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A case–control study between interleukin-10 gene variants and periodontal disease in dogs

Carlos Albuquerque [a,b,*], Francisco Morinha [a,c], João Requicha [b,d], Isabel Dias [b,d,e], Henrique Guedes-Pinto [a], Carlos Viegas [b,d,e], Estela Bastos [a,f]

[a] Centre of Genomics and Biotechnology-Institute for Biotechnology and Bioengineering (CGB-IBB), University of Trás-os-Montes e Alto Douro (UTAD), P.O. Box 1013, 5001-801 Vila Real, Portugal
[b] Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), P.O. Box 1013, 5001-801 Vila Real, Portugal
[c] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro (UTAD), P.O. Box 1013, 5001-801 Vila Real, Portugal
[d] 3B's Research Group: Biomaterials, Biodegradables and Biomimetics, University of Minho, Avepark, Taipas, 4806-909 Guimarães, Portugal
[e] ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal
[f] Department of Genetics and Biotechnology, Life and Environmental Sciences School, University of Trás-os-Montes e Alto Douro (UTAD), P.O. Box 1013, 5001-801 Vila Real, Portugal

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ABSTRACT

Periodontal disease (PD) refers to a group of inflammatory diseases that affect the periodontium, the organ which surrounds and supports the teeth. PD is a highly prevalent disease with a multifactorial etiology and, in humans the individual susceptibility is known to be strongly determined by genetic factors. Several candidate genes have been studied, namely genes related with molecules involved in the inflammatory response. Interleukin-10 (IL-10) is a cytokine with important anti-inflammatory and immunomodulatory roles, and several studies indicate an association between *IL10* polymorphisms and PD. In dogs, an important animal model in periodontology, PD is also a highly prevalent naturally occurring disease, and only now are emerging the first studies evaluating the genetic predisposition. In this case–control study, a population of 90 dogs (40 dogs with PD and 50 healthy dogs) was used to study the *IL10* gene, and seven new genetic variations in this gene were identified. No statistically significant differences were detected in genotype and allele frequencies of these variations between the PD cases and control groups. Nevertheless, one of the variations (*IL10*/2_g.285G > A) leads to an amino acid change (glycine to arginine) in the putative signal peptide, being predicted a potential influence on IL-10 protein functionality. Further investigations are important to clarify the biological importance of these new findings. The knowledge of these genetic determinants can help to understand properly the complex causal pathways of PD, with important clinical implications.

1. Introduction

Periodontal disease (PD) is a highly prevalent inflammatory condition developed in periodontal tissues in response to the oral bacterial biofilm, and includes gingivitis, an initial gingival inflammatory reversible stage, and periodontitis, an advanced stage that leads to destruction of alveolar bone, periodontal ligament and cementum, and, ultimately, leads to teeth exfoliation (Pihlstrom et al., 2005). Besides these important local consequences, PD is also associated with serious systemic health concerns, namely preterm delivery, diabetes, cardiovascular and osteoarticular diseases (Kuo et al., 2008). The severity of PD progression depends on the interplay of several factors, including biofilm bacteria, host immune status, genetic and environmental factors (Van Dyke and Dave, 2005). A host susceptibility profile, mainly related with individual inflammatory response, is the principal determinant of PD expression (Yoshie et al., 2007).

In human periodontology, several scientific papers have described the association between single nucleotide polymorphisms (SNPs) and PD susceptibility (Yoshie et al., 2007). Chronic periodontitis appears to have a 50% hereditability rate, meaning that half of the variation of the disease within a population results from genetic factors (Michalowicz et al., 2000). Cytokines, a group of immune-regulation molecules, develop an important and central role in the immunopathology of PD (Page, 1991). The presence of polymorphisms in cytokine genes often has an effect on the cytokine expression profile and, thus, may have an important role in resistance and susceptibility to PD (Stabholz et al., 2010). Nevertheless, as in other complex diseases, genetic variants associated with PD are multiple with synergic contributions to the overall effect (Yoshie et al., 2007). Thus, more studies with different approaches are still needed to obtain definitive conclusions.

Animal models have been extremely important to the current knowledge in periodontology, and dog has been one of the most widely

Abbreviations: A, Adenine; Arg-R, Arginine; C, Cytosine; G, Guanine; Gly-G, Glycine; *IL10*, Gene coding for interleukin-10; IL-10, Interleukin-10; PCR, Polymerase chain reaction; PD, Periodontal disease; T, Thymine.

* Corresponding author at: Centre of Genomics and Biotechnology, University of Trás-os-Montes and Alto Douro, Institute for Biotechnology and Bioengineering (CGB-UTAD/IBB), P.O. Box 1013, 5001-801, Vila Real, Portugal. Tel.: +351 259 350 735; fax: +351 259 350 480.
E-mail address: cmralb@hotmail.com (C. Albuquerque).

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Table 1
Main characteristics of the population under study.

PD stage	Frequency *n* (%)	Age (years)	Weight (kg)	Sex (males/females)
PD 0 (healthy periodontium)	50 (55.6)	3.9 (2–5)	12.2	19/31
PD1 (gingivitis)	5 (5.6)	3.4 (2–4)	11.7	2/3
PD2 (early periodontitis)	15 (16.6)	4.1 (3–6)	10.5	6/9
PD3 (moderate periodontitis)	11 (12.2)	4.2 (3–8)	11.3	5/6
PD4 (advanced periodontitis)	9 (10.0)	5.4 (4–8)	11.2	4/5
Control group	50 (55.6)	3.9 (2–5)	12.2	19/31
Cases group	40 (44.4)	4.3 (2–8)	11.1	17/23

used species, mainly because it presents some anatomical, clinical and epidemiological ideal characteristics (Albuquerque et al., 2012a). In dogs, PD is also a naturally occurring disease emerging with a high prevalence; being found in approximately 80% of dogs aged 2 years or older (Niemiec, 2008). Nevertheless, as in humans, the advanced disease stages with severe clinical signs appear only in a few dogs (Kortegaard et al., 2008). Unlike human periodontology, the role of genetics in dog PD susceptibility still remains unknown and only now it starts to be studied (Morinha et al., 2011, 2012). In this context, a comparative genomic approach should be today a very useful tool to study the genetic basis of complex diseases, like PD (Mellersh, 2008). For both species, a better characterization of genetic factors behind the PD susceptibility would contribute to early signaling of predisposed individuals, set up personalized therapies and develop more effective preventive measures (Yoshie et al., 2007).

Interleukin-10 (IL-10) plays a central immunomodulatory role, stimulating the production of protective antibodies and down-regulating pro-inflammatory cytokines (Moore et al., 2001). In periodontology, IL-10 is essential to maintain the periodontal health and stability, with a protective action against disease progression (Bozkurt et al., 2006; Cutler et al., 2000). In humans, the *IL10* gene is well described and the 5′-flanking promoter region contains a number of polymorphisms associated with periodontitis risk; nevertheless some inconsistent results arise from the studies (Albuquerque et al., 2012b). These polymorphisms also play an important role in other diseases, such as endometriosis (Zhang et al., 2007), Alzheimer's disease (Ma et al., 2005), aortic stenosis (Gaudreault et al., 2011), Crohn's disease (Fowler et al., 2005), Graves' disease (Liu et al., 2011) and sepsis (Stanilova et al., 2006).

Canine IL-10 shows strong sequence homology and similar cellular expression in relation to human IL-10 (Lu et al., 1995; Zucker et al., 1996). Anti-inflammatory properties of IL-10 were demonstrated in dogs with gram-negative bacteria sepsis (Ogasawara and Stokol, 2012). Additionally, an increased IL-10 expression was described in different canine diseases namely, recurrent demodicosis (Felix et al., 2013), inflammatory mammary cancer (Andrés et al., 2013), osteoarthritis (Maccoux et al., 2007) and leishmaniasis (Corrêa et al., 2007).

Short et al. (2007) developed an analysis of candidate susceptibility genes in canine diabetes, including the *IL10* gene, selected taking into account the previous associations described in human diabetes. These authors found various polymorphisms in the dog *IL10* gene associated with diabetes mellitus in the Cavalier King Charles Spaniel dog breed. This kind of study approach was also followed in other diseases shared by dogs and humans (Clements et al., 2010; Ollier et al., 2001). Therefore, recognizing the importance and similarity of PD in both species, this study was delineated hypothesizing that the dog *IL10* gene, as occurs in the human *IL10* gene, may present variations that can influence IL-10 regulatory role, and consequently the PD susceptibility.

2. Materials and methods

2.1. Study population

A population of 90 dogs was selected to be included in a case–control study. In the selection process, a general clinical examination was performed to assess the individual health status. After an informed consent of the dogs' owners and following all the animal welfare guidelines, the dogs were sedated by an intramuscular administration of butorphanol (Torbugesic 1%; Fort Dodge, The Netherlands) and acepromazine (Vetranquil; CEVA Sante Animal, France). Anaesthesia was achieved by an intravenous administration of diazepam (Diazepam MG; Labesfal, Portugal), ketamine (Imalgene 1000; Merial, France) and propofol (Lipuro 2%; Braun, Germany) and was maintained using isoflurane (IsoFlo; Abbott Animal Health, USA) administered in oxygen through an endotracheal tube. A systematic odonto-stomatological exam was performed to all dogs, to determine the presence or absence of PD and, when present, classified in the correct stage. The clinical periodontal examination was based on the American Veterinary Dental College classification of PD stages (AVDC, 2009).

The selection criteria included mesocephalic dogs with similar diet patterns (mix of home-prepared rations and commercial pet foods). All the included animals did not have history of any previous dental treatment or preventive measures (e.g. tooth brushing, dental diets). The dogs selected were unrelated/unfamiliar individuals. After the odonto-stomatological exam, dogs were distributed in two groups, namely 40 dogs with PD (ranging from gingivitis to advanced periodontitis), and a control group including 50 dogs with healthy periodontium. All animals were mixed-breed with body weight ranging from 9 to 18 kg and ages between 2 and 8 years. A more detailed characterization of the population is presented in Table 1.

2.2. Sample collection and DNA isolation

Blood samples for subsequent DNA extraction were collected in EDTA tubes and buffy coats were obtained by centrifugation at 2500 rpm for 15 min at room temperature. The Fujifilm QG-Mini 80 equipment was used for the DNA extraction following the instructions of QuickGene DNA whole blood kit S (DB-S) (Fujifilm). An initial volume of 250 μl was used and a final volume of 200 μl elution buffer (CDT) was obtained. The integrity of the genomic DNA was confirmed by 1% agarose gel electrophoresis. The concentration and quality of the DNA extracted were measured in a NanoDrop ND-1000 spectrophotometer (Thermo Scientific).

2.3. PCR conditions and genotyping

The data available in genome browsers for human and dog *IL10* genes were used to select the more appropriate regions of *IL10*. The 5′-flanking promoter region of the human *IL10* gene contains various polymorphisms associated with different diseases, including three important SNPs associated with PD (Albuquerque et al., 2012b). In the absence of previous studies in dogs, we used the human *IL10* gene information and the homology with the canine *IL10* gene to select two fragments. The fragment 1 (a target region in the 5′ flanking sequence) was obtained with the forward primer 5′-CTTAACTTCCTTATTATTCC-3′ and the reverse primer 5′-CTGAGATTGAGAAATAATTG-3′. The fragment 2 (includes 5′ untranslated region and exon 1) was obtained with the forward primer 5′-AAGCGAAGAACCTTAAAAAGTTAAA-3′ and the reverse primer 5′-AGGTGGCATTTTCTGCACAT-3′.