



**GENE /  
ENVIRONMENT  
STUDY**

**HOW AND WHY CLEFTS  
OCCUR?**

OPERATION SMILE INC. | Research and Outcomes Department.

## **INTRODUCTION**

For the past 27 years Operation Smile has been treating cleft patients around the world, with over 130,000 patients receiving surgery. During that period of cooperative work with local communities, Operation Smile has transferred medical and non-medical skills and knowledge to local counterparts to build their self-reliance in delivering services to patients in need. Today, Operation Smile is a network of 27 countries each at a different stage of evolution; ranging from countries where only international missions are run to countries where there are comprehensive care centers in which local volunteers are able to see patients all year long.

There will always be a need for surgical missions (local and international) to deal with the backlog and increasing number of patients with cleft lip and palate around the world. This amount of accumulated cases is huge because most patients need an average of three surgical procedures and highly specialized multidisciplinary treatment. Meanwhile, the incidence of oral clefts is increasing every day. If we want to win the battle against clefting we need to better understand the causative factors of clefts, in order to develop strategies to reduce incidence. It is necessary to focus more attention on genetic and environmental research.

Operation Smile, collaborating with several researchers, has contributed to the creation of some of the current literature on how and why clefts occur.<sup>1 2 3 4 5 6 7 8 9 10</sup> But there are still many questions to answer and the organization continues its quest for answers, especially in these two areas.

As we gain more knowledge it is very important that every person committed to cleft care, including patients, parents and health professionals have a basic understanding of the current theories about the causation of clefts. Families may want an explanation of why the cleft occurred and what the risk of recurrence is. Families and patients should have a good understanding of the problems faced by researchers looking for a cause and the potential implications (benefits-risks) of participating in research studies. Health care professionals and administrative personal could then use the information collected to develop and advocate for public health programs and health policies promoting the prevention of oral clefts and other congenital malformations.

With everyone from parents to patients to health professionals looking for answers and explanations, the lack of a uniform language is the main obstacle in ensuring that all audiences have a basic understanding of the current theories of cleft causation. We will try to simplify the "scientific" language in order to overcome this obstacle, but this is not an easy task. Pictorial examples of a variety of forms of CL/CP are provided to enhance understanding of the spectrum of this defect.

The other big challenge to overcome in explaining cleft causation is the fact that there is neither a single cause nor any single etiological model that explains the occurrence of oral clefts. We hope you will find this document useful.

## HOW

### DEVELOPMENT OF THE FACE

The study of the development of the face, lip, and palate, in uterus after conception is important to enhance the understanding of the timing, complexity, and factors that may influence the occurrence the oral clefts.

Facial development largely occurs between the fourth and eighth weeks of pregnancy, and the face has a clearly human appearance by 10 weeks. Four weeks after fecundation, the structures of the face can be recognized in the human embryo. The brain, eyes and stomodeum (mouth) are clearly recognized. Five facial prominences (groups of cells) one frontonasal, two maxillary and two mandibular bordering the stomodeum (mouth) are responsible for the development of adult facial features.

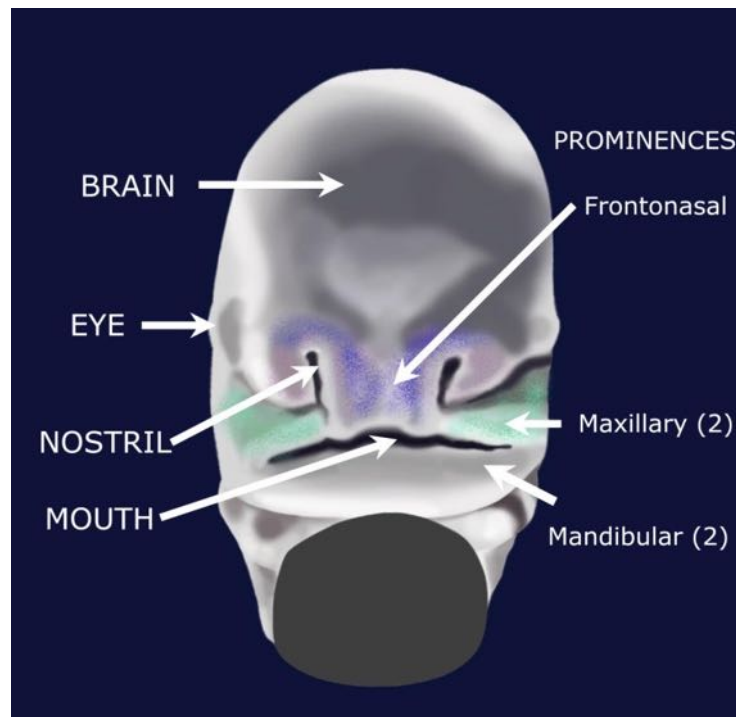


Figure 1. Facial schema of the face of the human embryo

Merging of the paired mandibular prominences produces the lower jaw, lower lip, lower cheek, and chin regions of the face. These are the first parts of the face to take a definitive form.

In the frontonasal prominence there is a further elevation of the margins of the nostrils. The sides develop into the medial and lateral nasal prominences, respectively. The paired maxillary prominences migrate medially, merging with the frontonasal prominence. Fusion of the medial nasal, lateral nasal, and maxillary prominences produces continuity between

the nose, the upper lip, and the palate. This process is complete by the end of the seventh week of gestation.

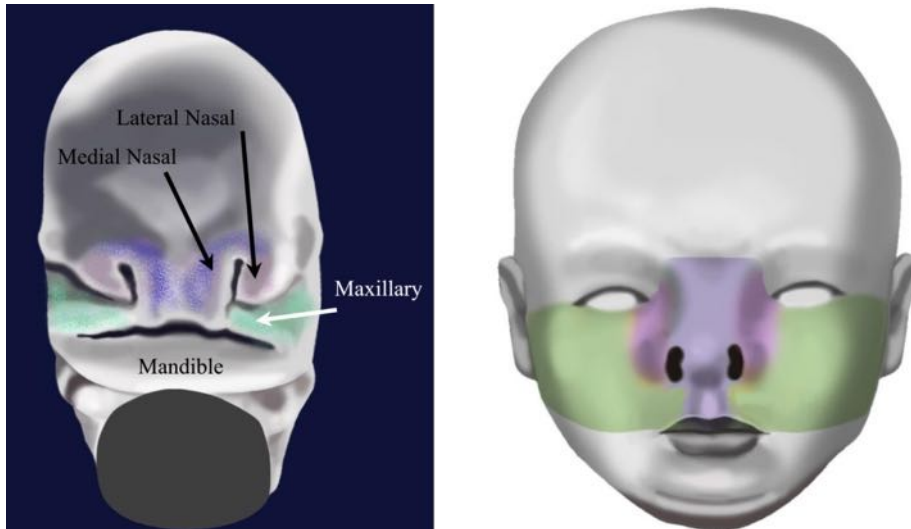


Figure 2. Merging of the medial nasal prominences forms the central part of the upper lip, the nasal tip, the premaxilla and primary palate. The lateral nasal prominences form the nasal alae. The maxillary prominence accounts for the major portion of the upper lip (excluding the central part) and the upper cheek regions.

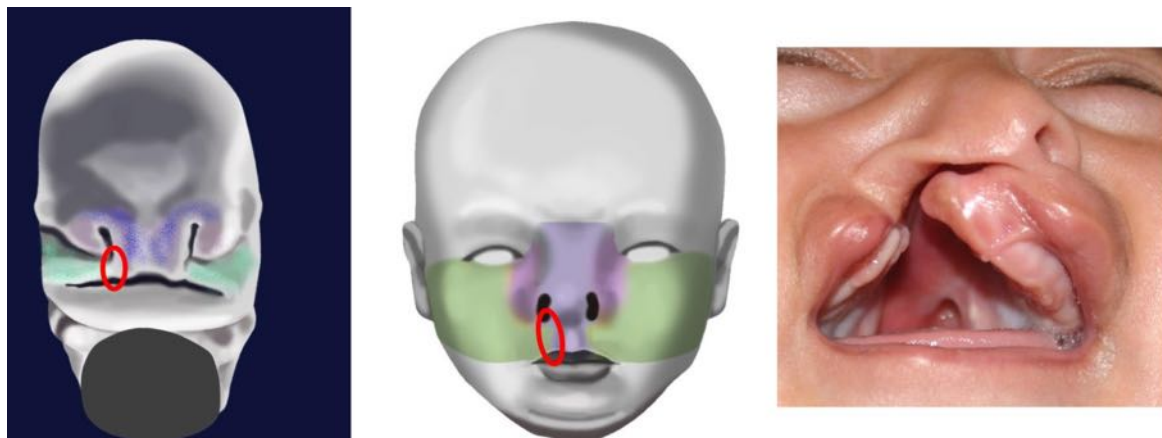


Figure 3. A complete unilateral cleft lip results from complete failure of fusion of the medial nasal prominence and maxillary prominence on one side. In this patient, the cleft involves the palate as well.



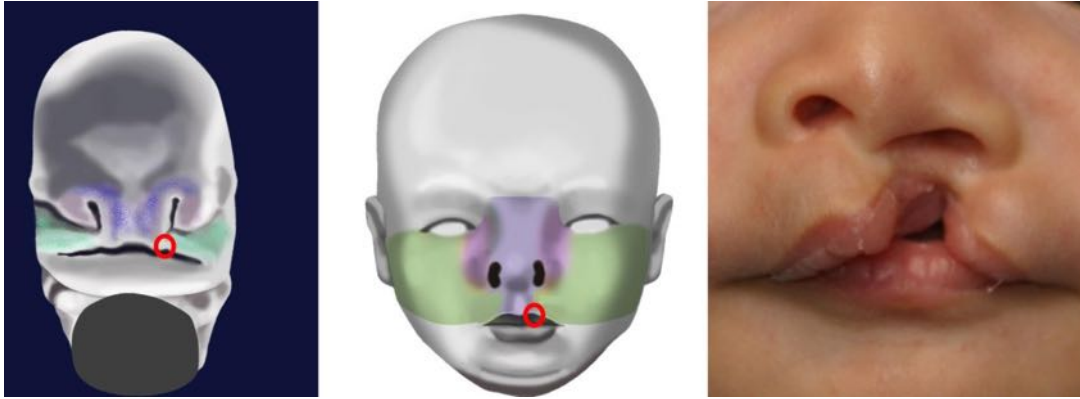


Figure 4. A partial unilateral cleft lip results from partial failure of fusion of the medial nasal prominence and maxillary prominence on one side. In this patient, the palate was not affected.

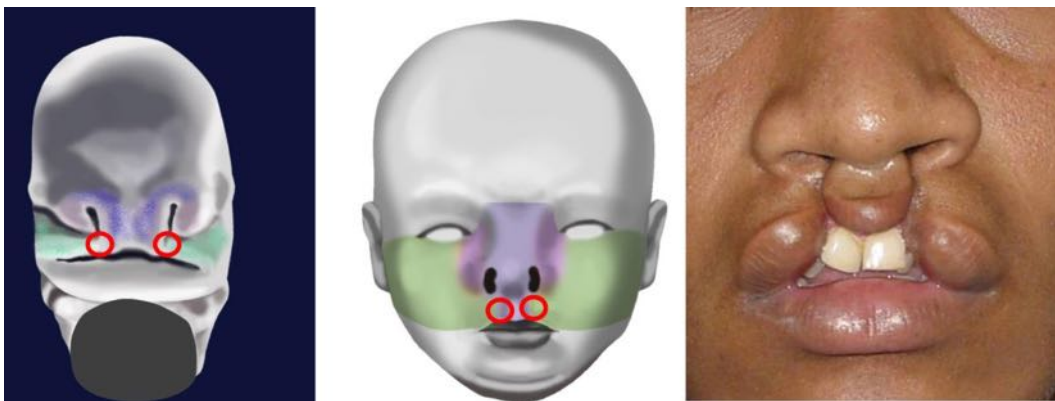


Figure 5. A complete bilateral cleft lip results from complete failure of fusion of the medial nasal prominences with the maxillary prominence on both sides. In this case, the palate was not compromised.

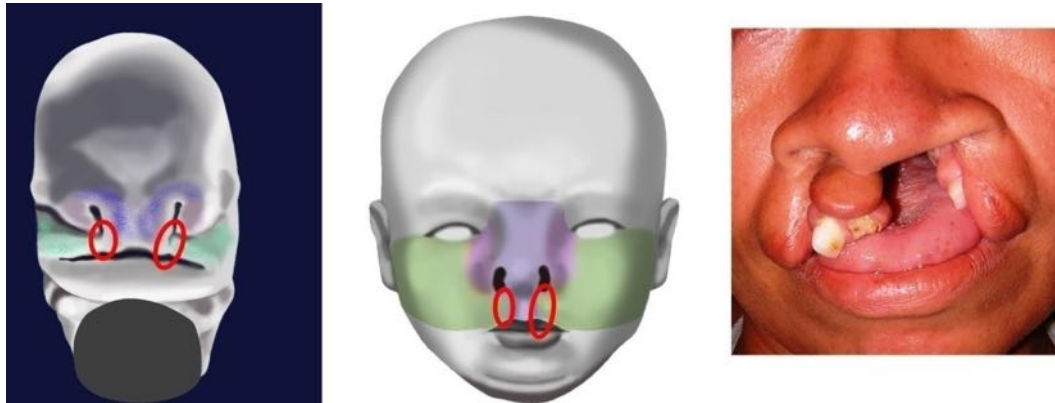


Figure 6. The severity of clefting varies from patient to patient and can be asymmetric. In this patient the cleft on the left side was more severe and there is a cleft palate. The same congenital defect is occurring, but with differing severity (expression) on each side.

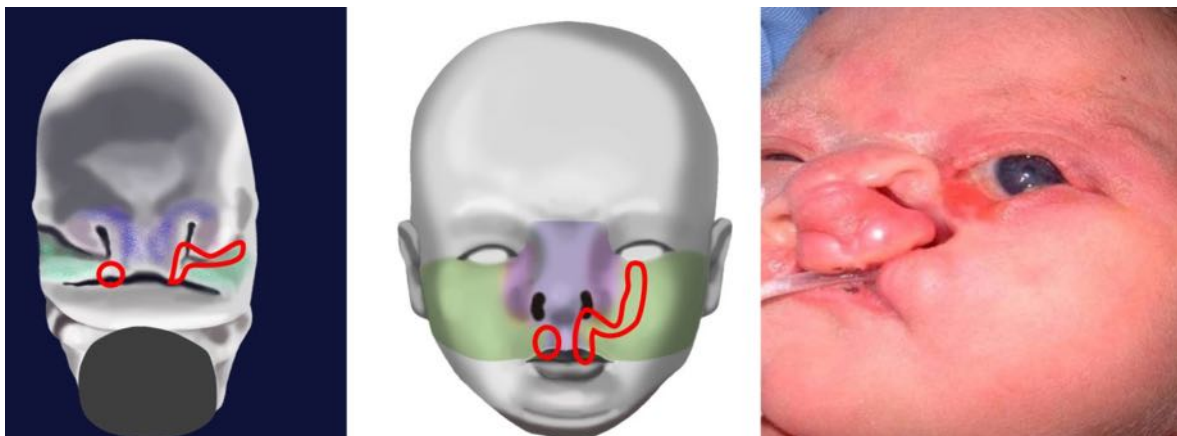


Figure 7. A oblique cleft or naso-ocular cleft results from the failure of union of the lateral nasal and maxillary prominences. In this patient, there was not a merging of the maxillary prominences with the medial nasal prominences, resulting in a bilateral cleft lip.

The palate results from the merging of both the frontonasal and maxillary prominences. The median palatine process is derived from the frontonasal prominence. The lateral palatine processes are derived from the maxillary prominences. All three elements are initially widely separated. During the eighth week of gestation, their fusion is initiated from anterior to posterior. By the end of the eighth week, the palate is completely formed.<sup>11 12</sup>

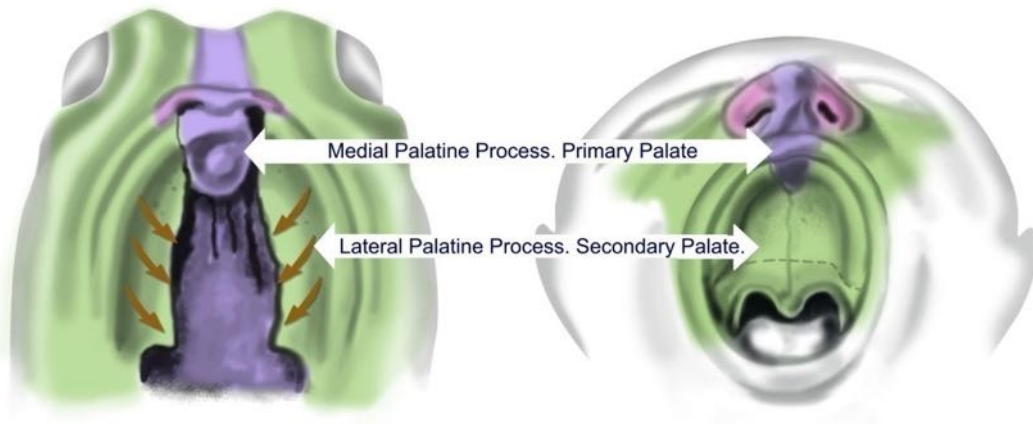


Figure 8 . The fusion of the lateral palatine processes (growing toward the midline) and the medial palatine process occurs at the end of the 8<sup>th</sup> week of gestation.



Figure 9. A complete unilateral cleft palate results from the failure of fusion of the merged lateral palatine processes and medial palatine process on one side.



Figure 10. A complete bilateral cleft palate results from the failure of fusion of merged lateral palatine processes and medial palatine process on both sides. As a result, the medial palatine process (premaxilla) is prominent (anterior over-projection) because it is not restrained by an attachment to the lateral palatine processes.

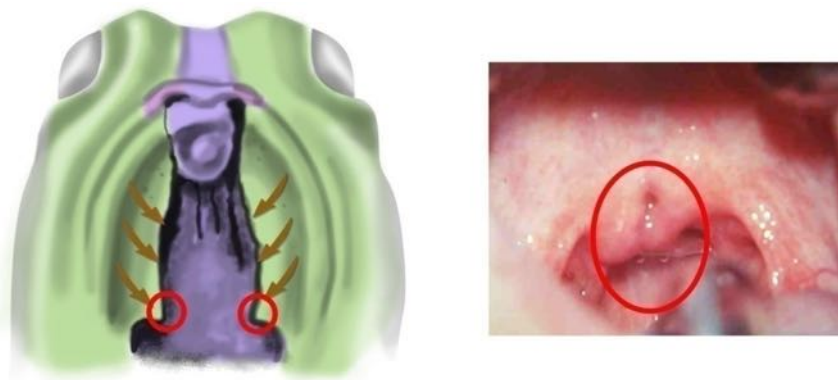


Figure 11. An incomplete cleft palate, like the uvula bifida, is a result of the failure of fusion of the palatine processes in the most posterior part of the palate. In this case, the anterior 95% percent of the palate was formed.

As we can see there are many degrees of severity of cleft lip and/or palate, from unilateral incomplete (Figure 4) or uvula bifida (Figure 11) to complete unilateral (Figures 3 and 9) or complete bilateral (Figures 6 and 10). This shows that the contact and fusion of the facial processes may cease at any point. The earlier the interruption or interference takes place, the greater the defect. Problems in development before the 4<sup>th</sup> week of gestation result in far more severe birth defects.

Normal and abnormal morphogenesis of the facial regions is a complex process dependent upon a myriad of cell types, signaling molecules, genes, growth factors and tissues. One of the most important cell types in understanding normal and abnormal craniofacial morphogenesis is the neural crest cell, because they migrate into the facial prominences directing all migration processes of the facial prominences. The identification of the exact molecular mechanisms and cellular events linked to the differentiation, proliferation and,



and especially, of the migration of crest cells into the facial prominences is not yet fully known.<sup>13</sup>

Once the prominences make contact there is a degeneration of the cells along the edges to allow the movement of cells from one prominence to the other. This process of cellular degeneration along with inter-prominences bridging of cells is called fusion.

We can conclude that oral clefts result from failure of migration or fusion of the facial prominences between the 4<sup>th</sup> and 8<sup>th</sup> weeks after conception, during the period of time when the embryo is most susceptible to environmental factors predisposing to clefts.

Why does the migration and fusion of the facial prominences cease? That is what we will discuss in the next chapter.

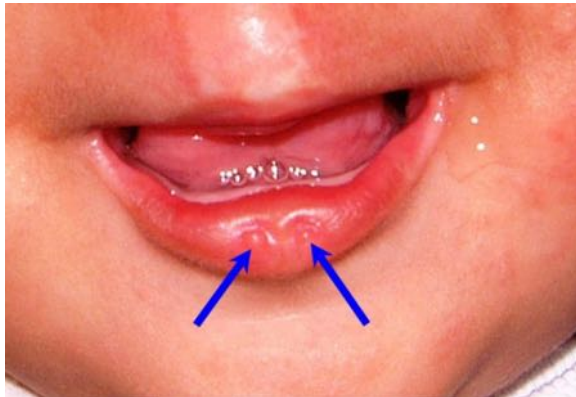
## **WHY**

Oral clefts are a heterogeneous group (cleft lip, cleft palate; unilateral, bilateral; complete, incomplete) of birth defects known to be multifactorial in origin, in that both genes and environmental factors contribute to their etiology. The genetic contribution to the causation of clefts is 20% to 50% and the remainder is attributable to environmental factors or gene-environment interactions. Most studies subdivide oral clefts into cleft lip (CL) with or without cleft palate (CL/P) and isolated cleft palate (CP) and, furthermore, into syndromic and nonsyndromic cases.

The overall incidence of oral clefts (excluding bifid uvula) is estimated to be 1 in 750 live births, making clefts the second most common congenital defect after clubfoot.<sup>14</sup> The most common type of oral cleft is a bifid uvula, occurring in 2% of births in some populations.<sup>15</sup>

When one or more additional features not related with the cleft itself are involved, clefts are referred to as syndromic. The majority are non-syndromic where CL/P occurs in isolation of other birth defects. Patients from figures 4, 5, 6, 9, 10 and 11 are examples of non-syndromic clefts because they did not have any associated birth defect. Approximately 30% of oral clefts are syndromic and 70% are non-syndromic.<sup>16</sup>

The subdivision into nonsyndromic and syndromic is important because non-syndromic CL/P and CP rarely occur again in the same family (2-6%)<sup>17, 18</sup> and many syndromic cases have a strong association with specific genetic mutations with a higher inheritance risk (passed down through families) .



Van der Woude Syndrome. Bilateral Cleft Lip.



Treacher Collins Syndrome. Cleft Palate.

Figures 11 and 12. Syndromic oral clefts with associated birth defects just in the face. The Van der Woude is the most common syndromic form of cleft lip and/or palate; the cleft is associated with pits or mucous cysts on the lower lip. The Treacher Collins Syndrome is a less frequent condition; the cleft palate is associated downward slanting eyes, micrognathia (a small lower jaw), hearing loss, underdeveloped cheekbone, drooping part of the lateral lower eyelids, and malformed or absent ears.



Apert Syndrome. Facial characteristics



Apert Syndrome Hand deformities. Syndactily (fusion of fingers 2, 3,4,5) and deformity of the thumb.

Figures 13 and 14. Patient with Apert Syndrome. In Apert Syndrome, a cleft palate is associated with deformity of the skull (high, prominent forehead with a flat posterior skull), shallow bony orbits and broadly spaced eyes, deficient growth of the midfacial bones with respiratory problems and, the most important characteristic, the syndactyly of the hands and feet. The thumb and big toe may also be broad and malformed.

## GENES

Genetics is the science that studies how living organisms inherit features from their ancestors. Genetics seeks to identify which features are inherited and explain how these features are passed from generation to generation. In genetics, a feature of an organism is called a trait. Some traits are features of an organism's physical appearance, for example, height, weight, or eye-color. It has been known for a long time that traits are inherited because children often look like their parents; however just last century, scientists realized where that information was in the human genome and how it was transmitted from generation to generation.

Genetic information is carried by a long molecule called DNA which is copied and inherited across generations. DNA is in the center (nucleus) of every single cell, compacted and folded making a structure called chromosome. Traits are carried in DNA as instructions for constructing and operating an organism. These instructions are contained in segments of DNA called genes.

All organisms have many genes corresponding to several different biological traits, some of which are immediately visible, such as eye color or number of fingers, and some of which are not, such as the increased risk for specific diseases like diabetes or heart disease.

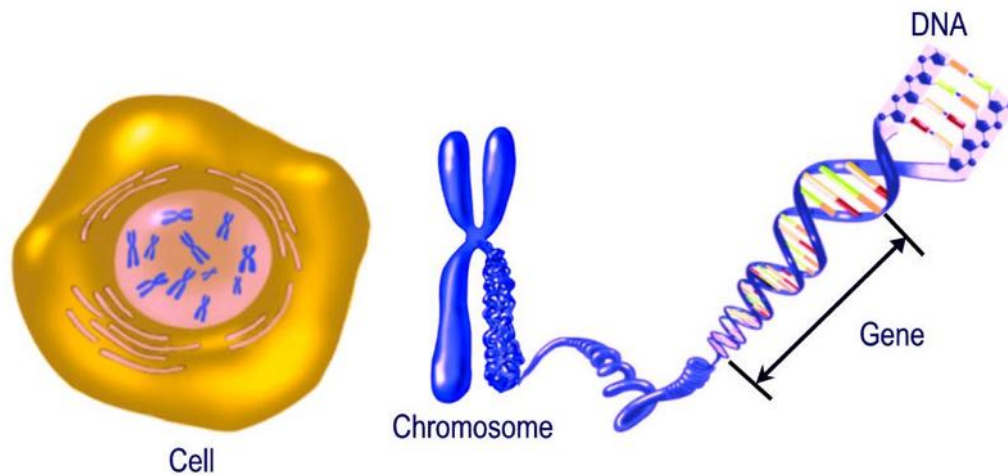


Figure 15. This diagram shows the chromosomes in the nucleus of the cell. Chromosomes are rodlike structures that are made of DNA and structural proteins. Human cells other than egg and sperm normally have 46 chromosomes (23 pairs). Each gene occupies a specific position on its chromosome and carries the instructions for each trait or function of the human body. Each person has two sets of information one from the mother and one from the father.

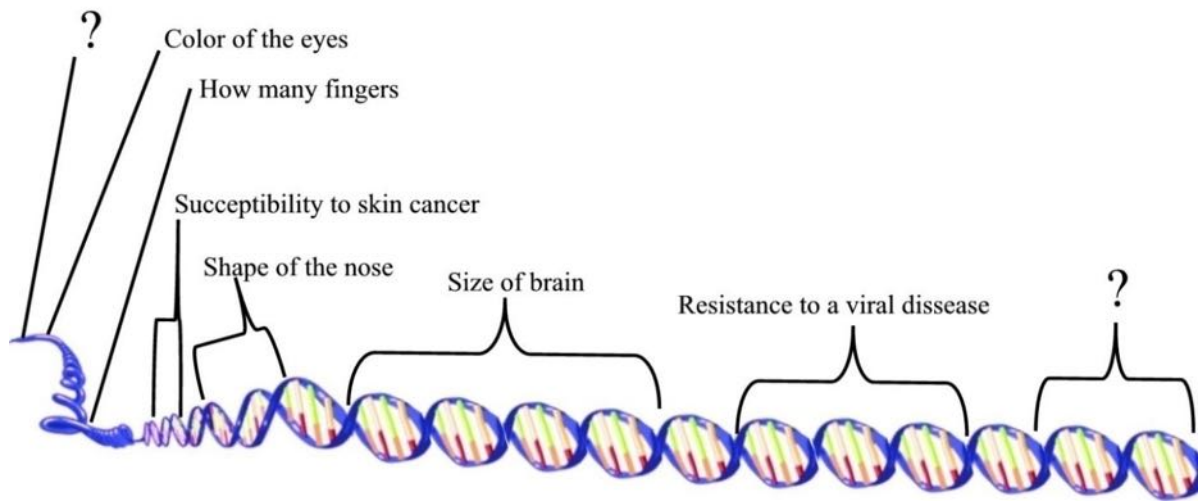


Figure 16. Schema of the information present in the DNA. The human genome is all the hereditary information encoded in DNA on one set of chromosomes. It contains all the information about function and physical characteristics of the body. There are still some unknown genes and more research is needed.

The genotype is the genetic makeup of an organism; the phenotype is the physical manifestation(s) of genes; expressivity is the severity of the trait in an affected organism, the degree to which the signs or symptoms of a given gene is manifested in the patient. Typically, clefts show variable expressivity, meaning that within the same family there are differences in the type and severity of clefts from one family member to other. Sometimes the expressivity is so small that is not possible to see the cleft with the naked eye.

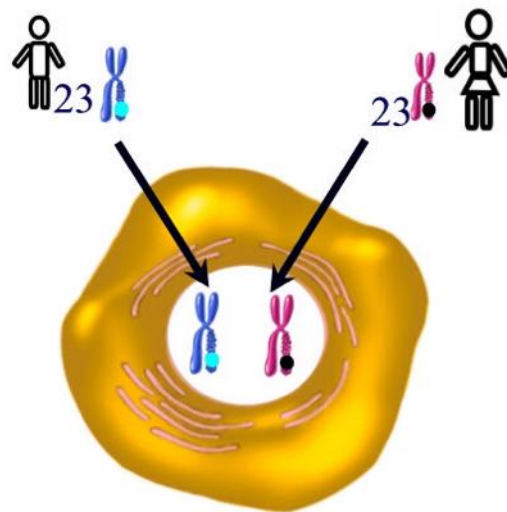


Figure 17 . Clinical example of different expressivity of genes (causing clefts) in two sisters. The patient on the left has a bilateral cleft lip, but her sister has a unilateral complete cleft lip and palate.



The beginning of a new human being requires the union of one sperm and one ovum. Egg and sperm cells have 23 chromosomes each; when they join they form the first cell called zygote with a total of 46 chromosomes (23 pairs). The zygote will undergo several divisions (called mitosis) forming the embryo, the fetus and finally the baby.

Twenty-two of the 23 pairs are autosomes (non-sex chromosomes) and they are numbered from largest to smallest as seen on a molecular picture or array called a karyotype. The twenty-third pair contains the sex chromosomes—XX for females, XY for males.



### 46 Chromosomes (23 pairs)

Figure 18. Fecundation, mother and father contribute with the 50% of the genetic material each. Each cell of the human being has two sets of information with versions of the same gene (allele). One version (allele) is on one chromosome and the other on the other chromosome. In this schema one gene was marked in each chromosome with a dot, let's say that one gene is for light blue eyes and other is for dark black eyes. In this scenario each cell of the new human being will have two alleles one for black eyes and one for blue eyes; that is the genotype. But the new person had dark black eyes; that is the phenotype.

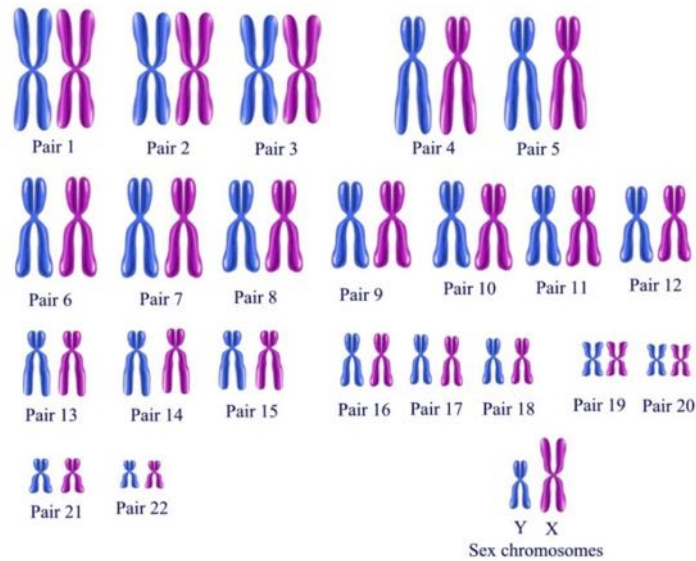


Figure 19. Schema of a normal Karyotype. Chromosomes are arranged by pairs. 50% of the chromosomes come from the mother and 50% from the father.

Sometimes there is a problem either in the ovule or in the sperm and the resulting zygote has an extra chromosome (all or part), and that extra chromosome will be present in all or some of the cells of the human body after birth. The extra material interferes with normal development.

**Trisomy** is a frequent genetic problem it can occur with any chromosome, but often results in miscarriage. The most common types of autosomal trisomy that survive to birth in humans are trisomy 21 (Down syndrome), trisomy 18, and trisomy 13. Only trisomy 13 is clearly related with oral clefts.

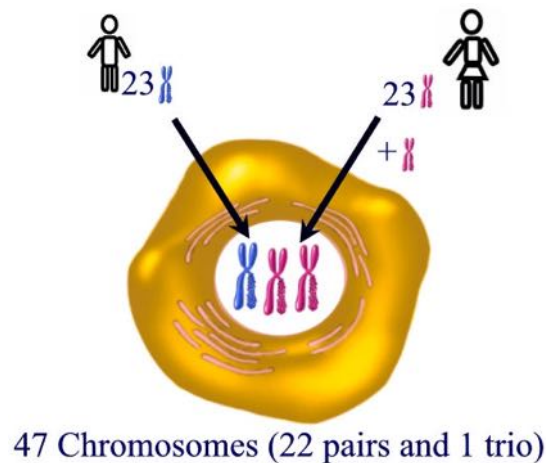


Figure 20. In full trisomy, an entire extra chromosome has been copied in the new zygote resulting in 47 chromosomes.

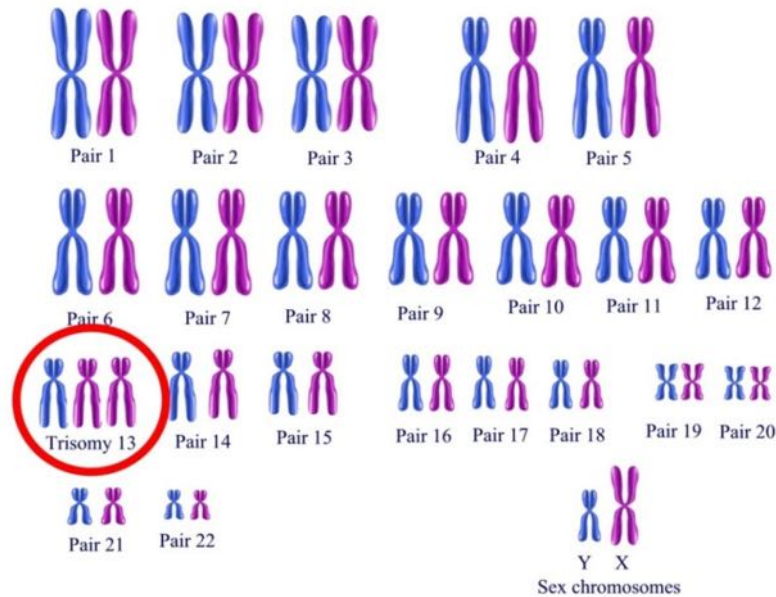


Figure 21. Schema of an abnormal Karyotype. Chromosomes are arranged by pairs, but there is an additional chromosome 13. Trisomy 13, also called Patau Syndrome involves multiple abnormalities, many of which are not compatible with life. Most children with trisomy 13 die in the first month.

**Chromosomal inheritance** (due to an abnormality in the number or structure of the chromosomes) is easy to diagnose because chromosomes can be seen with a microscope, but more technology is needed to identify single genes causing specific congenital defects. There are some genes already identified as causes of oral clefts, usually as part of syndromes, but to mention all of them is beyond the scope of this paper; however, some examples are listed in Table 1. While the location of the ‘problem’ gene is important to scientists, it is more important to understand why that one gene is mutated (the DNA code is changed), deleted (the DNA is lost) or translocated (the DNA is rearranged between chromosomes) and why the affected gene is the dominant one passed from generation to generation.

Syndrome	Gene
<b>Van der Woude Syndrome</b>	Mutation in the IRF6 gene, located on chromosome 1
<b>Treacher Collins Syndrome</b>	Mutation in the TCOF1 gene, located on chromosome 5.
<b>Apert Syndrome</b>	Defect on the FGFR 2 gene, located on chromosome 10.

Table 1. Examples of single genes that cause syndromic oral clefts.

There are three inheritance patterns of single genes: autosomal dominant, autosomal recessive and X-linked recessive. They were described by Mendel, in the 1860s, when studying inheritance in pea plants. At the time, DNA was not yet discovered, leading to the patterns being called Mendelian Patterns. Most of the single genes responsible for oral

clefting following a Mendelian pattern are inherited in an autosomal-dominant pattern; some are inherited in an autosomal-recessive pattern and rarely are any inherited as an x-recessive pattern.

In order to understand the heritage pattern, it is important to draw a diagram showing the genetic relationships between members of a family (pedigree), annotated with relevant medical information. Pedigrees are used to visualize inheritance patterns aiding in diagnosis and risk assessment. We will use pedigrees to explain these patterns of inheritance, the nomenclature in Table 2:





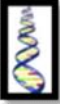
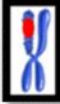

 Female Normal Phenotype	 Female Affected Phenotype	 Male Normal Phenotype	 Male Affected Phenotype
 Studied gene A or Mutant gene A	 Studied gene B or Mutant gene B	 Normal gene Normal allele	

Table 2. Nomenclature used to explain pedigrees in this document.

In the **autosomal dominant** inheritance pattern the gene is expressed phenotypically when it is present. Remember, there are two alleles of each gene, in this case the one expressed is the dominant one.

A patient with an autosomal dominant condition has a 50% chance of transmitting the dominant gene to the next generation, and that risk is the same for each pregnancy. Males and females are equally likely to be affected. Affected individuals are usually found in multiple successive generations. We have already mentioned three autosomal dominant syndromes with clefts: Van Der Woude Syndrome, Treacher Collins Syndrome, and Apert Syndrome.



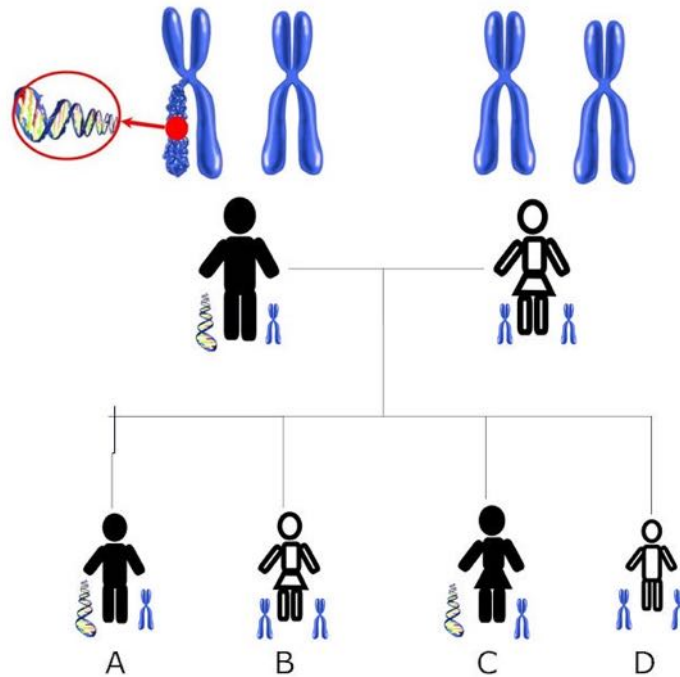


Figure 22. Schematic illustration of the transmission of the autosomal dominant gene. In this case there is a mutation in the father's DNA in one of the chromosome 1, the other one is normal. Mother's chromosomes 1 does not have the mutation. The mutated gene was inherited by the 50% of children, males and females were equally affected. Each child who inherited the gene from the father developed cleft lip and palate (A C). All received two different versions of the same gene, one from the mother and one from the father (alleles), but the mutated dominant gene was the gene expressed (phenotype) when it was present.

Children with the phenotype (A,C) of the birth defect have the mutated gene in their genotype and children without that phenotype (B,D) do not have the mutated gene in their genotype.

In the **autosomal recessive** inheritance pattern the gene is expressed phenotypically when both alleles on a chromosome pair are present. The child receives a mutated allele from each parent. Both parents must have the mutated gene. A consanguineous relationship (descending from a common ancestor) can increase the likelihood of occurrence of a recessive disorder.

The phenotypically affected individual is said to be homozygous for the mutated gene; heterozygous individuals have only a single copy of the abnormal gene and are not phenotypically affected. Because the parents each must have a mutated allele and a normal allele, each offspring has a 50% chance of being a carrier, a 25% chance of being affected, and a 25% chance of being neither affected nor a carrier.

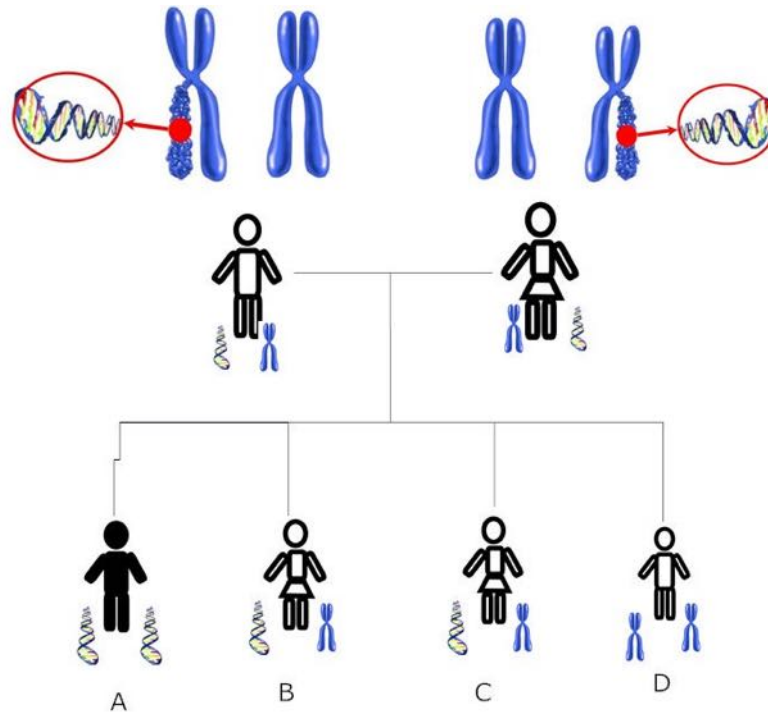


Figure 23. Schematic illustration of the transmission of the autosomal recessive gene. In this case there is a mutation in the father's DNA in one of the chromosomes, the other one is normal; there is also the same mutation in the mother's DNA in one of the chromosomes and the other one is normal. The mutated gene was inherited by the 75% of children, but it was phenotypically expressed in 25%; males and females were equally likely affected. Just the person (A) who got both mutated genes, one from the mother and one from the father, developed cleft lip and palate (filled with black). Every child got two different versions of the same gene one from the mother and one from the father (alleles). The mutated recessive gene was expressed phenotypically when both abnormal genes were present. The child with the abnormal phenotype (A) has both mutated genes in his genotype, the two children without the abnormal phenotype (B,C) have the mutated gene in their genotype and the one child without an abnormal phenotype (D) does not have the mutated gene in his genotype. Carriers are the individuals who have one recessive mutated gene; the carrier does not exhibit the associated trait, but can pass the mutated gene to his or her offspring.

Most of non-syndromic oral clefts do not exhibit the classical Mendelian recessive or dominant patterns attributable to any single locus, but show strong familial aggregation and have a substantial genetic component.<sup>19</sup> There is an elevated risk of recurrence in relatives of an affected individual. The risk of recurrence increases with the more affected relatives an individual has. The risk for oral cleft recurrence is greater in relatives of patients that are severely affected than in relatives of mildly affected patients<sup>20</sup> The Polygenic/Multifactorial Inheritance pattern has been the most common pattern used to explain the cause of oral clefts<sup>21</sup> (Tables 3, 4, and 5). However, there are a very large number of truly sporadic cases (those who have a "negative" family history after a very good study) reported.<sup>22</sup>

Siblings/Relatives	Parents	Cleft Lip with or without Cleft Palate
<b>No sibs</b>	Neither parent	0.1
	One parent	3
	Both parents	34
<b>One sib</b>	Neither parent	3
	One parent	11
	Both parents	40
<b>Two sibs</b>	Neither parent	8
	One parent	19
	Both parents	45
<b>One sib and one second degree relative</b>	Neither parent	6
	One parent	16
	Both parents	43
<b>One sib and one third degree relative</b>	Neither parent	4
	One parent	14
	Both parents	44

Table 3. Recurrence risks (%) for cleft lip with or without cleft palate.<sup>23</sup> Recurrence risks calculated before the widespread introduction of maternal folic acid supplementation during pregnancy in the USA. (From Bonati-Pellie C, Smith C. Risk tables for genetic Counseling in some common congenital malformations. J Med Genet 11:374-311, 1974.)

Phenotype / Severity of cleft	Incidence in sibs of cleft lip with or without cleft palate
<b>Unilateral cleft lip without cleft palate</b>	4.0
<b>Unilateral cleft lip and palate</b>	4.9
<b>Bilateral cleft lip without cleft palate</b>	6.7
<b>Bilateral cleft lip and palate</b>	8.0

Table 4. Risk for cleft lip with or without cleft palate in siblings of patients affected with clefts of increasing severity. (From Nussbaum R, Mc Innes R, Willard H. Thompson & Thompson Genetics in Medicine. Saunders; 2007)

Population affected	Incidence of cleft lip with or without cleft palate (%)
<b>General population</b>	0.1
<b>First-degree relatives</b>	4.0
<b>Second -degree relatives</b>	0.7
<b>Third-degree relatives</b>	0.3

Table 5. Empirical risks for cleft lip with or without cleft palate in relatives of affected patients. (From Nussbaum R, Mc Innes R, Willard H. Thompson & Thompson Genetics in Medicine. Saunders; 2007)

Basically **polygenic/multifactorial inheritance** means that a trait is caused by a combination of genetic and environmental factors. Examples of such traits include height, weight, intelligence, and blood pressure. Other multifactorial traits like oral clefts are discontinuous in distribution in that the trait is either present or absent (with differing

severities). If there is a sufficient number of predisposing factors (genes and environmental), a threshold is reached and the trait will appear.

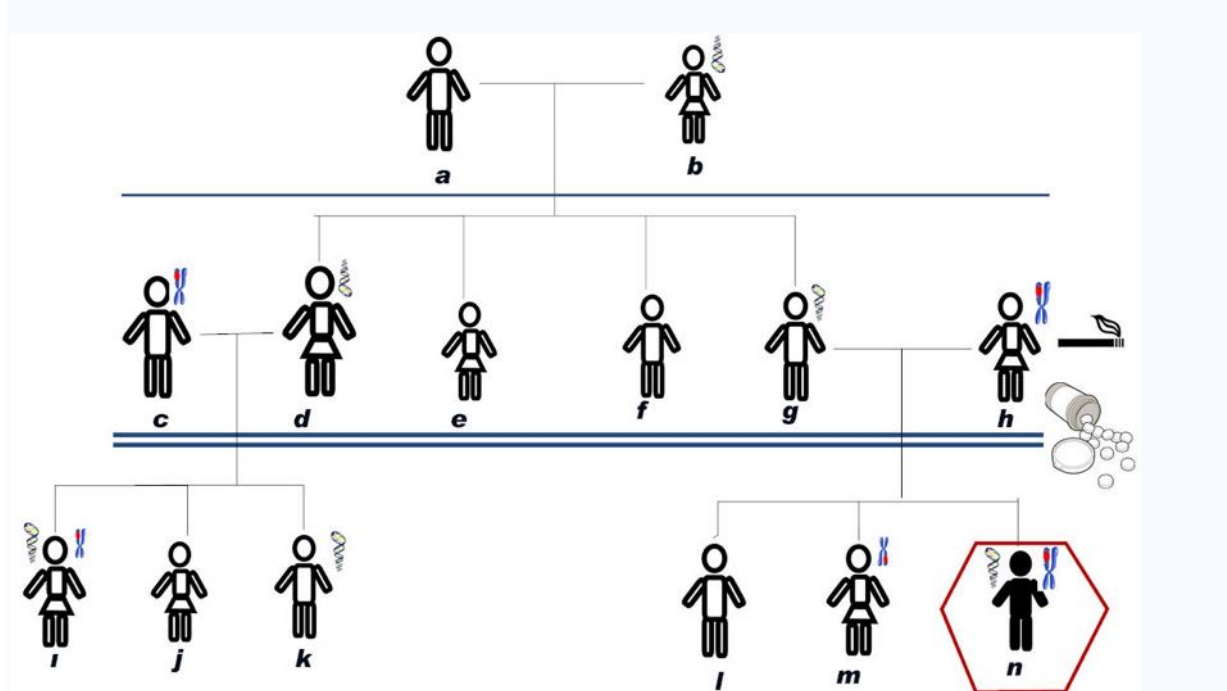


Figure 24. Schematic illustration of the polygenic multifactorial inheritance pattern. In this model in order to appear phenotypically the oral cleft, three factors need to be present at the moment of fusion of the facial prominences (4 to 8 weeks of gestation): mutated gene A, mutated gene B, and environmental factors like smoking or certain medications. The pedigree shown here includes 3 generations and three couples (a-b, c-d, g-h). Just one individual (n) was phenotypically affected by a cleft because he was exposed to all three predisposing factors. In the first generation (couple a-b) nobody was phenotypically affected, but the mother was carrying predisposing gene A. In the second generation two children got the predisposing gene A (d and g) and married individuals carrying predisposing gene B, widely present in their community. In the third generation some individuals did not receive a predisposing gene (j, l); some received just one predisposing gene (m and k); one got both predisposing genes but was not exposed to smoking (i); and one got both predisposing genes and was exposed to smoking during the first 8 weeks after gestation (n). He was the one with the oral cleft.

In recent years, scientists have identified candidate genes (a gene, located in a chromosomal region suspected of being involved in the expression of a trait) that seem to contribute to oral clefts.<sup>24</sup> Most of the today's investigation is centered on those candidate genes. To summarize, "no one locus (position that a gene occupies on a segment of DNA) has clearly emerged as a 'necessary' locus for development of oral clefts. On the contrary, the genetic etiology of oral-facial clefts appears more complex, with several loci showing significant results."<sup>25</sup> More research about these genes and their interaction with environmental factors is needed.



Full analysis of the DNA and patient's information is the cornerstone of current investigations, however the ability to sequence an individual's entire genome allows for the production a huge amount of detailed genetic information about the condition being studied (in this case oral clefts). However not only that can be uncovered. Traits regarding susceptibility to skin cancer, or size of the brain can be mapped, bringing up a lot of ethical challenges about how to protect privacy and respect autonomy.<sup>26 27 28</sup> Imagine that you live in a sunny place and that you donated your DNA (Figure 16) for a research project about clefts, 10 years later you want to get health insurance but the insurance company finds out that you participated in a genetic study and that your genetic information is accessible to them. After accessing your genome information the insurance company realizes that you have a higher risk of skin cancer and they decide not to cover you or to bill you extra for your policy.

Full genome studies are very important to understand better the cause of clefts and volunteer research participants are needed. But every research project that uses full genome needs to be authorized by a Research Committee or Institutional Review Board ensuring that all the standards of modern ethics are followed. Also there is a need to have robust oversight mechanisms.<sup>29</sup>

## ENVIRONMENT

Many environmental factors have been examined in epidemiologic studies of risk factors for oral clefts. Some implicate risk factors include exposure to medications during pregnancy, maternal alcohol consumption, maternal smoking, dietary and vitamin deficiencies, maternal metabolic factors like diabetes,<sup>30</sup> exposure to environmental toxins, altitude,<sup>31</sup> birth order,<sup>32</sup> socioeconomic status,<sup>33</sup> and parental age.<sup>34</sup> For many of these factors, no conclusive trend in cleft incidence has been demonstrated across populations because there are several sources of bias and it is not easy to prove their roles in the cleft formation.

The best evidence to date is for maternal alcohol consumption, smoking, folic acid deficiency and certain medications during pregnancy.

### Maternal Smoking

Maternal tobacco smoking during pregnancy is associated with a variety of adverse outcomes like: low birth weight, preterm<sup>35</sup> weight and presence of oral cleft defects in newborns. The increased risk is around twofold.<sup>36 37 38 39</sup>

Potential teratogens in tobacco smoke include nicotine, aromatic hydrocarbons (PAH), N-nitrosamines, and carbon monoxide. These compounds are absorbed into maternal blood and reach the developing fetus, yet the mechanisms through which tobacco smoke may cause abnormal development remain poorly understood. The presence of developmental abnormalities in infants whose mothers smoked during pregnancy is likely associated with the level of fetal exposure to these teratogens<sup>40</sup> (any agent that causes a structural abnormality following fetal exposure during pregnancy).

## Alcohol Consumption

Fetal alcohol syndrome is a congenital malformation found in children of mothers with chronic alcoholism. The face is characteristic in this syndrome, with abnormal shaped eyelids, nose, cheekbones and lip (from thin upper lip to cleft lips). Neural crest cells (the ones inducing the merging and fusion of the facial prominences during the embryonic period of life) are damaged by the alcohol and that is why these deformities occur.<sup>41</sup>

There is no increased risk of cleft with relatively low quantities of maternal alcohol consumption, but there is increased risk of clefting with higher quantities of alcohol consumption. Women who consume 5 or more alcoholic drinks per week have an increased risk of having a child with isolated oral cleft.<sup>42</sup> Scientists have found that the interaction of at least one candidate gene with alcohol increases the odds to have a child with an oral cleft.<sup>43</sup>

## Folic Acid Deficiency

Folic acid plays an important role in early fetal development. It has been proven that folic acid supplementation during the first 4 months of pregnancy provides significant protection against cardiovascular defects, neural tube defects (anencephaly, spina bifida), CL/P, and CP.<sup>44</sup> The protective effect of folic acid against neural tube defects is higher than the one against oral clefts; however, the protective effect of folic acid against oral cleft is still important.<sup>45 46 47</sup>

Data is strong enough to justify the recommendation of folic acid supplementation for pregnant woman, especially during the first three months of pregnancy.<sup>48</sup>

## Medications

*Retinoids.* Isotretinoin better known as accutane or roaccutane, is a form of vitamin A used for treatment of severe acne. Oral administration of isotretinoin during the first month of human pregnancy can induce severe congenital malformations. Isotretinoin induced facial malformations in humans, include rudimentary external ears, absent or imperforate auditory canals, deformed and small skull, cleft palate, depressed midface, and anomalies of the brain, jaw, and heart.<sup>49</sup>

*Anticonvulsants.* Maternal use of anticonvulsants is associated with an increased risk of congenital defects. Epileptic mothers managed with a multidrug anticonvulsant regime had a 10-fold increased risk of infants with cleft lip/palate when compared to non-epileptic mothers.<sup>50 51</sup>

*Steroids.* Consumption of steroids during the first 3 months of pregnancy has been associated with clefts, increasing the risk 3-5 times.<sup>52 53</sup>

Practical efforts to translate scientific advances related to birth defects into improved community outcomes must proceed on several fronts.

The first step is to gain scientific knowledge about the causation of oral clefts.

Translating research knowledge into public health actions should be also our responsibility. These efforts usually start with policy-making initiatives that establish scientifically based prevention programs and are followed by an evaluation of their success or failure. In other areas of maternal and child health, public health prevention programs have proven successful. An oral cleft prevention program of this magnitude would be a hard but worthy goal and an amazing achievement if successful.

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## **GLOSSARY**

**Adverse outcome**- an unfavorable result

**Allele**- any varying form of a gene that may occur at a given locus

**Anencephaly**-occurs when the cephalic (head) end of the neural tube fails to close, usually between the 23rd and 26th day of pregnancy, resulting in the absence of a major portion of the brain, skull, and scalp

**Anticonvulsants**- medications used to control the occurrence of seizures

**Apert Syndrome**- syndrome in which a cleft palate is associated with deformities of the skull, shallow and broadly spaced orbits, deficient growth of midfacial bones, respiratory problems and syndactyly (two or more digits are fused together)

**Asymmetric**- not the same or equal on both sides

**Autosome**- a chromosome not involved in determining an organism's sex; 22 of the 23 pairs of human chromosomes

**Autosomal dominant disorder**- a disease or trait that develops when only one copy of the abnormal autosomal gene (from one parent) is present

**Autosomal recessive disorder**- a disease or trait that develops when two copies of an abnormal autosomal gene (from both parents) are present

**Bifid uvula**- cleft occurring when the uvula is split into two; the most common type of oral cleft

**Bilateral cleft lip**- cleft occurring on both sides of the lip

**Candidate gene**- a specific gene believed to be responsible for the expression of a given trait

**Cell**- the basic structure of all living organisms; in masses by type, they make all parts of the human body

**Cellular degeneration**- breakdown of cells to allow fusion of prominences

**Chromosome**- rod like structures of DNA and structural proteins in a cell's nucleus that contains all genetic material; humans have 22 pairs plus 2 sex chromosomes

**Cleft lip**- fissure or opening of the lip, it is a birth defect resulting from the failure of the maxillary and nasal prominences to fuse

**Conception**- union of the sperm and egg to produce a new organism of the same species

**Congenital defect**- defect existing at birth. It may be the result of genetic abnormalities, the intrauterine (uterus) environment, errors of morphogenesis, or a chromosomal abnormality

**Cleft palate**- fissure or opening of the palate, it is a birth defect resulting from the failure of the medial and lateral palatine processes to fuse

**Complete cleft lip**- the sides of the lip do not connect at any point

**Consanguinity**- involving persons of the same origins or ancestry

**Diabetes**- a disease characterized by the body's inability to produce or regulate insulin and glucose (sugar) in the blood system

**DNA**- the genetic code contained in every cell that serves as instructions for constructing and operating an organism

**Dominant gene**- a strong gene that is always expressed phenotypically when it is present.

**Encode**- transforming genetic information from one format into another

**Etiology**- the cause of a disease

**Embryo**- a developing human after fertilization, until the eighth week of pregnancy

**Epileptic**- a person afflicted with epilepsy (a seizure disorder)

**Exposure**- being subjected to an outside effect or influence

**Facial prominences**- anatomical structures of the face that fuse together during pregnancy

**Fecundation**- process of producing a new organism through fertilization or joining of the egg and sperm

**Fetal alcohol syndrome**- a congenital malformation in children of mothers who drank during pregnancy, characteristic facial features can be associated with other mental defects

**Folic acid**- a member of the B vitamin family, used to help the body metabolize complex carbohydrates and proteins; consumption during pregnancy is necessary for proper growth and development of the fetus

**Fusion**- joining of two parts into one structure

**Gene**- the basic unit of heredity in a living organism; collectively creating an organism's DNA

**Genetics**- the study of how genes are inherited from one's ancestors and how they manifest



**Genome**- the full inherited DNA sequence of an organism

**Genotype**- the genetic identity of an individual that does not show as outward characteristics

**Gestation period**- the nine months of pregnancy

**Growth factors**- substances capable of stimulating or influencing cellular growth

**Heterogeneous**- different, diverse, varied

**Heterozygous individual**- one who has two different alleles occupying a gene's position on the homologous chromosomes

**Homozygous individual**- one who has identical alleles of present on both homologous chromosomes

**Incidence**- the number of cases in a population during a given period of time

**Incomplete cleft lip**- part of the lip is fused together; the cleft does not reach the nasal cavity

**Inheritance pattern**- the way in which genes are passed from one generation to another

**Institutional Review Board (IRB)**- an oversight committee responsible for determining if research studies are ethical and protect the rights and well beings of research subjects

**Karyotype**- is an organized profile of a person's chromosomes; chromosomes are arranged and numbered by size, from largest to smallest.

**Locus**- position/location on a gene, plural form is loci

**Mandibular**- relating to the lower jaw bone

**Maxillary**- relating to the upper jaw bone

**Molecule**- the simplest structural unit of a compound that retains and exhibits all the properties of the compound

**Midface**- the portion of the face containing the nose, upper jaw and cheek bones, as well as their surrounding muscle and tissue

**Migration**- movement

**Miscarriage**- a spontaneous end to a pregnancy at a stage where the embryo or fetus is incapable of surviving (prior to 20 weeks of gestation)

**Mitosis**- division of cells during pregnancy

**Morphogenesis**- development and transformation; is the biological process that causes an organism to develop its shape.

**Mutation**- an alteration in genetic structure or sequence

**Neural crest cell**- an embryonic cell from the neural tube that migrates through prescribed regions of the embryos where they differentiate into most of the peripheral nervous system as well as the facial skeleton and pigment cells

**Neural tube defect**- defects involving abnormalities in the development and growth of the brain and spinal cord

**Non-syndromic cleft**- not associated with a recognized syndrome (no associated birth defects)

**Palate**- the bone and soft tissue making up the roof of the mouth

**Palatine processes**- sections of the palate that fuse during pregnancy to form the roof of the mouth; can be medial (front center) or lateral (sides)

**Pedigree**- chart used to show the genetic relationships between individuals

**Phenotype**- the physical traits of an individual or organism that result from its genotype, environment and their interactions

**Polygenic/multifactorial inheritance**- inheritance pattern where a trait is caused by a combination of genetic and environmental factors

**Preterm**- a baby born at less than 37 weeks of gestation

**Prominence**- elevation of an anatomical structure

**Protein**-organic compounds made of amino acids, considered the building blocks of cells; essential in muscle and tissue growth and development

**Recurrence**- the expression of a given trait multiple times in different people

**Recurrence risk**- the chance that a defect will occur again

**Retinoid**- a form of vitamin A often used to treat acne; can interfere in normal embryonic and fetal development causing birth defects; accutane and roaccutane are examples

**Risk factor**- any internal or external influence on a body or cell that would increase the likelihood of an event occurring

**Sequence**- order DNA markers occur in

**Spine bifida**- occurs when the caudal end (lumbar, sacral) of the neural tube fails to close; some vertebrae overlying the spinal cord are not fully formed and remain unfused and open. If the opening is large enough, a portion of the spinal cord sticks out between the openings in the bones.

**Steroids**- a group of drugs used to minimize tissue swelling and relieve inflammation

**Syndromic cleft**- associated with a recognized syndrome (associated birth defects)

**Syndactily**- when two or more digits are fused together

**Teratogens**- any agent or substance that interferes with normal embryonic and fetal development causing birth defects

**Trait**- a distinguishing feature of an organism

**Treacher Collins Syndrome**- syndrome in which a cleft palate is associated with downward slanting eyes, a small lower jaw, hearing loss, underdeveloped cheek bones, drooping lateral lower eyelids and malformed or absent ears

**Translocate**- when a DNA sequence is rearrange or a chromosomal segments is transferred to a new position

**Trisomy**- genetic condition in which a third chromosome is inherited in addition to the traditional pair; often results in miscarriage

**Unilateral cleft lip**- a cleft occurring on only one side of the lip

**Van der Woude Syndrome**- the most common syndrome associated with cleft lip and palate, characterized by the cleft, pits in the lower lip, or both

**Zygote**-the cells resulting from the union of a sperm and egg

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