The genetic basis of cleft lip and cleft palate

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ABSTRACT: Cleft lip and cleft palate (CLP) together or as isolated incidents constitute a large proportion of orofacial deformities which are observed during birth. It is a medical condition widely researched in the scientific community primarily because of health effects but also social effects on the everyday life of the affected children. There is wide speculation and much scientific data about the genes which are implicated on its genesis. CLP can be a clinical feature in many syndromes, but they can also be observed as isolated incidents in cases considered as non syndromic. Especially in non syndromic forms genetic heterogeneity and the differences among studied populations provide us with sometimes conflicting results about the implicated genes. It is also widely accepted that CLP is a multifactorial medical condition and in its pathogenesis, environmental factors also play a crucial role.

Key Words: cleft, lip, palate, genetics

INTRODUCTION

Cleft lip and cleft palate are deformities of the face that are immediately recognized at someone's birth. Although they do not consist a fatal health issue in developed countries, cleft lip and palate (CLP) increase morbidity of affected children and impose a social stigma on the patients and their families. People with CLP experience problems with feeding, and communication such as with speaking, hearing and social integration[1]. Hearing issues occur more often in children with cleft palate. The eustachian tubes cannot function effectively and the children have conductive hearing loss, because of middle ear effusion. In that case, when the air in the middle ear is absorbed, negative pressure forms leading to secretion of fluid into the middle ear. This issues can be addressed either primarily by surgical correction of the deformity or by hearing devices later[2]. Other subsequent issues that arise can also be corrected to a large degree by surgery, dental rehabilitation, speech therapy and psychosocial support[1].

The etiology of CLP is genetically very heterogeneous and this makes it difficult to understand what biological factors are implicated in its genesis[1]. Moreover, we need to further investigate how these factors interact with the environment. We know that several environmental factors play a crucial role and increase the prevalence of CLP by interacting with genetic factors. These include dietary habits, smoking, alcohol consumption, drug consumption etc[1,4,5]. As far as the genetic basis is concerned, there are many syndromes which contain cleft lip and/or palate as a component of their phenotype, including Walker-Warburg syndrome, van der Woude syndrome, Kallmann syndrome, Crouzon syndrome, DiGeorge syndrome, Muenke syndrome, Pierre Robin Sequence and others. This is only one side of the story though, since there are many cases of CLP which cannot be attributed to a specific syndrome and occur because of single mutations in the human genome. Many genes are implicated, such as IRF6, VAX1, ABCA4, FGFR2, PDGFC, PVRL1, SUMO1, TGFA, TGFB3, MSX1

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and many more[27]. Recent breakthroughs in genome-wide linkage and association studies lead to the discovering of new genetic loci which are associated with CLP. Researchers currently strive to identify and understand the developmental disturbances that can under circumstances lead to CLP. This knowledge shall eventually enable us to improve prevention, treatment and prognosis for individuals with cleft lip and palate and lead to new approaches regarding clinical care[1]. Last but not least, it is of great importance to integrate genetic counseling in the process of treatment. This will answer questions of parents as far as the reason of the appearance of CLP in their family is concerned. It will also address the concerns about how to raise their children correctly without medical and/or social implications, the medical resolution of their situation and the future possibilities for recurrence in their family[3].

PREVALENCE

In a population based study of almost 8 million births, the prevalence of CLP was 9.9 every 10,000 births worldwide. These data were collected from birth defect registries from 30 countries between the years 2000 and 2005[3]. This study was consistent with a study conducted in the USA, according to which the prevalence of CLP was 10.2 per 10,000 births in the United States. The prevalence of CLP in Western Europe (12.1 per 10,000 births) was similar to the prevalence in the United States, but the prevalence in Japan (20.0 per 10,000 births) was twice that of the United States. 31% of the cases of CLP in the United States were CL without CP. Moreover, the same study showed that 75% of children with CLP during this period were non-syndromic (no other deformity or only a minor defect), 8% of cases were associated with a known syndrome and 17% of cases had several malformations[4]. These observations were similar also in Canada, Western Europe, Japan and Australia[3].

SEX, AGE AND RACE

The prevalence of CLP is different depending on sex, race, and maternal age. The prevalence of CLP among males is almost twice as large as that of females. The prevalence of CLP is lower in non-Hispanic blacks when compared with non-Hispanic white people. African Americans have a 44% lower likelihood of having CLP compared with non-Hispanic whites. Maternal age younger than 25 years and older than 29 years is associated with an increased risk of CLP com-pared with mothers aged 25 to 29 years. In ages younger than 25 or older than 29 no increased risk is observed compared to the general population[3].

ENVIRONMENTAL INFLUENCES

There are many environmentally-derived factors that are considered responsible for CLP. Maternal smoking during pregnancy has been associated with increased risk of CLP and meta-analysis data strongly support an odds ratio (OR) of 1.3 for having CLP among children of mothers who smoke during gestation[1]. In a study conducted in California among women, 70% of whom were smoking during the 15th to 18th week of gestation, it was observed that smoking exposures, as defined by measured cotinine (a nicotine derivative) levels, were associated with increased risk of CLP. The study also suggests that the effect may be more severe in causing bilateral clefts[5]. It is believed that genes in certain metabolic pathways may play a role in the development of CLP, especially during the first months of intrauterine development of the fetus. Specifically, *GSTT1* (glutathione S-transferase theta) or *NOS3* (nitric oxide synthetase 3) genes appear to increase the risk of CLP development when combined with maternal smoking. The *GSTT1* markers are gene deletion variants, a fact which suggests that deficiencies in intracellular detoxification pathways may increase susceptibility to CLP. Smoking has also been associated with a joint risk with markers in the *IRF6* gene. These findings underline the importance of the synergy between environmental and genetic factors[1]. Furthermore, there are other mechanisms, such as carbon monoxide hypoxia, nicotine teratogenic, cadmium or several amines and alterations to folate metabolism are considered to be a part of the environmental etiology of CLP[5].

Alcohol consumption during pregnancy has also been suggested as a risk factor. It is suggested that 'binge' drinking patterns (i.e. drinking large amounts of alcohol over a short period of time which leads to acute intoxication) increase risk, and it is believed that variations of the *ADH1C* alcohol dehydrogenase gene are implicated. These links to alcohol consumption need further confirmation[1].

Maternal nutritional habits also seem to play an important role in the development of CLP in their children. A link has been demonstrated by many studies between intake of multivitamins or folic acid during pregnancy and a reduced risk for CLP[3]. Several

medications widely prescribed have been linked to CLP when consumed during the first trimester. Folate antagonists and certain other drugs that exhibit antifolate actions are associated with an increased risk for CLP. These drugs include anticonvulsants, such as carbamazepine, valproic acid, phenytoin and phenobarbital among others. Retinoic acid and corticosteroids are also considered to be associated with increased CLP risk[3].

Some maternal and obstetric diseases are linked to CLP in the babies of affected women. Diabetes mellitus, but not gestational diabetes increases the risk for several congenital deformities, including CLP. Maternal obesity (body mass index> 30) is also associated with an increased risk for CLP, but the risk is relatively small, with an odds ratio around 1.3[2].

Last but not least, CLPs may be caused by mechanical conditions, such as amniotic bands that deform and disrupt the fetal facial structures[2].

SYNDROMES WITH CLEFT LIP AND/OR CLEFT PALATE

Walker-Warburg syndrome (WWS)

Walker–Warburg syndrome (WWS) is a congenital muscle dystrophy syndrome caused by a-dystroglycan deficiency. It is associated with brain and eye abnormalities. Other abnormalities include cleft lip and palate (CLP). Mutations in several genes, such as POMT1, POMT2, fukutin, FKRP and LARGE are found in 20–30% of children with WWS and are associated with its deformities[6]. In a study conducted on two brothers with WWS and CLP (both were born from healthy parents), sequence analysis revealed a new mutation previously not known-. The children's phenotype included hypotonia, hydrocephalus, cataracts, hydronephrosis and CLP. Sequence analysis of the proband identified mutations in exons 5 and 20 of the POMT1 gene. One mutation, c.291delC, was not previously reported and is believed to cause a frameshift resulting in premature stop codon, 124 nucleotides downstream of the deletion. The second mutation, c.2167insG, which has been reported in other studies previously, causes a frameshift that is predicted to remove the 25 aminoacids following Gly722. These two mutations in exons 5 and 20 were inherited from the mother and the father, respectively[6].

Van der Woude syndrome (VWS)

This is an autosomal dominant cleft syndrome which includes bilateral midline lower lip pits, cleft lip, and cleft palate together with hypodontia. VWS is a rare congenital syndrome. The lower lip pits which are described in patients occur on paramedian portion of the vermillion border of the lip. Congenital lip pits occur along with cleft lip and/or cleft palate and represent the most common clinical manifestation which is described in 80% of the patients[7]. There are other clinical features of VWS which may or may not co-exist, such as hypoplasia, ankyloglossia, high arched palate, limb anomalies and congenital heart defects among others. Most cases of VWS have been associated with deletion of chromosome 1q32–q41, but an extra chromosomal locus at 1p34 chromosome has also been identified[8]. Many mutations have been identified in the gene which encodes interferon regulatory factor 6 as well. Approximately 30–50% of all cases though happen because of de novo mutations. The expression of the syndrome is highly variable; all of the deformities can be present, either alone or in combination, or even no abnormalities can be observed clinically in some cases[9].

Kallmann syndrome

Kallmann syndrome's estimated prevalence is 1/10,000 in males and at 1/50,000 – 1/70,000 in females[10]. Sporadic cases are more frequent than familial. Kallmann syndrome is genetically heterogeneous and is expressed by three modes of inheritance: X-linked due to a mutation in the KAL1 gene (Xp22.3), autosomal dominant due to mutations in fibroblast growth factor receptor 1 (FGFR1), in KAL2 genes and (8p12) locus and autosomal recessive which is less usual and for which no mutation has been identified till now. KAL1 and KAL2 mutations are included in only 20% of Kallmann syndrome cases. In Kallmann syndrome due to KAL1 mutation, a specific phenotype (almost always hypogonadism and various degrees of anosmia) is often seen with many associated deformities, such as unilateral renal agenesis and bimanual synkinesis. On the contrary, in Kallmann syndrome due to FGFR1 and KAL2 mutations, the phenotype is highly variable with several degrees of hypogonadism and/or anosmia. Many malformations have been described in patients with FGFR1 mutation, such as CLP, teeth agenesis, bimanual synkinesis, corpus callosum agenesis, hearing loss, fusion of the fourth and fifth metacarpal bones (or syndactylia), unilateral nasal cartilage agenesis and iris coloboma[11].

Crouzon syndrome

Crouzon Syndrome appears with an incidence of 12.5 per million births and is associated with gain-of-function mutations in FGRF2 gene[12]. Craniofacial manifestations are its most obvious feature and include craniosynostosis, hypoplasia of the middle face, oral anomalies such as an increased incidence of cleft palate and a constricted dental arch. More than 30 different FGFR2 mutations may lead to Crouzon Syndrome, most of which refer to the FGFR2c isoform and are mesenchymally expressed. All patients with Crouzon Syndrome are heterozygous for the mutation, since homozygous mutations are considered to be lethal[13].

In a xenograft study by Alison K. et al cleft palate occurred in nearly all mice that were homozygous for the C342Y mutation in the mesenchymal splice form of FGFR2, which is associated with CS. Mutant embryos presented delayed palate elevation, changes in palate mesenchymal proliferation, and lower levels of mesenchymal glycosaminoglycans (GAGs). Lower levels of feedback regulators of FGF signaling were also observed and they suggest that this gain-of-function mutation in FGFR2 is similar to the loss of FGF function in palate tissue[13].

DiGeorge syndrome

DiGeorge syndrome, also called 22q11.2 deletion syndrome (22q11DS) is the most commonly observed microdeletion syndrome[14]. Its incidence is estimated at 1/2,000–1/4,000 live births. Approximately 9–11% of patients with this disorder have cleft palate (CP), but the genetic factors responsible for CP in the 22q11DS are yet to be found[15]. The TBX1 gene, a member of the T-box transcription factor gene family, is located within the 22q11.2 which is associated with DiGeorge syndrome. In experiments conducted in mice, inactivation of one allele of Tbx1 does not lead to CP, but inactivation of both alleles does[16]. Common DNA variants in TBX1 may also cause CP in patients with 22q11DS. Genes elsewhere on the remaining allele of 22q11.2 or in the genome could be relevant. Although many candidate genes have been reported from time to time, it is hard and complicated to establish certain correlations between genetic mutations and the cleft phenotype, because of genetic and allelic heterogeneity, incomplete penetration, and variable phenotypes[14].

Margarita island syndrome

Margarita Island syndrome represents a syndromic form of cleft lip/palate with ectodermal dysplasia. Its phenotype includes cleft lip/palate, ectodermal dysplasia (CLPED), and abnormal fingernails[17]. Although that syndrome is rare in general population, CLPED occurs with a high frequency of nearly 1/2000 among the inhabitants of Margarita Island, an island located in the Caribbean Sea of the coast of Venezuela[18]. There is a mutation associated with it, which is found in exon 3 (W185) of poliovirus receptor-like 1 (PVRL) gene in people from Margarita Island. PVRL1 encoding nectin-1 is an immunoglobulin – related transmembrane cell-to-cell adhesion molecule which constitutes a part of the cell adhesion system[17].

Muenke syndrome

Individuals with Muenke syndrome have the lowest incidence of cleft palate among craniosynostosis syndromes. Muenke syndrome is also the most common, with an incidence of 1 in 30,000 births. It is an autosomal dominant syndrome caused by a gain-of-function point mutation in the FGFR3 gene, c.749 C9G, which results in p.P250R (proline to arginine substitution at amino acid 250). Patients with Muenke syndrome clinically have craniosynostosis, carpal and/or tarsal bone fusion delay in development, and sensorineural hearing loss. It includes several craniofacial anomalies such as mild hypoplasia of the middle face, high-arched palate, and hypertelorism[19]. Muenke syndrome accounts for 24% of cases of craniosynostosis with a known genetic cause. Still the frequency of oral and palatal anomalies including cleft lip with or without cleft palate has not been documented till now[20]. Furthermore, cleft lip with cleft palate has not yet been reported in a patient with Muenke syndrome[19].

Pfeiffer syndrome

The frequency of cleft palate is known to be high in some craniosynostosis syndromes which are linked with fibroblast growth factor receptor 2 (FGFR2), such as Apert syndrome. However, little do we know about the frequency of CP in the also FGFR2-linked deformity called Pfeiffer syndrome. This is an autosomal dominant craniosynostosis syndrome, caused by activating mutations of the FGF receptor 1 (FGFR1) or FGF receptor 2 (FGFR2) genes[21]. Apart from CP which can be present, its clinical features consist of craniosynostosis, hypoplasia of the middle face, large, medially deviated thumbs and big toes, syndactyly, radiohumeral synostosis of elbow, and increased airway obstruction risk due to tracheal stenosis[22].

Pierre Robin Sequence (PRS)

The Pierre Robin Sequence (PRS) is a clinical entity which includes Cleft Palate. Its etiology is generally unknown but it is widely believed to have a genetic basis. Clinically PRS is characterized by cleft palate and micrognathia which leads to glossoptosis, which means that the tongue tends to obstruct the airway and therefore causes feeding and respiratory problems during the early years after birth[23]. PRS is observed with a higher frequency in siblings than in general population (9% versus 1%). This could lead to the assumption that PRS is a result of the twinning process. Moreover, the hypothesis that the causative factor is a mechanical intrauterine constraint also exists. PRS has the same prevalence in both sexes[24].

There are factors that connect PRS syndrome with a genetic background. Patients with PRS quite often have also other family members which present cleft lip or palate in their medical history (13.0%–27.7%) and PRS is also present in other clinical entities, such as Stickler syndrome, DiGeorge syndrome, Marshall syndrome, Treacher Collins syndrome, Catel-Mancke syndrome, teratogene syndromes and others[23]. The most common syndrome which co-exists with PRS is Stickler syndrome[25]. In a molecular level, deletions, duplications translocations and mutations affecting chromosomes 1 to 6, 10 to 13, and 16 to 18 are involved. Various loci in chromosome 2 (2q24.1-33.3), chromosome 4 (4q32-qter), chromosome 11 (11q21-q23.1), and chromosome 17 (17q21- q24.3) are also involved. There is a large number of possible candidate genes which are implicated in PRS pathogenesis, such as GAD67, IDH1, ITGAV, DLX2, PDGFC, PVRL1 and SOX9, too. It has been suggested that GAD67 which is based on chromosome 2q31 is involved in the etiology of CLP in the Japanese population. Also there is evidence that the SATB2 gene on 2q32-q33 is responsible for CP. This gene is involved in transcriptional control and is expressed in the palate during the stages of its development[23]. Moreover it has been found that a heterozygote mutation in the PVRL1 gene Margarita Island syndrome). Furthermore, the transcription factor SOX9 might be an interesting gene. On chromosome 11q23-q24 is a moderate genetic risk factor for CLP in Venezuela[26]. Observations till now show that mutations in the SOX9 gene and approximately 1 Mb upstream the SOX9 gene, cause campomelic dysplasia[23]. There are many syndromes which contain CLP as a clinical manifestation, as presented in the following table (Table 1).

Table 1. Syndromes with CLP as a clinical manifestation[1].

Cleft Type	Syndrome	Gene	Reference
Cleft lip +/− cleft palate	Autosomal dominant developmental malformations, deafness, and dystonia	*ACTB*	*1*
	Familial gastric cancer and CLP	*CDH1*	*2*
	Craniofrontonasal	*EFNB1*	*3*
	Roberts	*ESCO2*	*4*
	Holoprosencephaly	*GLI2*	*5*
	"Oro-facial-digital"	*GLI3*	*6*
	Hydrolethalus	*HYLS1*	*7*
	Van der Woude/popliteal pterygium	*IRF6*	*8*
	X-linked mental retardation and CL/P	*PHF8*	*9*
	Gorlin	*PTCH1*	*10,11*
	CLP – ectodermal dysplasia	*PVRL1*	*12*
	Holoprosencephaly	*SHH*	*13*
	Holoprosencephaly	*SIX3*	*14*
	Branchio-oculo-facial	*TFAP2A*	*15*
	Holoprosencephaly	*TGIF*	*16*
	Ectrodactyly-ectodermal dysplasia-clefting	*TP73L*	*17*
	Ankyloblepharon-ectodermal dysplasia-clefting	*TP73L*	*18*
	Tetra-amelia with CLP	*WNT3*	*19*

Cleft palate only	Oculofaciocardiodental	*BCOR*	*20*
	CHARGE	*CHD7*	*21*
	Lethal and Escobar multiple pterygium	*CHRNG*	*22*
	Stickler type 1	*COL2A1*	*23*
	Stickler type 2	*COL11A1*	*23*
	Stickler type 3	*COL11A2*	*23*
	Desmosterolosis	*DHCR24*	*24*
	Smith-Lemli-Opitz	*DHCR7*	*25*
	Miller	*DHODH*	*26*
	Craniofrontonasal	*EFNB1*	*3*
	Kallmann	*FGFR1*	*27*
	Crouzon	*FGFR2*	*28*
	Apert	*FGFR2*	*29*
	Otopalatodigital types 1 and 2	*FLNA*	*30*
	Larsen syndrome; atelosteogenesis	*FLNB*	*31*
	Hereditary lymphedema-distichiasis	*FOXC2*	*32*
	Bamforth-Lazarus	*FOXE1*	*33*
	"Oro-facial-digital"	*GLI3*	*6*
Cleft Type	**Syndrome**	**Gene**	**Reference**
	Van der Woude/popliteal pterygium	*IRF6*	*8*
	Andersen	*KCNJ2*	*34*
	Kabuki	*MLL2*	*35*
	Cornelia de Lange	*NIPBL*	*36,37*
	X-linked mental retardation	*PQBP1*	*38*
	Isolated cleft palate	*SATB2*	*39*
	Diastrophic dysplasia	*SLC26A2*	*40*
	Campomelic dysplasia	*SOX9*	*41,42*
	Pierre Robin	*SOX9*	*43*
	DiGeorge	*TBX1*	*44*
	X-linked cleft palate and ankyloglossia	*TBX22*	*45*
	Treacher Collins	*TCOF1*	*46*
	Loeys-Dietz	*TGFBR1*	*47*
	Loeys-Dietz	*TGFBR2*	*47*
	Saethre-Chotzen	*TWIST1*	*48,49*
Midline cleft lip	Opitz G/BBB	*MID1*	*50*
	Oro-facial-digital type I	*OFD1*	*51*

Non-syndromic Cleft Lip and Cleft Palate (NSCLP)

Several genome-wide linkage studies and meta-analyses have proposed that NSCLP might be connected with many genetic regions, each one of which has a different relative risk in different populations. These candidate loci include IRF6 (1q32.3-q41), VAX1 (10q26.1), ABCA4 (1p22.1-21), BMP4 (14q22q23), FGFR2 (10q26), FOXE1(9q22), MAFB (20q11,2-q13.1), MSX1 (4p16.3-p16.1), MYH9 (22q13.1), CRISPLD2 (16q24.1), FGF8 (0q24),GSTT1 (22q11.23), MTHFR (1p36.3), PDGFC (4q32), PVRL1 (11q23.3), SUMO1 (2q33), TGFA (2p13), TGFB3(14q24) and others[27].

In a study conducted in the Taiwanese population 22 types of gene variants within 10 studied genes in individuals with non-syndromic CLP were observed. Single nucleotide variations were found (ABCA4, MYH9, MTHFR, CRISPLD2, FGF8, PVRL1, FOXE1 and FGFR2), deletions were estimated (CRISPLD2 and IRF6 gene) and one duplication was identified (VAX1). The most frequent risk loci in the Taiwanese population have been found to be the MYH9 and the ABCA4[28].

MYH9, or myosin heavy chain 9, is associated with non-syndromic CLP in several populations[29]. High expression of MYH9 was observed in the palatal shelves prior to fusion during the developmental process. MYH9 is considered to be the most frequent risk loci in the Taiwanese population, providing scientists with further evidence that MYH9 is involved in the etiology of nonsyndromic CLP. Also studies revealed markers in and near the ABCA4 gene, which indicates a susceptibility locus for CLP, especially in the Honduran and Colombian populations. In the Brazilian population, ABCA4 rs540426 is associated strongly with CLP, unilateral or bilateral, while the SNP rs481931 exhibited borderline involvement in the pathogenesis of CLP. However, in a Chinese population, ABCA4 was not found to be associated with CLP[28]. As far as European populations are concerned Birnbaum et al described the localization of a major susceptibility locus for NSCLP on 8q24.21. The region of strongest association is a B 1.7-Mb gene which does not encode any known protein[30]. In a study conducted by Scapoli et al in the Italian population a strong correlation between loci of IRF6 gene and NSCLP was established. Individuals with rs2013162 and rs2235375 markers were found to have higher prevalence of NSCLP compared with those who did not have these two markers[31]. There

are many other genes, which are believed to be associated with non-syndromic CLP, as the following table suggests (Table 2). Subsequently we present some of the most important genes and loci which are considered to be etiologic factors of CLP.

10q25

Non-syndromic cleft lip with or without cleft palate (NSCLP) is a common human defect. A polymorphism, rs7078160 in 10q25 loci, has been reported to be involved in the etiology of the condition but results are highly variable and conflict with each other. Results show that rs7078160 on 10q25 loci, the minor allele A, had a higher risk of NSCLP than the major allele G. Overall, the results showed that the 10q25rs7078160 polymorphism was involved in the pathogenesis of N-SCLP. The magnitude of this involvement between 10q25 rs7078160 polymorphism and risk of NSCLP varied among white, Asian, and mixed populations, a fact suggesting that ethnic heterogeneity and environmental factors can have different influences in 10q25 rs7078160 loci when it comes to NSCLP[32].

Bcl3

BCL3 is believed to play a role in the etiology of N-SCLP as an allele or a modifier locus. BCL 3 (B-cell leukemia/lymphoma-3) is a proto-oncogene located at 19q13 that encodes a transcription factor involved in cell cycle regulation. An association of the BCL3 locus or a nearby locus with NSCLP has been presented by several studies. More specifically there is association between the 19q13.2 region which contains the BCL3 gene and this deformity. Moreover an excess transmission of the 3 allele (135 bp) at the BCL 3 gene was observed[33].

OFC1

Different groups have investigated the localization of CLP gene on chromosome 6 (named OCF1) with conflicting results though. It is noticed that NSCLP has been associated with chromosomal aberrations involving the short arm of chromosome 6 (6p). The involved loci were included within the 6p24.3 region near the HGP22 and AP2 genes. The HLA locus, located in 6p21.3, has offered both negative and positive results. A linkage to the 6p24 at F13A locus has also been found. However, no linkage between NSCLP and this 6p region was found in other groups[34]. In an investigation on 21 nonsyndromic CLP Italian families, linkage to the 6p23 region was observed[35]. There is

Table 2. Genes implicated in non-syndromic CLP 1.

Class/Gene	Evidence°	Refs
Confirmed*		
IRF6	GWA, LD, L, M	Zucchero et al., 2004 (41); Rahimov et al., 2008 (12); Birnbaum et al., 2009 (3)
8q24 locus	GWA, LD	Birnbaum et al. 2009 (3); Grant *et al.*, 2009 (4); Beaty et al. 2010 (6)
VAX1	GWA, LD	Mangold et al., 2010 (5); Beaty et al., 2010 (6)
Likely**		
MSX1	LD, M	Lidral et al., 1998 (); Van den Boogaard et al. 2000 (17); Jezewski et al., 2003 (51); Vieira et al., 2004 (); Suzuki et al., 2004 ()
FOXE1	L, LD, M	Vieira et al., 2005 (); Moreno et al., 2009 (50); Venza et al., 2006 ()
MYH9	LD	Martinelli et al., 2007; Chiquet et al., 2009; Birnbaum et al., 2009 (3); Jia et al., 2010
MAFB	GWA	Beaty et al. 2010 (6)
ABCA4 (locus only)	GWA	Beaty et al. 2010 (6)
17q22 locus	GWA	Mangold et al., 2010 (5); Beaty et al. 2010 (6)
BMP4	M	Suzuki et al., 2009 (36); Jianyan et al, 2010
FGFR2	M	Riley et al., 2007; Riley and Murray, 2007 (52); Osoegawa et al, 2008 (45)
Intensively Studied***		
TGFA	LD	Ardinger et al., 1989 (40); Vieira, 2006; Carter et al, 2010
TGFB3	LD, M	Lidral et al., 1998; Beaty et al., 2002; Vieira et al., 2003; Suazo et al, 2010
MTHFR	LD	Mills et al., 2008; Jagomagi et al, 2010
GSTT1	LD	Shi et al., 2007 (81)
PDGFC	LD, M	Ding et al., 2004; Choi et al., 2009; Jugessur *et al.*, 2009 (24)
BMP4	M	Suzuki et al., 2009 (36); Jianyan et al, 2010
FGFR2	M	Riley et al., 2007; Riley and Murray, 2007 (52); Osoegawa et al, 2008 (45)
Intensively Studied***		
TGFA	LD	Ardinger et al., 1989 (40); Vieira, 2006; Carter et al, 2010
TGFB3	LD, M	Lidral et al., 1998; Beaty et al., 2002; Vieira et al., 2003; Suazo et al, 2010
MTHFR	LD	Mills et al., 2008; Jagomagi et al, 2010
GSTT1	LD	Shi et al., 2007 (81)
PDGFC	LD, M	Ding et al., 2004; Choi et al., 2009; Jugessur *et al.*, 2009 (24)
FGF8	M	Riley et al, 2007; Riley and Murray, 2007
PVRL1	M, LD	Sozen et al., 2001; Avila et al., 2006; Sozen et al., 2009.
SUMO1	M	Alkurayra et al., 2005; Shi et al., 2009 (47); Mostowska et al., 2010; Carter et al, 2010
CRISPLD2	LD	Chiquet et al, 2007; Letra et al, 2010

*At least two independent studies reaching conservative levels of significance

**At least one study with conservation/compelling data and other supportive studies.

***Multiple studies, no consensus or convincing meta-analysis

°GWA= Genome-wide association, LD=Candidate Gene Association, L = Linkage, M = Mutation Detection

also evidence in xenograft models that endothelin-1 (EDN1) could be involved in NSCLP[36].

IRF6

Interferon regulatory factor-6 (IRF6) is a member of a family of nine transcription factors which regulate the expression of IFNa and IFNb after viral infection. Mutation in the IRF6 gene can cause van der Woude syndrome (VWS) which is associated with CLP. Some VWS cases, especially those who lack lip pits, are not easily distinguished from nonsyndromic CLP, raising the question of whether allelic variety in the VWS locus could imply nonsyndromic CLP[34]. A significant association with the V allele at a val274-to-ile polymorphism in the IRF6 gene was found[37].

TGFB3 (locus 14q24)

An etiologic role for TGF- b3 was proposed because linkage disequilibrium was found in several studies. Vieira et al. proved an association between cleft palate alone and TGF- b3, and subsequently a joint analysis of MSX1 and TGF- b3 together suggested that these two loci might interact and increase susceptibility to NSCLP. The distribution of the SfaN1 polymorphism of TGFb3 is strongly associated with an increased risk of NSCLP in males, but not in females[34].

RARA (locus 17q21.1)

There is a significant existing difference between nonsyndromic CLP cases and unrelated controls in the frequency of alleles at the retinoic acid receptor alpha (RARa), located at 17q21.1. Genes in the region of RARa, or its genetic variations are involved in the formation of CLP. RARa, or a nearby locus, is primarily considered as a modifier of the NSCLP severity[34]. On the contrary, other study groups did not find linkage between RARa and non syndromic clefts. Peanchitlertkajorn et al. investigated RARa, in Chinese families and found that genetic variety within the RARa locus is involved in the etiology of non syndromic clefts[38].

Cleft palate X-linked (TBX22 gene)

The implicated gene is located in Xq21.3, and the flanking markers are DXS1196 and DXS1217. It has been proven that cleft palate chromosome X-linked is caused by mutations in the gene encoding the T-box transcription factor TBX22. Members of this family play essential roles in early vertebrate development. An analysis of the TBX22 gene in patients with cleft palate showed that mutations within families could result in either cleft palate alone or ankyloglossia alone, or both[34].

OFC5 (MSX1 gene)

It is believed that there is interaction between environment factors and MSX1. Indeed, the risk of CLP related to maternal smoking and alcohol consumption during pregnancy is increased because of the interaction between such exposure and specific allelic variants of MSX1[34]. A missense mutation in the MSX1 gene in Vietnamese and Filipino CLP patients has also been found[39].

MTHFR (locus 1q36)

Methylenetetrahydrofolate reductase (MTHFR) is located on 1q36 locus and is an important enzyme of folic acid metabolism. The C677T mutation of MTHFR encodes a thermolabile enzyme with reduced activity. This characteristic leads to elevated plasma homocysteine levels and lower plasma folate, because of reduced MTHFR activity. Fetal homozygosity in C677T is found to be three times more frequent in patients with NSCLP than found in controls. Homozygosity for the common folate-related polymorphism associated with the thermolabile form of MTHFR is more frequently observed in patients with non syndromic CLP. Homozygosity for either the T or C allele of C677T polymorphism in females is an important susceptibility factor for the development the deformity[34].

NOG polymorphisms

In a study conducted in the Chinese population it is suggested that NOG rs227731 polymorphism constitutes decreased risk to NSCLP in a Northern Chinese population. The NOG rs227731 CC genotype was found to be uncommon among NSCLP cases[40]. NOG's localization is found on 17q22 and is generally considered a candidate gene for NSCLP, because inactivation of NOG leads to development of the cleft palate in xenograft rat models[41].In addition, NOG may cause NSCLP through the BMP signals, which play an important role in formation of the upper lip or primary palate. As a result the BMPs are also important candidate genes for NSCLP[42].

SUMO1

The SNP marker rs7580433 is considered to be associated with NSCLP. Given its location within the

SUMO1 gene, it is not possible that SNP rs7580433 creates a functional change, but it could be possibly a marker for other unknown SUMO1 polymorphisms. Also genetic variation that results in SUMO1 haploinsufficiency may contribute to NSCLP. Also animal model studies support that SUMO1 plays an important role in CLP development[43].

TGFA

A significant association has been observed between transforming-growth-factor-a (TGFA) locus and N-SCLP. It is believed that either the TGFA gene itself or DNA sequences in a nearby region contribute to the development of NSCLP in people. A significant association between the 2.7 kbp Taq I and the 4.0 kbp Bam HI fragments of the TGFA probe and nonsyndromic CLP has been observed. This association suggests that an abnormality in this gene may cause a predisposition for CLP or that a still unidentified clefting gene may be tightly linked to the TGFA locus. Furthermore, EGF/TGF-a and glucocorticoids are believed to play a regulating role in the proliferation and differentiation of palatal epithelial cells. The continuous presence of EGF inhibits the fusion process as well and TGF-a might also have similar effects. The mutations that affect the timing of the tissue-specific expression of this gene might be the reason for the NSCLP deformity. Examinations of the haplotype distributions also show that there is overrepresentation of the C2A2B2 and C1A2B1 haplotypes[44].

Genetic counseling

Most parents with a child with either cleft lip or cleft palate wonder why this happened to their family and if they are to be blamed for. They may also be worried about the chances of future reappearance of the deformity in other pregnancies. Patients with CLP may have similar concerns about the health of their own children. The first step for genetic counseling is taking medical history and examination. In addition to that a thorough gestational history is necessary to look for possible teratogenic factors that have contributed to the appearance of CLP as well as a detailed family history to identify genetic factors. In this way, it is possible to determine if patients have a syndromic or nonsyndromic CLP, a familial or nonfamilial CLP. Genetic counseling also contains recommendation to avoid the appearance of CLP and other deformities such as the uptake of 400 mg of folic acid every day during pregnancy. This mainly prevents malformations regarding the neural tubes but it also protects from orofacial clefts. Genetic counseling is important for families of children with CLP. Professionals can help the parents of an unborn child with CLP understand their baby's birth defect and prepare themselves for a variety of issues that might arise during the child's life. Medical advice offered should be discrete non-judgmental and accurate. This has a huge impact on the ability of these families to adapt to the needs of their children and to manage their future expectations[3].

CONCLUSION

Cleft lip and cleft palate along with other orofacial clefts are a clinical condition which causes facial deformities on the affected children and is still a factor of major morbidity and social stigma both in developed and developing countries. There are many factors which contribute to its appearance, both genetic and environmental. As far as genetic research is concerned, there is still a lot to be done in order to identify the mutations which are responsible for CLP development and possibly create prenatal diagnostic methods. Genetic counseling and surgery also play a crucial role in understanding and managing the problems that arise in the everyday lives of the affected children and their families, improving their quality of life.

Γενετική της Χειλεοσχιστίας και της Υπερωιοσχιστίας

Χρήστος Βέρος, Ζαφειρούλα Ιακωβίδου-Κρίτση

ΠΕΡΙΛΗΨΗ: Η χειλεοσχιστία και η υπερωιοσχιστία μαζί ή σαν μεμονωμένες περιπτώσεις αποτελούν ένα μεγάλο τμήμα των σχιστιών του προσώπου, που παρατηρούνται κατά τη γέννηση. Πρόκειται για μια ιατρική κατάσταση, που έχει τύχει εκτεταμένης έρευνας από την επιστημονική κοινότητα λόγω των επιπτώσεων στην υγεία καθώς και των κοινωνικών επιπτώσεων στην καθημερινή ζωή των ασθενών. Υπάρχουν πολλές υποθέσεις και πολλά επιστημονικά δεδομένα για τα γονίδια που εμπλέκονται στη γένεσή τους. Η χειλεοσχιστία και η υπερωιοσχιστία μπορούν να αποτελούν κλινικές εκφράσεις σε πολλά σύνδρομα, αλλά μπορούν επίσης να εμφανιστούν και σαν μεμονωμένες περιπτώσεις και σε αυτή την περίπτωση θεωρούνται μη συνδρομικές. Ειδικά στις μη συνδρομικές μορφές η γενετική ετερογένεια και οι διαφορές ανάμεσα στους μελετώμενους πληθυσμούς μας παρέχουν πολλές φορές αντικρουόμενα αποτελέσματα για τα εμπλεκόμενα γονίδια. Είναι επίσης ευρέως αποδεκτό ότι η χειλεοσχιστία και η υπερωιοσχιστία είναι μια πολυπαραγοντική ιατρική κατάσταση, στην παθογένεση της οποίας και οι περιβαλλοντικοί παράγοντες παίζουν σπουδαίο ρόλο.

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