothers from 71 CL±P families and 75 sporadic cases was tested using linkage and linkage disequilibrium analyses. This investigation evidenced only a silent mutation in FOLR1 in a mother and her child that does not support a role for FOLR1 and FOLR2 genes in the onset of L±PC (20, 28, 29).

In addition was verified the involvement in CL±P aetiology of four genes belonging to the folate pathway: 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 collections (20, 28, 29).

Moreover, in an Italian study were evaluated genetic variants of key genes in folate and homocysteine metabolism in influencing the risk of orofacial clefts; no significant level of association between betaine-homocysteine methyltransferase (BHMT and BHMT2) and cystathionine beta-synthase (CBS) variants with CL±P were found (20, 28, 29).

As previously reported, the common MTHFR 677T variant leads to reduced folate availability to mother and consequently to the embryo, which who uses maternal reserves. In fact, this genetic variant is an important factor associated to increased risk of CL±P (40).

Several studies have tested the interaction between methylenetetrahydrofolate reductase (MTHFR) and fetal ABCB1 genotypes (20, 28, 29). ABCB1 gene codes for P-glycoprotein (P-gp), 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 feto-maternal genetic interaction between ABCB1 and MTHFR, but no evidence of association was detected (23, 24, 40).

Lack of association could mean that the sample size was not sufficient to detect a very low effect on CL±P. A sample selection criteria including drugs or medication assumption during pregnancy may help to increase the power of the study, thus identifying the possible feto-maternal interaction between ABCB1 and MTHFR genotypes (23, 24, 40).

Drugs

For some drugs, as for example diazepam and diphenylhydantoin, the teratogenic effect on CP and CL±P development was demonstrated (22, 42), CP and CL±P malformation are related to the morphogenetic processes of extracellular matrix composition, which is important both into cell activities and in gene expression. Diphenylhydantoin may influence cytoskeletal structure and extracellular matrix-cell adhesion related to the expression of genes involved in CP and CL±P malformation (41, 43, 44). Steroids are drugs widely used for the treatment of a variety of conditions in women of childbearing age, and the relation between clefting and steroids in animal models is well known (45, 46). Recently, some studies have investigated the association between women's steroids use during the periconceptional period (1 month before conception-3 months after conception) and the CL±P malformation. Other studies have been shown that other factors, such as the transforming growth factor-β (TGF-β), retinoic acid (RA) and γ-aminobutyric acid (GABA)ergic are potentially involved in the malformation (27, 47-63).

Conclusions

It is well established that CL±P is a congenital disease that may affect the lip with or without the involvement of the palate or just the palate. CL±P may have a genetic aetiology, but environmental factors play an important role in the onset of these malformations also.

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

In CL±P several loci have been identified. In CP, one gene has been identified, but many more are probably involved (64, 65). More studies are needed to have a multidisciplinary idea of primary factors affecting CL±P since it is important not only for non-syndromic (1-7, 9-24, 26-32, 40, 42-44, 47-57, 59, 60, 66-74) but also in syndromic condition (61-63, 75-92). However, despite all the studies to identify the genetic and environmental aetiology of CL±P, published data are controversial and more studies are needed to define CL±P causes.

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