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Cleft Lip and Palate in the Dog: Medical and Genetic Aspects

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Abstract

The same types of cleft lip and/or cleft palate (CL/P) that affects humans also naturally affect dogs. Therefore, the dog has become an important spontaneous animal model for the study of human oral clefts. In order to provide an overview of CL/P in dogs to people with an interest in this area, we present in this chapter the main medical aspects, ranging from the etiology to the prevention, and also the main genetic aspects, including inheritance mechanisms and highlighting the homology between the two species, and the most recent molecular findings.

Keywords: dog, cleft lip, cleft palate, cleft lip and palate, oral clefts, genetics

1. Introduction

In the last 20 years, the domestic dog has become one of the main animal models for the study of genetic disorders and congenital defects due to advances in genetics and genomics. The frequent occurrence of birth defects in dogs, with the cleft lip and palate being among the most common, is a byproduct of breeding practices. Since the mechanisms responsible for the morphogenesis of mammals are highly conserved and the genomic similarity between dogs and humans is high, in addition to sharing the same environment, spontaneous cases of cleft lip and palate in dogs are exceptionally useful for studies on the pathogenesis and genetics of oral clefts and the morphogenesis of the face [1–3].

In this chapter, we present an overview of the medical and genetic aspects of cleft lip and palate in dogs, in the hope that it will be useful to veterinary clinicians, researchers, and other professionals interested in genetics and developmental biology.

2. General considerations

2.1. Considerations on homology

It is easy even for a layperson to see that human anatomy and physiology have their equivalence throughout the zoological scale of vertebrates, especially when it comes to tetrapods. It is also not difficult to deduce that the mechanisms of development are similar or even identical, especially when we compare eutherian mammals. However, when we think of genes, genotypes, and their mechanisms of action, there is a tendency to conclude that everything is quite different. Nevertheless, in reality, “our genome” is not as exclusively ours as we generally imagine. Dogs and mice share over 90% of our genes [4], enabling us to suppose that genetic programs that control embryonic development are similar in the three species. Genes with a common evolutionary origin, maintaining the same function in different species, are known as orthologs (**Figure 1**). They are clear evidence that the homology of structures among species also have a molecular base. For instance, the *ADAMTS20* gene is one of the necessary genes for the normal palatogenesis of mice, to the extent that homozygous individuals for a mutation with loss of function have a palatal cleft [5]. Recently, a recessive mutation in the canine ortholog was identified in dogs with a cleft palate [6].

Knowledge of the developmental biology and genetics of one species helps us to understand those of another. Much has been learned regarding craniofacial morphogenesis by studying chickens and mice [7–9]. The dog, which has contributed so much to the development of

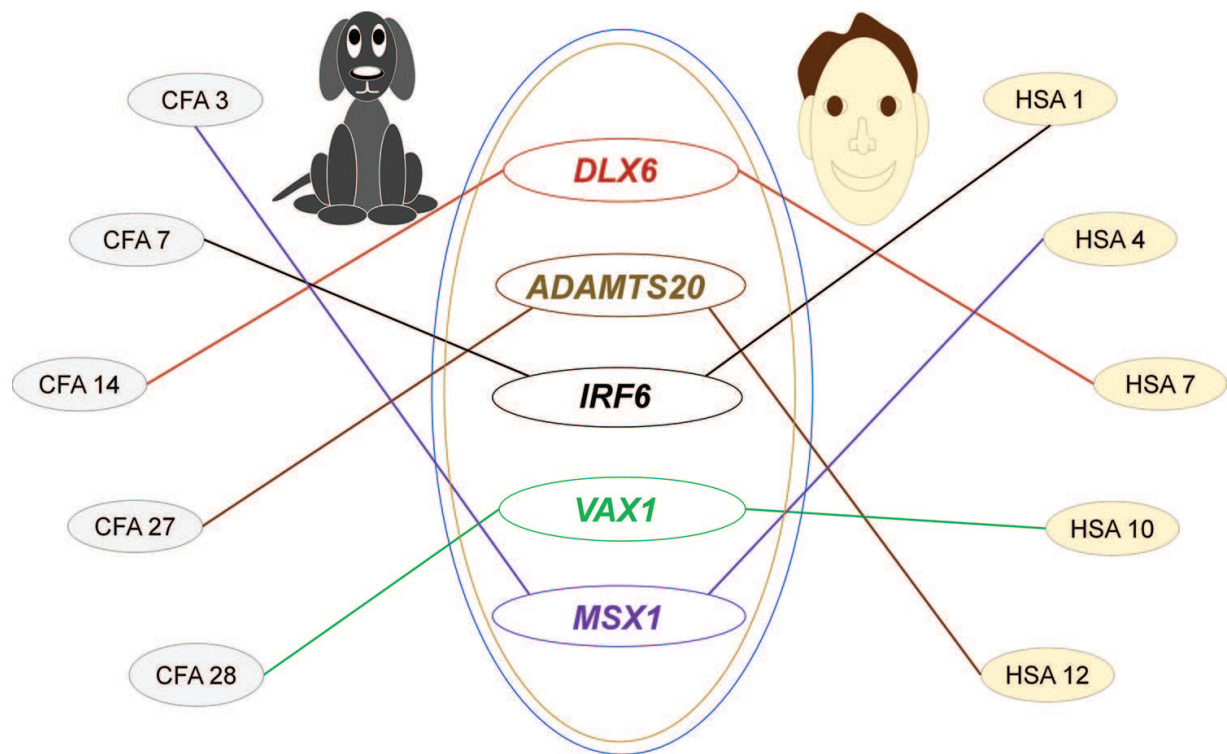


Figure 1. Shared genome. Examples of orthologs with the respective chromosomal assignment in the dog (CFA) and man (HSA). In two of them (*ADAMTS20* and *DLX6*), mutations are known that cause cleft lip and palate in a breed of dogs, while in the other three, mutations are known that have been associated with cleft lip and palate in humans.

surgical techniques used today to correct oral defects, can also help to expand our knowledge of the pathogenesis and genetics of orofacial defects.

2.2. Considerations on the morphogenesis of the lip and palate

Orofacial development is a sequence of events in space and time that involve cellular multiplication, migration and differentiation, tissue fusion, and apoptosis and are dependent on the action of various signaling molecules and transcription factors [10].

The primitive mouth is called the stomodeum. It emerges as a slight depression on the ectodermal surface, delimited by mesenchymal structures where cells from the neural crest proliferate. Although these cells are ectodermal in origin, they settle and integrate with the mesenchyme of the head of the embryo. They are fundamental to the development of the craniofacial structures. Five structures surround the stomodeum: frontonasal prominence, from which the primary palate will originate; right and left maxillary prominences, from which the secondary palate will originate; and right and left mandibular prominences, from which the mandible will originate (**Figure 2**). The maxillary and mandibular prominences are derived from the first branchial arch [10, 11].

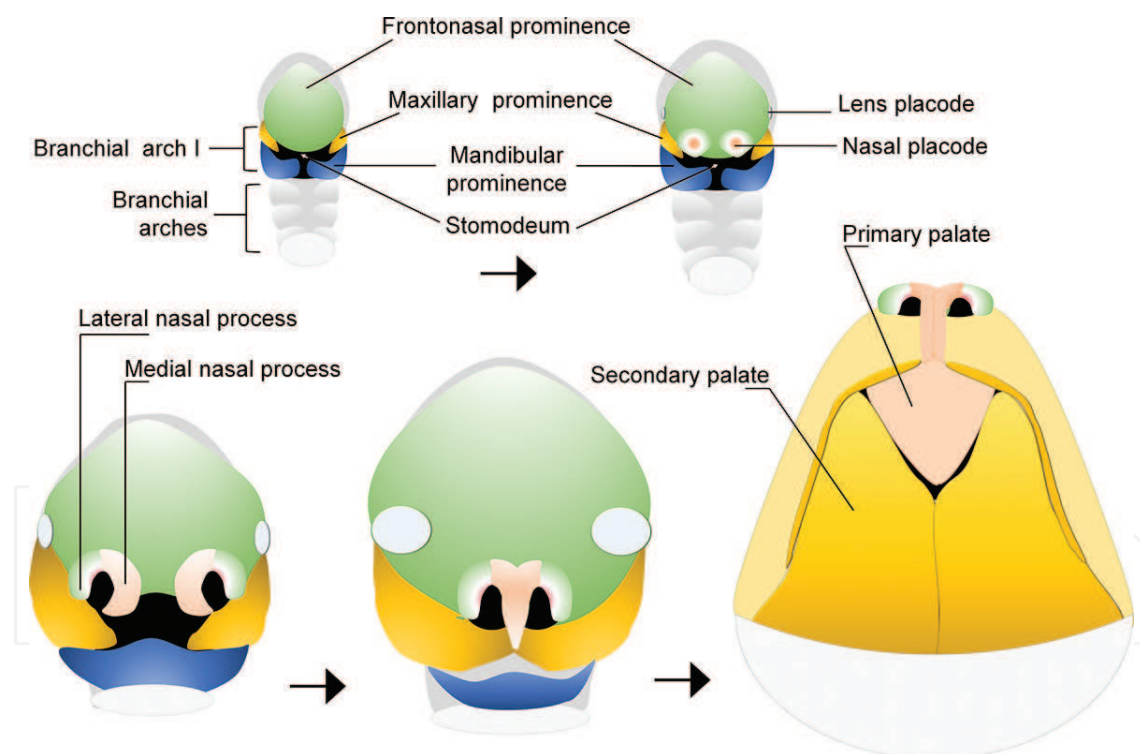


Figure 2. Palatogenesis. Semischematic drawing representing the formation of the primary and secondary palate in dogs.

2.2.1. Formation of the primary palate

The primary palate is the primordium of the hard palate (incisive bone) rostrally located at the incisive fissures (incisive foramen in humans). During development, the frontonasal prominence forms a pair of lateral and medial nasal processes. The fusion of the lateral and medial parts of each process delimits the nasal cavities that are forming. The medial processes

are then lengthened and projected between the maxillary prominences, are fused with them, and transformed into the primary palate and medial part of the upper lip [11].

2.2.2. Formation of the secondary palate

The secondary palate is the primordium of the palate caudally located at the incisive fissures (hard palate and soft palate, so-called because its formation is completed after the formation of the primary palate). Initially, the maxillary prominences are projected vertically by the sides of the tongue, and are then raised and projected horizontally on the tongue until they meet. A fusion then occurs between the two in the medial line forming a continuous epithelial seam, which will subsequently disappear. Rostrally, the secondary palate is also fused with the primary palate and, dorsally, with the projection (nasal septum) formed by the united medial nasal processes. The maxillary prominences also form the lateral parts of the upper lip [11].

At approximately 23 days of gestation, in the canine embryo it is possible to see the frontonasal, maxillary, and mandibular prominences. At approximately 28 days, the first ossification of the maxilla and mandible occurs [12].

3. Medical aspects

3.1. Frequency

A cleft lip and/or palate can affect purebred dogs or mongrels. Any canine breed can be affected, especially if we consider cleft lip and palate caused by environmental teratogens. However, the relatively frequent occurrence in some breeds indicates a strong contribution of genetic factors [13].

Indeed, certain breeds of dog are more likely to have cleft lip and palate, especially brachycephalic dogs [14]. At least, this is the clear impression of numerous veterinary practitioners who work with small animals worldwide. Unfortunately, no statistics are yet available that enable definitive statement regarding frequency in different breeds, nor in canine species as a whole.

In boxers, a frequency of 0.6% has been recorded, while in beagles has been 0.11%, and in Pyrenees shepherd dogs, 2.2%. In Portuguese water dogs, cleft palate has been reported in 2.3% of litters [15–18].

In some cases, the high frequency observed at veterinary clinics in certain breeds may be due to the popularity of those breeds at a given time. It is also possible that the frequency is high in certain lines due to constant inbreeding, but not high in the breed as a whole. In a lineage of old Spanish pointer dogs a frequency of 15–20% was found [19].

Table 1 shows the breeds that are considered as having a predisposition to oral clefts or for which cases have been registered.

Affenpinscher	Chihuahua	Nova Scotia duck-tolling retriever
Akita inu	Collie	Old Spanish pointer dog
American cocker spaniel	Dachshund	Papillon
American pit bull terrier	English bulldog	Pekingese
American Staffordshire terrier	English pointer	Poodle
American water spaniel	English toy spaniel	Portuguese water dog
Australian shepherd	Finnish spitz	Puli
Australian terrier	Fox terrier	Pug
Basset hound	French bulldog	Pyrenees shepherd dog
Beagle	German shepherd dog	Rottweiler
Bearded collie	Giant schnauzer	Samoyed
Bernese mountain dog	Golden retriever	Schipperke
Bichon frise	Great Pyrenees	Scottish terrier
Boston Terrier	Irish setter	Shetland Sheepdog
Bouvier des flandres	Italian greyhound	Shih Tzu
Boxer	Labrador retriever	Silky terrier
Brittany spaniel	Maltese	Staffordshire bull terrier
Brussels griffon	Manchester terrier	Swiss sheep dog
Bull terrier	Mastiff	Welsh corgi, cardigan
Bullmastiff	Miniature pinscher	West Highland white terrier
Cairn terrier	Miniature schnauzer	Yorkshire terrier
Cavalier King Charles spaniel	Norwegian elkhound	
Refs. [13, 14, 17–23].		

Table 1. Breeds with records of CL/P.

3.2. Classification

Successful communication between professionals (veterinary practitioners, geneticists, surgeons, dentists, etc.) who treat patients with CL/P depends on an appropriate and correct registration of these abnormalities adhering to common criteria by everyone involved. Thus, the adoption of a classification is highly important. Furthermore, a consistent register based on a classification helps to establish the cause, planned treatment, prognosis, and studies of comparative anatomy [24].

The different classifications used in human medicine can be adapted for use with dogs, as has been done by some researchers based on the first classifications of human oral clefts [24, 25]. Many of the classifications of human clefts are modifications of the classification of Kernahan and Stark [26], which will be adopted here for the purposes of this chapter. It is based on the

morphology and pattern of embryonic development of mammals. The clefts are clustered into three groups, each with three subgroups, with all of them considering the degree of impairment of the structures as total or partial (**Figures 3 and 4**):

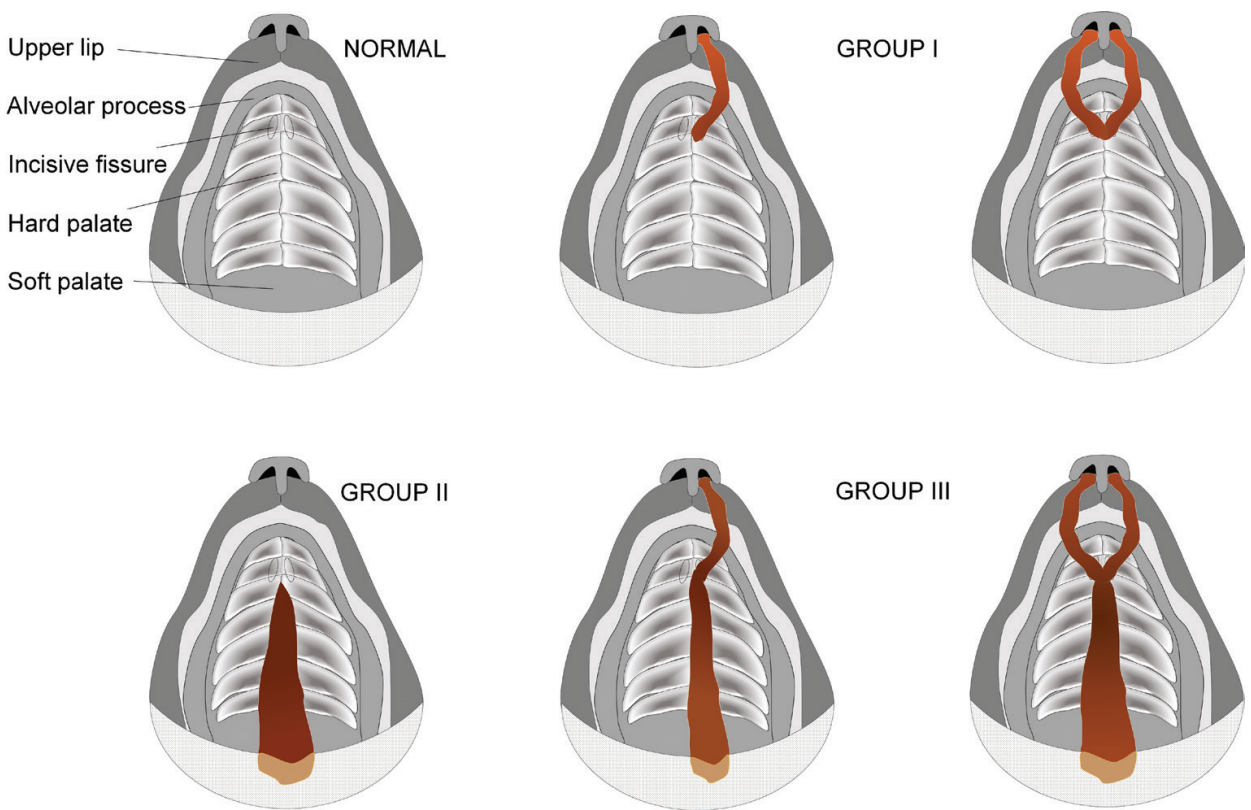


Figure 3. Types of cleft. In each group, complete unilateral or bilateral clefts are shown. However, a cleft from Group I can be left- or right-sided and affect only the lip, the lip and the alveolar process, or include the entire extension of the primary palate, as shown in the illustration. Likewise, a cleft from Group II may affect only the soft palate or the soft palate and the hard palate.

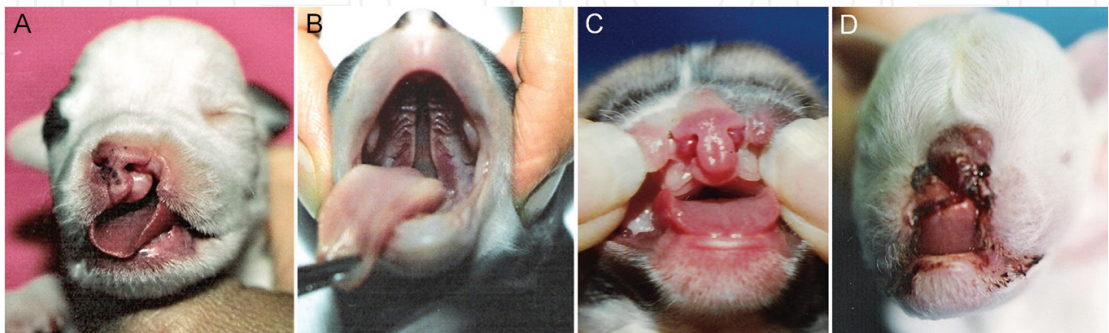


Figure 4. Dogs with nonsyndromic (A–C) and syndromic (D) clefts. (A) Left-sided unilateral cleft, affecting the upper lip, alveolar process, and incisive bone (primary palate); (B) cleft palate only; (C) bilateral cleft (upper lip, hard palate, and soft palate); (D) anophthalmia and CLP. Photographs (A–C) reprinted from Moura and Pimpão [35].

Group I. Primary cleft palate (total or partial impairment)

1 – unilateral left or right; 2 – medial; 3 – bilateral

Group II. Secondary cleft palate only

1 – total; 2 – partial; 3 – submucous

Group III. Primary and secondary palate (total or partial impairment)

1 – unilateral left or right; 2 – medial; 3 – bilateral

The criteria for defining a cleft as partial (incomplete) or total (complete) is subjective. Thus, with broader objectives, especially for epidemiological studies and minute comparison with human clefts, we suggest using the numerical system adapted by Schwartz et al. [27] from the striped Y of Kernahan [28], known as the RPL system, or one of the others that are available.

3.3. Etiology

Cleft lip and/or palate (CL/P) in dogs, as in humans, are etiologically heterogeneous, and can be caused by genetic factors, environmental factors, or a combination of these two groups of factors [29, 30].

Mutations in different genes, both in murine models and human beings, have been associated with CL/P [29]. As these genes have the respective homologs that are also present in the canine genome, the same situation is expected to occur in dogs (see Section 4).

The environment of an embryo is represented by the amniotic sac, uterus, maternal body, and the place where the mother lives. Thus, the potentially negative influences of this environment include amniotic abnormalities, uterine abnormalities, maternal metabolic disease, viruses, chemical substances swallowed by or administered to the mother, and maternal exposure to chemical or physical environmental pollutants [31]. Few studies of dogs associate a given environmental factor to oral clefts. Furthermore, these studies focus on substances administered to the mother of the affected dogs during gestation, such as 6-diazo-5-oxo-L-norleucine, aspirin, and vitamin A [32–34]. However, it should be remembered that in the case of aspirin and vitamin A, excessive doses were used, much higher than therapeutic doses. Based on the data obtained in other species (mice, rats, cats, goats, etc.), or personal impressions, it has been suggested that maternal exposure to various substances such as hydroxyurea, griseofulvin, anabasine, metronidazole, primidone, sulphonamides, and corticosteroids can cause oral clefts in dogs [30]. Indeed, as the morphogenic processes are highly conserved [35], the same causes of oral dysmorphogenesis known in man can also be found in other species of mammals, including dogs, and vice versa (**Table 2**).

The interaction between genetic and environmental factors is a known underlying phenomenon of the development of certain phenotypes [38]. Evidence has already been found in humans, linking certain genetic markers to CL/P. For example, maternal smoking in combination with the variants of the *GSTT1* and *IRF6* genes increases the risk of clefts [29]. It should be remembered once again that dogs and humans have high genomic homology and share the same environment [1]. Therefore, similar or even identical interactions may occur.

Amoxicillin	Maternal hyperthermia
Anticonvulsants (diazepam, phenytoin, phenobarbital, topiramate)	Maternal obesity
Cholesterol deficiency	Retinoic acid
Corticosteroids	Smoking
Folate deficiency	Stress
Fluconazole	Viral infections
High parental age	Vitamin B complex deficiency
Hyperglycemia	Zinc deficiency
Ionizing radiation	
Maternal alcohol consumption	Others (occupational exposures, environmental pollutants)

Obs.: Not all the risk factors presented in this table are definitely associated with CL/P, and further studies are required. Several factors (amoxicillin, corticosteroids, maternal obesity, stress, etc.) have not shown a consistent association and there are discrepancies between the studies.

Refs. [29, 36, 37].

Table 2. Presumed or confirmed risk factors that have been associated with CL/P in humans.

3.4. Pathogenesis

Due to its etiology, a cleft lip or palate may be the result of an originally abnormal development process or negative interference in a normal development process, corresponding to the concepts of malformation and disruption, respectively, used in dysmorphology [35].

The heterogeneous etiology, in cases of malformation and disruption, assumes varied mechanisms in the development of CL/P. While some mechanisms impair the morphogenesis of various structures in addition to the palate, resulting in syndromic clefts, others act only in the palatogenesis, resulting in nonsyndromic clefts [29, 35].

Developmental field (or morphogenic field) theory aids understanding because different factors can cause the same type of defect. In the early stages, the whole embryo represents a developmental field (primary field). Later, a developmental field is a region or part of the body of the embryo which responds as a coordinated unit to embryonic induction and gives rise to multiple or complex anatomic structures [39, 40]. The induction depends on influences, both physical and chemical, that one developing tissue has on another (or others) in embryogenesis [39]. Developmental fields are systems that control the progressive differentiation of the structure and size, in addition to the temporal and spatial distribution of complex organ components [40]. During blastogenesis, the interactions of the primary field (embryo) generate the progenitor fields (primordia of the final structures) that, in turn, create the secondary fields that produce the final structures during organogenesis [41].

Defects in a structure or in part of the body result from disturbances in one or more secondary fields and are known as monotopic field defects, such as nonsyndromic oral clefts. Multiple defects are the result of disturbances in the primary field or progenitor fields, as occurs in

individuals with various defects, including CL/P (syndromic clefts). Correlated defects that emerge early during blastogenesis and affecting structures in different parts of the body are polytopic field defects [41].

At any time during embryogenesis, disturbances in the developmental fields can reflect negatively on fusion mechanisms between the lateral and medial nasal processes, and the medial nasal processes with the maxillary processes (Group I clefts); and/or the mechanisms of development, elevation, and fusion of the palatal shelves and the disappearance of the midline epithelial seam (clefts in Groups II and III).

3.5. Patient evaluation

The diagnosis is conducted by visual inspection of the entire extension of the oral cavity, from the premaxilla (incisive bone) to the soft palate. Without this precaution, smaller clefts may go undetected, especially those that affect the soft palate only.

Cleft lip is evident, however, it indicates the need for a thorough and detailed examination of the oral cavity of the patient and the entire organism in search of other congenital abnormalities to determine whether the cleft is an isolated (nonsyndromic) defect or part of a larger (syndromic) condition.

In newborns, difficulty in nursing, nasal reflux of milk, and fault in development are frequent clinical signs. In older patients, in addition to delayed development, choking, coughing, and sneezing during feeding are common. Nasal discharge is also frequent, but the existence of one or more clinical signs and their intensity depends on the location and gravity of the cleft. It is important to be attentive to clinical manifestation resulting from complications, especially signs of pneumonia, a condition that requires immediate treatment.

Detailed record of the oral cleft is essential for adequate planning of treatment, evaluation of postsurgery progress, and studies with different purposes.

Evaluation of the general condition of the patient may include routine laboratory tests and X-rays. Computerized tomography may be useful for planning surgical treatment [3]. The simultaneous existence of oral cleft and other congenital defects justifies a karyotype test.

Irrespective of the existence of obvious abnormalities or clinical signs, inspection of the oral cavity should be part of the physical examination of all newborns.

3.6. Complications

Cleft lip in general means no complications or complications limited to suction problems. However, clefts that affect the incisive bone and, above all, those that affect the secondary palate cause problems of feeding, breathing, and malocclusion. They cause rhinitis, rhinosinusitis, and occasionally otitis media [42, 43]. They can also cause aspiration pneumonia with risk of death. Malnutrition, dehydration, and accumulation of food in the cleft are commonplace.

Unlike in humans and for obvious reasons, difficulty in emitting sounds is not important in dogs and speech defects do not exist.

3.7. Treatment

Cleft lip and palate require corrective surgery to enable adequate function and for esthetic reasons. However, the decision to undergo surgery falls to the owner of the dog. Although many opt for euthanasia, every day, people seek veterinary clinics to inquire about treatment for a dog born with a CL/P.

If the owner opts for treatment, it is necessary for him to be fully aware of the intensive work involved before the patient is old enough for surgery. It is also important to give the owner careful guidelines regarding feeding and cleaning procedures for his dog. He should also be warned of the need to be constantly on the lookout for possible complications. Clefts that affect only the lip or the lip and the alveolar process require little of the owner, but the more extensive clefts may require a lot of dedication.

An efficient and minimally invasive technique for feeding dogs with a cleft palate was described by Martínez-Sanz et al. [19] using baby bottle nipples and customized palatal prostheses made of dental thermoplastic plates. During the breastfeeding period, dogs were fed with a commercial maternal milk substitute using a baby bottle with a customized nipple. After weaning, which occurred during the fifth week of life, palatal prostheses were made every week in keeping with the development of the dogs. The palatal prosthesis was kept in the mouth during the day and removed at night. The technique did not impede oral development and the materials used are easily obtained from dental suppliers. The cost is relatively low and accessible to most veterinary clinics [19].

In cases of severe clefts, it is necessary for the newborn to be fed through a stomach tube to ensure its height and weight development and good nourishment. It may even be necessary to create an esophageal or gastric stoma for feeding and hospitalize the patient [30]. These procedures can be found in several textbooks of veterinary hospital techniques.

In any situation, the owner must be duly trained to deal with the patient's condition and clean the oral cavity adequately after feeding. Alternatively, the owner should take the dog to a veterinary clinic every day for adequate care. A collaborative, patient, and well-informed owner is essential for dogs with cleft lip and palate to develop and be ready for a surgical procedure.

The age that most surgeons consider appropriate for the first corrective procedure is between 4 and 6 months, i.e., it is advisable to await permanent dentition eruption. Before this time, dental development may be harmed. It is also important to consider that oral clefts tend to diminish with growth and become stable at around 6 months [30, 44, 45].

The surgery should be carefully planned and all preoperative care should be taken, including stabilization of the nutritional status and the solution of any complications that may arise. Rhinitis or rhinosinusitis should be treated with antibiotics and secretolytic agents. The same medication is used to treat aspiration pneumonia together with oxygen, bronchodilators, and, in some cases, corticosteroids [30].

Several techniques are available to correct cleft lip and cleft palate, ranging from those that use a mucosal flap or mucoperiosteal flap to autologous bone grafts and prostheses in the case of larger clefts. There are also promising procedures that use mesenchymal stem cells of the

iliac bone with hydroxyapatite particles [44, 46–49]. When the correction is done in stages, the functional rehabilitation and esthetic results are better [50]. Although the main purpose is the rehabilitation of the patient, veterinary procedures in plastic surgery and dentistry are now available and would provide a really good esthetic effect in a final step.

Like all surgery, postoperative care is essential for success. Thus, supportive measures and the administration of antibiotics, analgesics, and antiinflammatory medicine should be followed strictly. Care should also be taken regarding the patient's feeding and hygiene.

3.8. Prevention

The prevention of oral clefts in dogs follows the same principles as prevention in humans. In other words, educating people regarding the risk factors and genetic counseling, with appropriate adaptations.

Pregnant dogs should be given a balanced diet and their health should be monitored. They should also be protected from viral agents. The environment where they live should be free of chemical products. Breeders and owners should be warned of the risk to the embryo/fetus from the administration of certain medicines. Before prescribing medicine, veterinary practitioners should check the teratogenic potential of the drug.

In humans, advanced parental age is linked to an increased probability of oral clefts in offspring [51]. However, in dogs, there are not studies on this aspect. Assuming that this is the case with dogs, a preventive measure is to use good sense and avoid crossing very young animals or much older ones.

As in human medicine, in veterinary medicine, mineral and vitamin supplements have been recommended, especially folic acid and vitamins B6 and B12 [52, 53]. However, the results are not definitive and there have been discrepancies between studies [29, 54].

A daily supplement of 5 mg of folic acid in pregnant French bulldogs, beginning on the 15th day and ending on the last day of pregnancy, reduced the frequency of cleft palate by 48.54% in a research period of 18 months [53]. In Boston terriers, a reduction of 76% was observed [52]. In pugs and Chihuahuas, there were reductions of 60 and 66.67%, respectively. A supplement of 5 mg/day was given to pugs and 2.5 mg/day to Chihuahuas from the beginning of estrus to the 40th day of gestation [55].

Considerations on genetic counseling will be given later in Section 4.3.

4. Genetic aspects

The genetic basis of cleft lip and palate is extremely complex due to the potential number of genes involved, their behavior (mode of inheritance, gene interaction, penetrance, expressivity, etc.), number of alleles in each gene, independent segregation (two or more genes), epistasis, and gene linkage, in addition to environmental factors that might cause phenocopies. This complexity, added to the difficulties of maintaining and handling the affected animals,

has severely limited clinical and genetic studies of orofacial clefts in dogs. Consequently, few are available and these will be summarized as follows.

4.1. Syndromic and nonsyndromic clefts

Canine oral clefts may be isolated abnormalities, affecting the lip, lip and palate, or only the palate. They may also coexist with abnormalities in other areas of the body. The former are nonsyndromic clefts and the latter are syndromic clefts. The term “syndromic,” as used here, is well established and corresponds to a syndrome in a general sense, i.e., a set of abnormalities that occur jointly, but does not necessarily correspond to the concept used in clinical genetics, in which a set of abnormalities can indeed be a syndrome, but also an association or sequence [39].

In dogs, there are no conclusive data on the frequency of each of these two groups. However, the clear perception of veterinary practitioners is that the nonsyndromic forms are far more common than the syndromic. In humans, approximately 70% of cleft lip and palate are isolated abnormalities, while 30% are part of multiple abnormalities due to chromosome aberrations, monogenic inheritance, teratogens, or unknown causes [56].

In veterinary clinics, the common procedure for dogs with multiple abnormalities is immediate euthanasia. This is often performed by the owners or breeders, with no records or study. Consequently, little is known about the syndromic forms of cleft lip and palate.

4.1.1. Syndromic clefts

We have seen bilateral anophthalmia and cleft lip and palate in mongrels, omphalocele, and cleft palate in Siberian huskies, and anencephaly and cleft palate in Yorkshire terriers, to name three examples. Most of the few reports available have to do with cases in which it was not possible to identify a cause. However, in four cases, a hereditary pattern was established or presumed and, in two cases, the mutation that was responsible was identified [6, 57–59].

In 2015, Wolf et al. [6] studied 13 cases of CL/P with a phenotypic spectrum ranging from bilateral cleft in the nasal wings to complete CLP in Nova Scotia duck tolling retrievers. Furthermore, 10 of the affected animals had syndactyly in the third and fourth toes, varying from incomplete in only one paw to complete in all four paws. As for the other three dogs, whether they had syndactyly was not known. These abnormalities were the result of autosomal recessive inheritance and were a syndromic form of CL/P with variable expressivity. A mutation in the *ADAMTS20* gene was associated with this phenotype. In 2014, Wolf et al. [57] had already identified another mutation in the same breed: an insertion of a LINE-1 in the *DLX6* gene, causing CP and brachygnathia with a pattern of autosomal recessive inheritance. More details on these mutations are given in the section on molecular aspects.

In 1998, Villagómez and Alonso [58] described four individuals from a litter of six Saint Bernard dogs, the offspring of normal parents. They had a cleft palate, bilateral anotia, supernumerary vertebrae and ribs, bifid tongue, and bilateral pedal preaxial polydactyly. In two of these dogs, there was also a cleft lip and one did not have polydactyly. The parents, in four previous gestations, had 28 offspring, 22 of which were normal and 6 had the same clinical phenotype as the

four affected individuals. As the parents were normal and had affected male and female offspring, the authors of this report concluded that the abnormalities could be a recessive mutation of an autosomal gene, although the action of teratogens could not be discarded.

In 1985, Sponenberg and Bowling [59] studied a family of Australian shepherds in which there was a syndrome lethal only to the males. The affected animals had a cleft palate and multiple skeletal defects (scoliosis, brachygnathia, short tibia and fibula, polydactyly, syndactyly). In the females, the defects were less severe and there was no cleft palate. The authors of this report raised the hypothesis of X-linked inheritance.

There are also brief reports of omphalocele and bilateral cleft of primary palate in Yorkshire terriers [23], cleft lip and unilateral left-sided anophthalmia in a French bulldog [60], and bilateral cleft of the primary palate, anencephaly, and macroglossia in a dog of unspecified breed [61].

4.1.2. Nonsyndromic clefts

Most genetic nonsyndromic clefts occur in families in accordance with the multifactorial inheritance model. However, there are cases in which a Mendelian pattern of inheritance has been documented.

Monogenic inheritance. Monogenic inheritance is one that depends on a single gene and the type that has so far been confirmed in dogs is autosomal recessive. In other words, the phenotype only manifests if the individual has two copies of the mutant allele. Like all monogenic inheritance, it has a characteristic pattern as follows and is shown in **Figure 5** [62]:

- The phenotype occurs approximately with the same frequency in males and females;
- The parents of an affected individual are generally heterozygotes ($Aa \times Aa$) and thus phenotypically normal; although there is the possibility of an affected individual having one or both parents affected, such situations are improbable;
- The phenotype tends to skip generations;
- The risk of recurrence in descendants of the parents of an affected individual is 25%;
- There is a 50% chance of the parents of an affected having heterozygous descendants like them;
- Normal siblings of an affected individual have a chance of approximately 67% of being heterozygotes; and
- Consanguineous unions increase the chance of the phenotype occurring.

This pattern of inheritance was registered in cases of nonsyndromic CL/P in dogs of the Brittany spaniel, Pyrenees shepherd, and boxer breeds.

In Brittany spaniels, Richtsmeier et al. [63] studied dogs belonging to an intensely inbred colony. In 12 litters, 52 individuals were born, 14 of which had a cleft palate (CP). One of them also had a cleft lip (CL). In 10 of these 12 litters, the number of males and females was

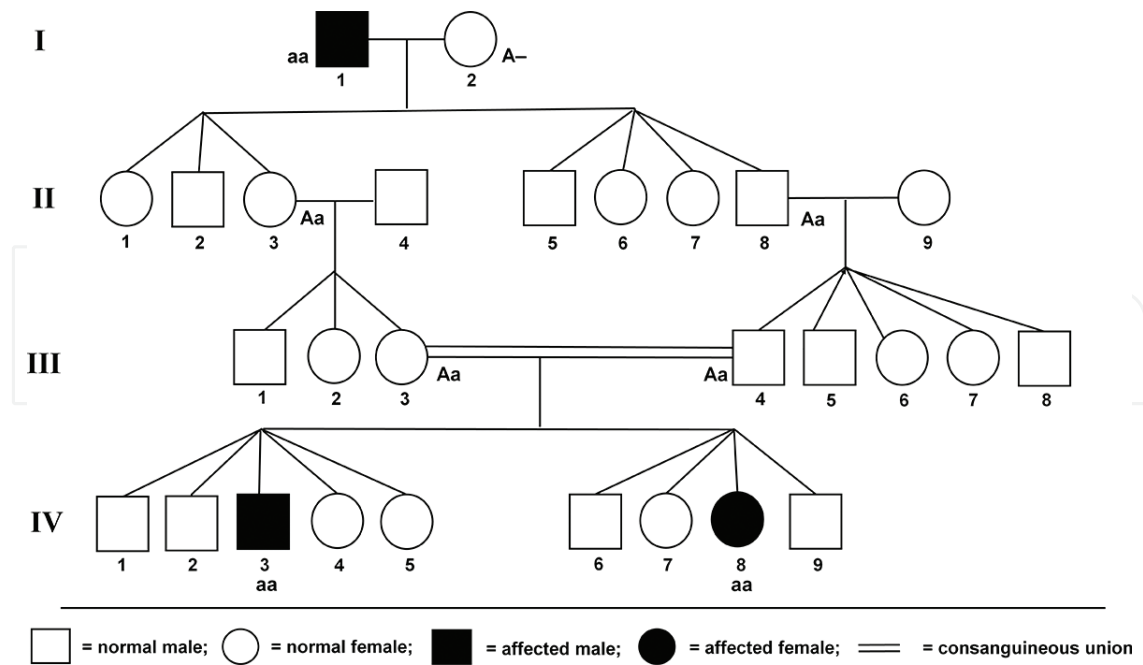


Figure 5. Autosomal recessive inheritance. Consanguineous unions increase the probability that both individuals are heterozygotes, such as couple III-3 X III-4. The risk of recurrence in the offspring of this couple is 25%. The likelihood of having more heterozygous descendants is 2/4 (50%). However, for any one of the normal descendants (male or female) that have already been born, the likelihood is 2/3 (67%).

registered (15 males and 29 females). Of those affected (11), there were more females than males (9 females and 2 males). In all crossings, the parents were normal.

In Pyrenees shepherd dogs, Kemp et al. [17] analyzed the records of a club for this breed over a 20-year period (1984–2004), corresponding to a population of 2104 dogs. They found 47 cases (24 males and 23 females) born in 37 litters with a total of 163 pups and normal parents. Some were only affected by a CP, while others had a cleft lip with or without a cleft palate (CL ± P).

In boxers, Moura et al. [64] found four affected dogs (two males and two females) in two litters with 11 pups born of a consanguineous union (uncle and niece) between normal individuals. All the dogs had essentially the same phenotype (bilateral CLP). Previously, Turba and Willer [15] had raised the hypothesis that in this breed, CLP had a monogenic autosomal recessive pattern of inheritance.

Bleicher et al. [65] reported a case of cleft palate in a beagle together with its pedigree, which is suggestive of autosomal recessive inheritance. There were five affected individuals of both sexes and, in all crossings, the parents were normal.

An older report on cleft palate is suggestive of autosomal recessive inheritance in bulldogs. It presents 33 pups (24 normal and nine affected) born in six litters of a supposedly heterozygous couple [66].

Regarding autosomal dominant inheritance, two reports have described possible cases in which there was nasal cleft, cleft lip, and cleft palate, occurring separately or in association in Bernese mountain dogs (Bernese sennenhund). An affected male that crossed with a normal

female and then with a female German shepherd fathered 26 pups, 11 of which were affected [67, 68]. An abnormality with some similarity was also observed in a Portuguese pointer [69]. However, no further data were published to confirm the mode of inheritance in these dogs.

It should be remembered that, in principle, clefts with different patterns of inheritance could be present in the same lineage, which would hinder the interpretation of the gene segregation mechanism.

Multifactorial inheritance. Nonsyndromic clefts are normally distributed in families without following any monogenic pattern of inheritance, but recurrence in generations is undeniable evidence of a genetic basis. The theoretical model that explains this inheritance assumes the contribution of several genes (polygenic inheritance) with an additive effect. The presence of a determined number of liability alleles would create a critical threshold and different degrees of expression of the phenotype, which can also depend on the influence of environmental factors. For instance, if we represent four genes, segregating independently and with the liability alleles identified by the number 2, and that from five number 2 alleles the critical threshold emerges, then several genotypes would be possible ($A_1A_2 B_1B_2 C_1C_2 D_1D_2$; $A_2A_2 B_1B_2 C_1C_2 D_1D_2$; $A_1A_2 B_2B_2 C_1C_2 D_1D_2$; $A_1A_1 B_2B_2 C_1C_2 D_1D_2$; etc.). Thus, with any combination of five number 2 alleles, the cleft would occur, and the higher the quantity of these alleles, the more serious it would be, with environmental factors also contributing to this. **Figure 6** illustrates this example. There may also be a principal gene that would have a greater effect than the others. In real situations, the number involved is probably much higher than four genes.

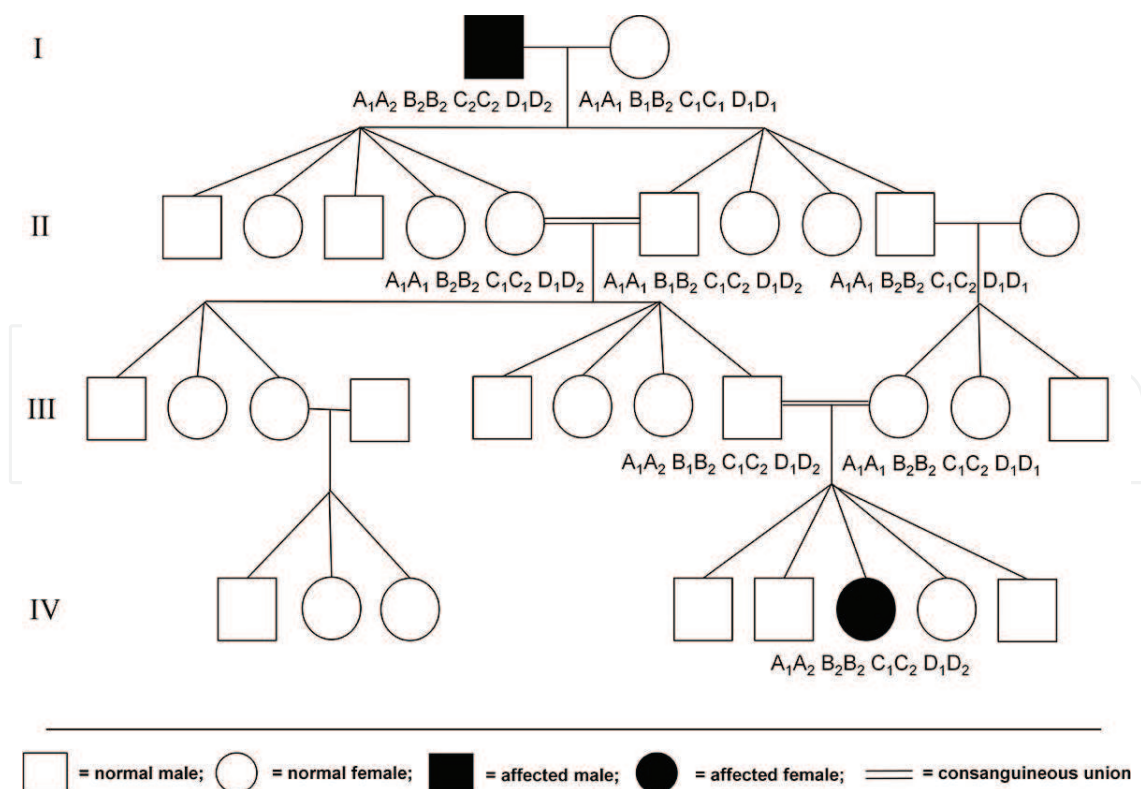


Figure 6. Polygenic inheritance. In this hypothetical pedigree, the individual who inherited at least five number 2 alleles shows the clinical phenotype.

When canine families with high degrees of consanguinity are considered, the critical threshold is more frequent than in families with less or no inbreeding (**Figure 7**). Likewise, the artificial selection process that formed certain breeds led to an increased frequency of liability alleles, making the critical threshold closer than in other breeds and, consequently, leading to a higher frequency of CL/P. As stated previously, there may be a principal gene that increases the risk, as occurs in brachycephalic breeds [70].

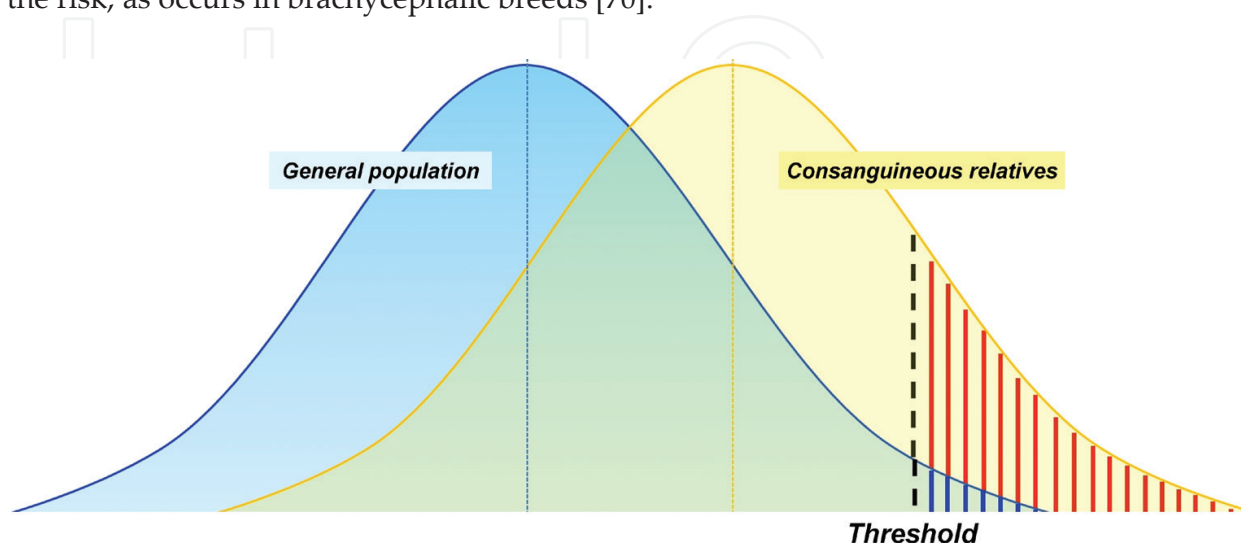


Figure 7. Distribution of genotypes in polygenic inheritance. Comparison of the threshold between the general population and consanguineous relatives or inbred lines.

4.2. Molecular aspects

Modern molecular biology techniques and the use of murine models have enabled the identification of many genes that may be associated with CL/P, and, with each new study, the number of candidate genes grows. The evidence suggests that mutations in these genes, in addition to environmental factors, can act alone or interact with several signaling pathways, negatively interfering in the development of the lip and palate [10]. These genes, and the complex signaling pathways with which they interact, are generally highly conserved in vertebrates and therefore a high degree of homology between man and dog is expected. The identification of mutations in canine genes opens up possibilities for identifying human genes and vice versa, as has happened with the discovery of mutations in mice genes [71]. **Table 3** shows several examples of candidate genes related to CL/P in humans and, potentially, in dogs.

Recently, in Nova Scotia duck tolling retrievers (NSDTR) with a cleft palate and other abnormalities, mutations have been identified in two genes: *DLX6*, located in chromosome 14 of the dog (CFA 14), and *ADAMTS20*, located in chromosome 27 (CFA 27).

In the *DLX6* gene, a LINE-1 insertion was found in the intron 2 jointly segregating with the phenotype (CP and brachygnathia) and obeying an autosomal recessive pattern of inheritance. The presence of the LINE-1 insertion disrupts the transcription of the *DLX6* gene in such a way that only 25% of the normal levels of expression occur, which is not sufficient to prevent CP and mandibular abnormalities. It is located in a noncoding region that is highly conserved,

Gene (abbrev.)	Gene name	Chromosomal assignment (human)	Chromosomal assignment (dog)
<i>IRF6</i>	Interferon regulatory factor 6	1	7
<i>VAX1</i>	Ventral anterior homeobox 1	10	28
<i>BMP4</i>	Bone morphogenetic protein 4	14	8
<i>FGFR2</i>	Fibroblast growth factor receptor 2	10	28
<i>FOXE1</i>	Forkhead box E1	9	11
<i>MAFB</i>	MAF bZIP transcription factor B	20	24
<i>MSX1</i>	msh homeobox 1	4	3
<i>CRISPLD2</i>	Cysteine rich secretory protein LCCL domain containing 2	16	5
<i>FGF8</i>	Fibroblast growth factor 8	10	28
<i>GSTT1</i>	Glutathione S-transferase theta-1-like	22	26
<i>MTHFR</i>	Methylenetetrahydrofolate reductase (NAD(P)H)	1	2
<i>PDGFC</i>	Platelet derived growth factor C	4	15
<i>PVRL1</i>	Poliovirus receptor-related 1 (herpesvirus entry mediator C)	11	5
<i>SUMO1</i>	Small ubiquitin-like modifier 1	2	37
<i>TGFA</i>	Transforming growth factor alpha	2	10
<i>TGFB3</i>	Transforming growth factor beta 3	14	8
Refs. [29, 38, 75].			

Table 3. Examples of genes (human and dog orthologs) that have been associated with CL/P in humans.

disturbing a binding domain for SUZ12, a molecule that plays a significant regulatory role in the development of the embryo [57]. *Dlx* genes form an important family for the development of the first branchial arch, regulating genetic programs that direct the formation of the pattern of the maxilla and mandible [72]. The inactivation of *Dlx5* and *Dlx6* in mice causes serious defects in the craniofacial, axial, and appendicular skeleton, leading to perinatal death [73].

In the *ADAMTS20* gene, a deletion of two nucleotides (AA) was found, segregating together with the phenotype (CL/P and syndactyly) and adhering to an autosomal recessive pattern of inheritance. This deletion represents a frameshift mutation in the metalloprotease domain and should cause the truncation of 1461 amino acids of a protein of 1916 amino acids [6]. The *ADAMTS20* gene is a member of a gene family that encode zinc-dependent proteases. In mouse embryos, its expression is detected in the first branchial arch and between the medial nasal processes [74]. In the palatal mesenchyme, it directs the formation and extension of the palatal shelves [5].

In parallel with the study on NSDTR dogs, Wolf et al. [6] conducted a family-based genome-wide association analysis in a population of native Guatemalans. They identified a significant

association between cases of CL/P and the *ADAMTS20* gene, lengthening the list of candidate genes for the etiology of oral clefts in humans.

4.3. Genetic counseling

Like any genetic abnormality, the main recommendation in cases of CLP in dogs is that affected individuals should not be crossed, nor should normal couples with affected descendants ever be crossed again. As the majority of oral clefts in dogs appear to be multifactorial or recessive, it should be noted that owners of normal dogs who have had affected offspring are not always willing to follow this recommendation, especially when the dogs have characteristics of their breed that are highly valued. Therefore, if the owners/breeders decide to cross them again, and are sure that the cleft lip or palate is genetic in nature, the risk of recurrence should be seriously taken into consideration [35].

To avoid autosomal recessive clefts, an important strategy is never to cross individuals that are known to be heterozygotes one with another, such as those that have already had affected offspring. When there is a family history of recessive cleft and the zygosity of an individual is not known, consanguineous unions should be avoided. For X-linked recessive phenotypes, normal female offspring of affected father are all carriers, i.e., heterozygotes, and should not be crossed even when the males are normal. For multifactorial clefts, the main strategy is to avoid crossing dogs that have any relationship. This will reduce the probability of reaching the critical threshold [35].

5. Final considerations

Always bearing in mind that greater knowledge results in a correct diagnosis, suitable management of each case, and definition of criteria that give consistency to guidelines for prevention of CLP, the first step to expand knowledge is appropriate details when publishing new canine cases, using one of the classifications established in human medicine. This will facilitate international communication between professionals from the different fields in question.

Breeds, lineages, or families of dogs in which CLP occurs more frequently are a valuable source of information on the molecular biology and genetics of oral clefts. Genome-wide association studies (GWAS) with genotyping using arrays based on single nucleotide polymorphisms (SNP) are powerful means for mapping of regions of interest. The current technologies of next-generation sequencing (NGS), with increasingly robust platforms and increasingly expanded panels, facilitate the identification of candidate genes, allowing studies that confirm the role of these genes in the etiology of oral clefts.

It should also be remembered that a chromosomal analysis in syndromic cases should be routine. Analyses with fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (GCH) may identify chromosomal aberrations and describe new syndromes, as well as establishing a correlation with human syndromes.

An interface of knowledge on human and canine species opens up new paths in both veterinary and human medicine. This promotes quality and more humane and competent clinical practice. It is also clearly reflected in the fields of genetics, developmental biology, and evolutionary biology.

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