Introduction

• facial cleft is the second most common congenital deformity (after clubfoot)
• problems they cause are cosmetic, dental, speech, swallowing, hearing, facial growth, emotional
Epidemiology

• Cleft Lip +/- Palate- 2 male: 1 female
• Cleft Palate - 2 female: 1 male
• Cleft Lip +/- Palate- Native Americans > Oriental and Caucasians > Blacks
• Cleft Palate- Same among ethnic groups
Anatomy

• **Hard palate** (anterior 2/3)
  - made of bone
  - covered with mucosa

• **Soft palate** (posterior 1/3)
  - made of skeletal muscle
  - covered with mucosa
  - ends in the uvula
  - closes nasopharynx during swallowing
The primary palate represents only a small part lying anterior to the incisive fossa, of the adult hard palate.
Embryology - Development of the Palate

It involves the formation of:
- a primary palate
- a secondary palate
- fusion of their processes

**Primary palate** (forms during 4th to 7th week of Gestation)
- Two maxillary swellings merge
- Two medial nasal swellings fuse
- forms from an internal swelling of the intermaxillary process (fusion of medial processes)

**Secondary palate** (forms in 6th to 9th weeks of gestation)
- is formed by merging up primary palate with two lateral palatine shelves
- develop as internal projections of the maxillary prominences
- Palatal shelves change from vertical to horizontal position and fuse
- Tongue must migrate antero-inferiorly

Embryology - Upper lip formation

- during the fourth week
- fusion of the maxillary processes with each medial nasal process
- this partially forms the lateral sides of the upper lip – together with the intermaxillary segment which contribute to the medial aspect of the upper lip
- the maxillary processes also fuse with the lateral nasal processes – results in a nasolacrimal groove which extends from the medial corner of the eye to the nasal cavity

Cleft Formation

- Cleft is result of a deficiency of tissue
- Cleft lip occurs when an epithelial bridge fails
- Clefts of primary palate occur anterior to incisive foramen
- Clefts of secondary palate occur posterior to incisive foramen
- Secondary Palate closes 1 week later in females
- Cleft of lip increases likelihood of cleft of palate because tongue gets trapped.
The most common types of cleft affecting the palate

(a) Unilateral cleft lip with alveolar involvement; (b) bilateral cleft lip with alveolar involvement; (c) unilateral cleft lip associated with cleft palate; (d) bilateral cleft lip and palate; (e) cleft palate only.
Unilateral Cleft Lip

- Nasal floor communicates with oral cavity
- Maxilla on cleft side is hypoplastic
- Columella is displaced to normal side
- Nasal ala on cleft side are laterally, posteriorly, and inferiorly displaced
- Lip muscles insert into ala and columella
Palatal Clefts

• Soft palate muscles insert on posterior margin of remaining hard palate.

• Associated Dental Abnormalities
  – Supernumery teeth- 20%
  – Dystrophic teeth- 30%
  – Missing teeth- 50%
  – Malocclusion- 100%
Etiology - MULTIFACTORIAL

There are only two factors that cause any trait:

Genetics

Environment
GENETICS OF CLEFT LIP AND PALATE

Non-syndromic clefts – cleft is present like only congenital malformation
• Non-syndromic clefts of lip and palate
• Non-syndromic isolated clefts of palate

Syndromic clefts – cleft is part of the syndrome, one from the number of symptoms
• Syndromic clefts of lip and palate
• Syndromic isolated clefts of palate
Syndromic clefting

• **Examples of syndromes with CLP:**
  - **Apert** syndrome (OMIM 101200), **Crouzon** syndrome (OMIM 123500), **Hemifacial microsomia** (HFM, OMIM 164210), **Pierre Robin sequence** (RS, OMIM 261800) and associated syndrome, **Velocardiofacial** syndrome (VCFS; OMIM 192430), **X-linked Opitz G/BBB** syndrome(OSX, OMIM 300000)

• **Examples of syndromes with CP:**
  - **Holoprosencephaly** (HPE, OMIM 236100; 609637), **Smith-Lemli-Opitz** syndrome (SLOS, OMIM 270400), **Stickler** syndrome (OMIM 108300, 604841), **Treacher Collins** syndrome (TCS, OMIM 154500)
GENETICS OF CLEFT LIP AND PALATE

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Non-syndromic clefts

Candidate genes or loci implicated in the etiology of nonsyndromic cleft lip and palate, and the available evidence:

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*MTHFR - 5,10-methylenetetrahydrofolate reductase, IRF6 - Interferon regulatory factor-6, TGFA - Transforming growth factor-alpha, SATB2 - Special AT-rich sequence-binding protein-2, MSX1 - Drosophila msh homeobox homolog-1, ACOD4 - Acyl-coenzyme A desaturase-4, PVRL1 - Poliovirus receptor like-1m, TGFβ3 - Transforming growth factor beta-3, CLPTM1 - Cleft lip and palate-associated transmembrane protein-1, TBX22 - T-box transcription factor-22

Genes involved in development of orofacial clefts

- genes expressed in some parts of the embryo development – e.g. TGF–α, TGF-β2, TGF-β3

- genes encoded products indirectly associated with development of orofacial structures – e.g. receptor for RA, receptor for MTHFR, receptor for FA

- homeiotic genes MSX-1 and MSX-2

- genes involved with metabolism of xenobiotics – e.g. cytochrome system P-450
Some syndromic genes, like *IRF6, PVRL1, TP63* and *TBX*, contribute to the incidence of non-syndromic clefts.

- **Van der Woude syndrome** (VWS; OMIM 119300)
- **CLP with ectodermal dysplasia** (CLPED1, OMIM 225060)
- **EEC syndrome** (OMIM 604292)
- **X-linked Mendelian inherited form of CP** (CPX)
Syndromic models for non-syndromic CL/P

Van der Woude syndrome (VWS; OMIM119300)
- autosomal dominant disorder
- provides one of the best models for non-syndromic CLP since most patients have only minor additional phenotypes of lip pits and missing teeth (hypodontia), while 15% have isolated CL/P,
- typical mutations were identified in the interferon regulatory factor 6 gene IRF6.
Syndromic models for non-syndromic CL/P

CLP with ectodermal dysplasia (CLPED1, OMIM 225060)
- autosomal recessive disorder
- is generally rare but occurs with a much higher frequency on Margarita Island (north of Venezuela)
- mutations were identified in the cell adhesion molecule PVRL1 (Nectin-1), which is expressed in the developing face and palate
- on Margarita Island, CLPED1 is generally caused by homozygosity of the nonsense mutation W185X, while heterozygosity is high in the unaffected population
- it has been speculated that, since Nectin-1 is the principle cell surface receptor for α-herpes viruses, the high frequency of heterozygotes might have resulted from relative resistance to infection by viruses such as HSV1 and HSV2.
Syndromic models for non-syndromic CL/P

**EEC syndrome (OMIM 604292)**
- autosomal dominant disorder
- ectrodactyly, ectodermal dysplasia, CL/P
- heterozygous mutations were identified in the *TP63* gene
- missense mutation of the conserved DNA binding domain region gives CLP while C-terminal mutations give CL or CP
- mutation at the N-terminal end outside of the conserved domains gives rise to CP or no clefting at all
Syndromic models for non-syndromic CL/P

X-linked Mendelian inherited form of CP (CPX)

- the gene encoding T-BOX 22 (TBX22) was identified as the locus responsible for the disorder
Environmental influences

- Alcohol use
- Smoking
- Use of folic acid and multivitamins
- Steroids
- Anticonvulsants
Environmental influences

Alcohol use

• heavy maternal alcohol drinking causes fetal alcohol syndrome and increases the risk of CLP
• low-level alcohol drinking does not increase the risk of orofacial clefts
Environmental influences

Smoking

• the relationship between maternal smoking and CLP is not strong, but it is significant
• combination of maternal smoking with a positive genetic background significantly increases the risk of orofacial clefts
• maternal smoking and infant TGF-α genotypes acted together to increase the risk for CLP
• maternal glutathione s-transferase (GSTT1) genotype – antioxidant agent – combinated with smoking
Environmental influences

Use of folic acid and multivitamins

• if vitamins like folic acid and cobalamins were not taken during early pregnancy the risk for CLP could be tripled
• defective maternal vitamin-dependent homocysteine metabolism, that is linked with folic acid metabolism, is a risk factor for CLP in the offspring
• only a very high dose of supplementary folic acid (10 mg/day) could reduce the risk of CLP significantly (65% reduction was observed)
Environmental influences

Use of folic acid and multivitamins

- enzyme encoded MTHFR is the enzyme responsible for the folate metabolism pathway
- the MTHFR C677T single-nucleotide polymorphism (SNP) is thermally labile and considered a risk factor for neural tube defects
- in NSCLP, the MTHFR C677T genotype in the mother conferred a risk of CLP in offspring that was increased by 4.6 times
- in periconceptional folic acid deficiency, the MTHFR thermally labile variant could lead to a risk of CLP that was increased by 10 times
Environmental influences

Steroids

• the association between maternal corticosteroid use during the periconceptional period (1 month before conception to 3 months after conception) and congenital anomalies was described

• prednison a synthetic corticosteroid drug that is particularly effective as an immunosuppressant drug, was considered not to be a terratogene – however the use of prednison during the periconceptional period increases the risk for CO 3.4 times
Environmental influences

- **Anticonvulsants** (phenytoin/hydantoin, oxazolidinones, and valproic acid)
- women with epilepsy have an increased risk of having offspring with orofacial clefts, mostly because of teratogenic effects of antiepileptic drugs,
- other risk factors have also been suggested, including epilepsy *per se* or some underlying genetic defects associated with epilepsy.
1. The clinical evaluation of a CL/P patient starts with his/her classification in syndromic and nonsyndromic cases, based on the presence or absence of other dysmorphisms or malformations, together with an investigation of the occurrence of relatives with similar features.

2. Among the syndromic cases, it is first necessary to investigate the possibility of non-genetic causes (exposure to teratogens during the first trimester of gestation.)

3. Once the possibility of a teratogenic origin for CL/P is ruled out, the geneticist should raise the diagnostic hypothesis of genetic syndromes and recommend the most adequate test.

• The most commonly performed tests are the karyotype, Multiplex Ligation-dependent Probe Amplification (MLPA), Comparative Genomic Hybridization array (CGH-array), gene target sequencing, and exome sequencing. Whilst the karyotype is a cytogenetic technique which allows for detection of large structural and numeric chromosomal anomalies in a low resolution, MLPA and CGH-array are quantitative molecular tests that enable the investigation of gain or loss of genetic material at the submicroscopic level. MLPA is applied to investigate specific targets in the genome while CGH-array can be used to screen the whole genome with a very high resolution. MLPA or CGH-array are the recommended tests to be used for a first screening, depending on the available resources).

• Gene target sequencing is recommended when one or more genes are known to be causative of the disorder.

• Recurrence risk estimates for future children of the parents of one affected patient is dependent on the definition of the etiological mechanisms of the disease, evidencing the importance of selecting the appropriate test, combined with the clinical evaluation, for the establishment of the diagnosis.

• In nonsyndromic cases the recurrence risks have been empirically determined by epidemiological studies., As expected for a multifactorial model of inheritance.

• In NS cases, the identification of other individuals with CL/P in the family should be always interpreted with caution. Due to genetic heterogeneity associated with NS CL/P, a family with several affected individuals can actually represent the segregation of a single-gene disorder, which would not be promptly recognized based solely on clinical evaluation.
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