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Genetics of Syndromic and Nonsyndromic Cleft Lip and Palate

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Abstract: Cleft of the lip with or without cleft palate (CL/P) represents one of the commonest congenital malformations in Western countries. Based on their association with specific malformative patterns or their presence as isolated defects, CL/P can be classified as syndromic and nonsyndromic, respectively. Both forms of CL/P are characterized by a strong genetic component. Syndromic forms are in many cases due to chromosomal aberrations or monogenic diseases. Among these, the Van der Woude syndrome, caused by mutation of the *IRF6* gene, represents the commonest form of syndromic CL/P, accounting for about 2% of all cases. On the other hand, nonsyndromic CL/P is a multifactorial disease derived by the interaction between genetic and environmental factors. In recent years, great efforts have been made to identify the genes involved in the susceptibility to nonsyndromic CL/P and to disclose their relationship with specific environmental risk factors, to get information about the pathogenic mechanism leading to the malformation. In this article, we will review the most recent findings about the genes involved in the pathogenesis of syndromic and nonsyndromic CL/P, to provide information about the opportunity in the future to use specific genetic testing for the identification of at-risk mothers and the prevention of the disease based on a personalized approach.

Key Words: Cleft lip, cleft palate, *IRF6* gene, Van der Woude Syndrome, nonsyndromic cleft palate

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Cleft of the lip with or without cleft palate (CL/P [MIM 119530]) is a heterogeneous group of disorders affecting the lips and oral cavity and represents the most common congenital craniofacial defects, with an estimated prevalence ranging from 1 to 7 in 1000 newborns, based on the different ethnic and geographic group.[1] Although treatable, CL/P can produce during the growth deleterious effects on language and hearing abilities, as well as on the appearance, leading to psychologic problems for the patients and their families and eventually to difficulties in social integration. Children affected by CL/P need multidisciplinary care from birth to adulthood and show increased morbidity and mortality during lifetime as compared with the general population.[1] In addition to overt forms of CL/P, showing a variable severity ranging from notches in the vermillion to complete bilateral clefts of the lip and palate,[2] also less evident forms can be observed. These are represented by the so-called microforms, typically involving small defects of the lip, alveolar arch, or scar-like ridges above the lip, and by the even more subtle subepithelial defects of the orbicularis oris muscle.[3] These defects can be considered as part of the spectrum of orofacial cleft expression, as demonstrated by their increased frequency in subjects related to individuals with CL/P.[4,5]

Cleft of the lip with or without cleft palate is typically classified in syndromic and nonsyndromic forms. Syndromic forms of CL/P (5%–7% of cases) consists of more than 200 different conditions and is characterized by the presence of a specific malformation pattern involving the presence of other associated anomalies in addition to CL/P.[1] These syndromes are mostly due to monogenic diseases or chromosomal disorders, although in some cases, the origin of the disease is of environmental nature. On the other hand, nonsyndromic CL/P (93%–95% of cases) consists of isolated, nonspecific malformations and shows a multifactorial etiology due to the interaction of a genetic background of susceptibility with environmental factors. Recurrence risk of nonsyndromic CL/P has been evaluated in 4% to 10%.[6] Although the presence of a genetic component both in syndromic and nonsyndromic CL/P has been clearly demonstrated, only in a limited portion of cases the genes involved have been so far identified, and in many cases, only preliminary data are available about the mechanisms leading to the craniofacial defects in the presence of a specific gene alteration.

In this review, we analyze the genetics of syndromic and nonsyndromic CL/P, with the aim to demonstrate the relevance of the identification of the genetic factors underlying the development of these craniofacial disorders both for the improvement of the knowledge about their biologic basis and for their prevention.

SYNDROMIC CL/P

The commonest form of nonsyndromic CL/P is represented by the Van der Woude syndrome (VWS, MIM 119300), accounting for about 2% of all the cases of CL/P and occurring in about 1:35,000 to 1:100,000 in the white population.[2] This condition is characterized by congenital sinuses of the lower lip associated with

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CL/P and is inherited in autosomal dominant manner, with a penetrance of about 89% to 99% . The expression of the disease is highly variable even within the same family, ranging from the presence of pits in the lower lips to an overt CLP.[7] The gene responsible for VWS was mapped on chromosome 1q by Murray et al,[8] but because of high density of genes in the VWS critical regions, only 12 years later it was possible to demonstrate that mutations in the *interferon regulatory factor 6* (*IRF6*) gene were responsible for this disease.[9] *IRF6* is a member of a family consisting of 9 transcription factors, characterized by the presence of a highly conserved DNA-binding domain and of a protein-binding domain named SMIR (SMad-interferon regulatory factor–binding domain).[9] The majority of the IRF proteins plays a role in the regulation of the α and β interferon expression after viral infection. Expression analysis of IRF6 evidenced that this gene is broadly expressed in embryonic and adult mouse tissues as well as in human fetal and adult tissues, with the greater expression occurring in secondary palates dissected from days 14.5 to 15 mouse embryos and in adult skin.[9] Subsequently, several reports confirmed the association between *IRF6* mutation and VWS,[10–15] allowing to enlarge the spectrum of mutations of this gene and to discover specific genotype-phenotype associations, in some cases characterized by very unusual features of the disease.[16] More than 200 different mutations have been reported in the *IRF6* gene (human mutation database; http://www.hgmd.cf.ac.uk, accessed September 2010). Although the majority of IRF6 changes associated with VWS is represented by point mutations (missense, nonsense, or frameshift mutations) located within the gene coding region, it has been demonstrated that a portion of *IRF6* mutations consists of nucleotide substitutions localized in the exon-intron junctions and affecting the splicing efficiency.[17,18]

IRF6 mutations are also responsible of the popliteal pterygium syndrome (PPS; MIM 119500), characterized by the presence of bilateral popliteal webs, syndactyly, genital anomalies, ankyloblepharon, and nail abnormalities in addition to lower lip alterations and orofacial cleft.[17]

The distribution of mutations in the IRF6 exons is not random both in WVS and in PPS. In WVS, the majority of mutations is located in exons 3, 4, 7, and 9, with exons 3, 4, and 9 being the most frequently involved also in PPS. It has been suggested that mutations leading to VWS are mostly represented by stop mutations, missense mutations within the DNA-binding and SMIR domains, and splicing mutations producing an haploinsufficiency of the *IRF6* gene.[9] On the other hand, mutations leading to PPS are mostly missense mutations involving DNA-binding domain but not affecting the protein-binding activity or mutations leading to the creation of cryptic splicing sites, which would produce a dominant negative effect, thus explaining the major severity of the phenotype.[17,19]

Other examples of monogenic diseases producing syndromic CL/P include autosomal dominant Kallmann syndrome due to mutations in the FGFR1 gene,[20] EEC (ectrodactyly, ectodermal dysplasia, and clefting) syndrome due to mutations of TP63,[21] X-linked clefting and ankyloglossia due to mutations of TBX22,[22] and Gorlin syndrome due to mutations of PTCH1.[23]

TABLE 1. Environmental Risk Factors for Nonsyndromic CL/P

Maternal Exposure During Early Pregnancy to
Smoke
Alcohol
Diet
Viral infections
Drugs
Teratogen agents

TABLE 2. Genes Involved in Susceptibility to Nonsyndromic CL/P

- Growth factors
  - TGFA, TGFb3
- Transcription factors
  - MSX1, IRF6, TBX22
- Genes involved in the metabolism of xenobiotics
  - CYP1A1, GSTM1, NAT2
- Genes involved in the nutritional metabolism
  - MTHFR, RARA
- Genes involved in immune response
  - PVRL1, IRF6

NONSYNDROMIC CL/P

Nonsyndromic CL/P is a multifactorial disease derived from the interaction between genes and environment. The role played by environmental factors is demonstrated by the variable birth prevalence of the disease in different countries.[1] Several studies have focused their attention to the possible environmental risk factors for CL/P, and different possible agents have been identified, such as maternal exposure to smoke, alcohol, diet, viral infections, drugs, and teratogen agents during early pregnancy[24,25] (Table 1). Very recently, it has also been suggested that physical and/or emotional stress may be implicated in clefting.[26] In this view, great attention has been devoted to the identification of the genes related to the increased susceptibility to the above described environmental agents. These studies allowed the identification of several genes whose variants have been reported as associated to an increased risk of CL/P. Among these are growth factors (TGFA, TGFb3),[27,28] transcription factors (MSX1, TBX22),[29,30] genes involved in the metabolism of xenobiotics (*CYP1A1*, *GSTM1*, *NAT2*),[31–34] genes related to the nutritional metabolism (*MTHFR*, *RARA*),[35,36] and genes involved in immune response (*PVLR1*)[37] (Table 2). Interestingly, the strongest association with nonsyndromic CL/P has been demonstrated again for *IRF6* gene.[38–43] In fact, it has been suggested that variations at IRF6 account for 12% of the genetic contribution to CL/P and produce a 3-fold increased risk of recurrence in families who already had 1 affected child.[38] Other studies have demonstrated that the presence of a common polymorphism within an IRF6 enhancer confers an 18% attributable risk for isolated cleft lip.[44] All these findings have suggested the usefulness of sequence analysis of the *IRF6* gene also in nonsyndromic CL/P, because the detection of a point mutation or of a specific haplotype could increase familial risk of recurrence from the currently used empirical value of 3% to 5% to much higher values.[38] As a consequence, a point of discussion is represented by the selection criteria of patient with nonsyndromic CL/P who should undergo molecular analysis of the *IRF6* gene, also considering that about 14% of VWS cases do not show the typical lower lip pits and must be incorrectly classified as nonsyndromic CL/P, with a wrong recurrence risk calculation for the family.[45] Based on these findings, it has been suggested that families with apparent nonsyndromic CL/P eligible for the IRF6 screening are those with at least 1 affected parent-offspring pair and particularly those segregating CP and CL/P.[45]

Anyway, as previously described, IRF6 is not the only gene related to the increased risk of nonsyndromic CL/P. In addition to

association studies, also investigations based on the candidate gene approach (ie, the study of genes selected on the basis of their expression pattern and of the results shown in animal models) have provided good evidence for the presence of a poligenic model. In particular, it has been calculated that point mutations in 6 candidate genes (FOXE1, GLI2, MSX2, SKI, SATB2, and SPRY2) may account for about 5% of isolated CL/P, more likely those with more severe phenotypes and/or a positive family history.[46]

The most interesting issue in the study of susceptibility genes for CL/P is represented by the attempt to correlate specific gene variants with specific environmental risk factor. In this view, it has been suggested that some genetic variants could represent a risk background for the exposition to some, but not all, environmental agents, such as in the cases of TGFA (smoke, vitamin deficit),[47–50] TGFb3 (smoke, alcohol),[51–53] MSX1 (smoke, alcohol),[52–54] ADH1C (alcohol),[55] EPHX1, GSTM1, GSTT1, NAT1, NAT2, CYP1A1 (smoke, drug abuse, professional exposures),[52–54] RARA (vitamin A intake),[56] MTHFR, and RFC1 (folate assumption) (Table 3).[52,57–61] It must be stressed that in many cases these genetic variants of susceptibility are detected not in the affected child, but in their mothers. This could represent an important point in the prevention of nonsyndromic CL/P. In fact, based on the genetic variant detected in a pregnant woman, it could be possible to identify the specific environmental agent of risk, avoiding the exposure of that woman to that specific agent. It has been stressed that identification of modifiable risk factors for oral clefts is the first step toward primary prevention,[1] and in this view, it is of interest to note that it has been recently suggested that the risk induced by *IRF6* gene variants could be reduced by maternal multivitamin supplementation.[43]

Another interesting point is represented by the search of genes related to microforms and subepithelial forms of CL/P. In fact, it has been demonstrated that missense and nonsense mutations of the BMP4 gene are present with a significantly higher frequency in patients with microforms of CL/P and orbicularis oris muscle defects and compared with controls in agreement with data on animal models showing that mice with a conditional knockout for Bmp4 (MIM 112262) had an unusual "healed" cleft-lip phenotype.[3,62]

CONCLUSIONS

In the last years, a huge improvement in our knowledge about the genetic basis of CL/P has been achieved, both for syndromic and nonsyndromic forms, and studies aimed to the identification of novel genes involved in the pathogenesis of this disease are in progress.[63] In the future, these knowledge could represent the basis for setting up novel strategies for the prevention of these malformations. In particular, the identification of at-risk genotypes in women before pregnancy could help to identify the environmental factors potentially dangerous for the development of CL/P in the child and to avoid the exposure to such agents. Moreover, the vitamin supplementation in women carriers of at-risk alleles of specific genes such as IRF6 and MTHFR could represent a crucial point in the prevention of these diseases. Because the efficiency of this treatment is likely limited to the very early period of pregnancy, genetic counseling and testing should be carried out in a preconceptional period rather than during pregnancy. The identification of other genes related to the pathogenesis of CL/P will allow to establish a panel of genetic variants to be analyzed using high-throughput techniques able to simultaneously investigate the presence of several risk alleles and to organize a personalized prevention strategy for women carriers of such variants.

TABLE 3. Possible Gene-Environment Interactions in the Pathogenesis of CL/P

- *TGFA*
  - Smoke, vitamin deficiency
- *TGFB3*
  - Smoke, alcohol
- *MSX1*
  - Smoke, alcohol
- *ADH1C*
  - Alcohol
- *EPHX1*, *GSTM1*, *GSTT1*, *NAT1*, *NAT2*, *CYP1A1*
  - Smoke, drug abuse, occupational exposure
- *RARA*
  - Vitamin A intake
- *MTHFR, RFC1*
  - Folic acid intake

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