Elsevier Editorial System(tm) for Journal of Proteomics

Manuscript Draft

Manuscript Number: JPROT-D-15-00616R1

Title: CanisOme - The protein signatures of Canis lupus familiaris diseases

Article Type: Full Length Article

Keywords: CanisOme; Canis DiseasOme; Canis biomarkers; Canis Leishmaniosis.

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Abstract: Although the applications of Proteomics in Human Biomedicine have been explored for some time now, in animal and veterinary research, the potential of this resource has just started to be explored, especially when companion animal health is considered.In the last years, knowledge on the Canis lupus familiaris proteome has been accumulating in the literature and a resource compiling all this information and critically reviewing it was lacking. This article presents such a resource for the first time. CanisOme is a database of all proteins identified in Canis lupus familiaris tissues, either in health or in disease, annotated with information on the proteins present on the sample and on the donors. This database reunites information on 549 proteins, associated to 63 dog diseases and 33 dog breeds. Examples of how this information may be used to produce new hypothesis on disease mechanisms is presented both through the functional analysis of the proteins quantified in canine cutaneous mast cell tumors and through the study of the interactome of Canis lupus familiaris and Leishmania infantum. Therefore, the usefulness of CanisOme for researchers looking for protein biomarkers in dogs and interested in a comprehensive analysis of disease mechanisms is demonstrated.

*Graphical Abstract

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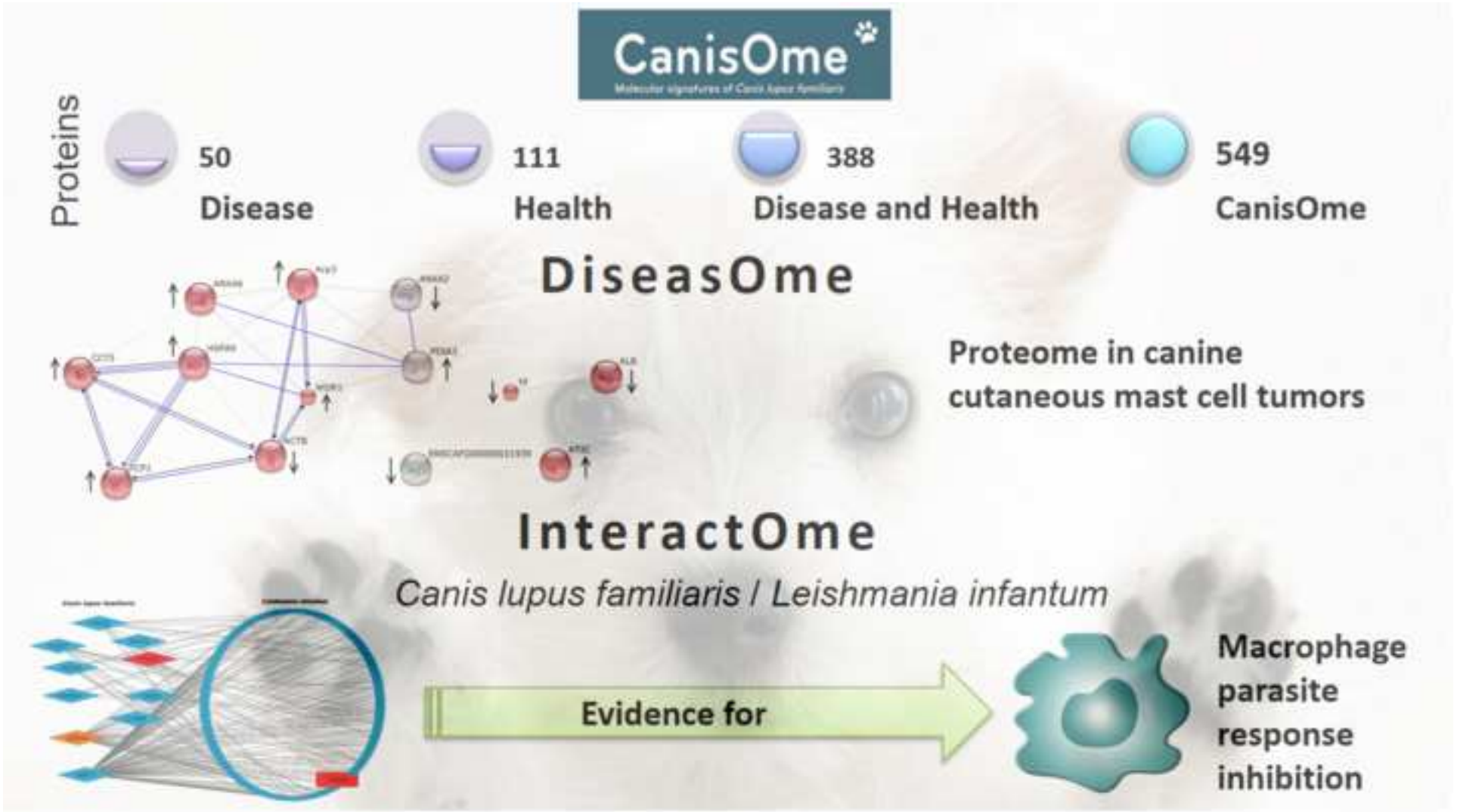

***Highlights (for review)**

- We built and present the database CanisOme with dogs annotated proteome.
- Proteome annotation includes quantification by disease and dog breed.
- Dogs tryptase-P15944 is a marker candidate for immune response in tumoral process.
- Two mechanisms of macrophage response inhibition by *Leishmania* are proposed.

CanisOme - The protein signatures of *Canis lupus familiaris* diseases

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1. ABSTRACT

Although the applications of Proteomics in Human Biomedicine have been explored for some time now, in animal and veterinary research, the potential of this resource has just started to be explored, especially ~~if~~ when companion animal health is considered. ~~Nevertheless, l~~In the last years, knowledge on the *Canis lupus familiaris* proteome has been accumulating in the literature and a resource compiling all this information and critically reviewing it was lacking. This article presents such a resource for the first time. CanisOme is a database of all proteins identified in *Canis lupus familiaris* tissues, either in health or in disease ~~samples~~, annotated with information on the proteins present on the sample and on the donors. This database reunites information on 549~~599~~ proteins, associated to ~~58~~ 63 dog diseases and 3~~1~~ dog breeds. Examples of how this information may be used to produce new hypothesis on disease mechanisms is presented both through the functional analysis of the proteins quantified in canine cutaneous mast cell tumors and through the study of the interactome of *Canis lupus familiaris* and *Leishmania infantum*. Therefore, the usefulness of CanisOme for researchers looking for protein biomarkers in dogs and interested in a comprehensive analysis of disease mechanisms is demonstrated.

Biological Significance

This paper presents CanisOme, a database of proteomics studies with relevant protein annotation, allowing the enlightenment of disease mechanisms and the discovery of novel disease biomarkers for *Ca~~n~~nis lupus familiaris.* This knowledge is important not only for the improvement of animal health but also for the use of dogs as models for human health studies.

Keywords: CanisOme; *Canis* DiseasOme; *Canis* biomarkers; *Canis* Leishmaniosis.

1. INTRODUCTION

Proteomics applications in veterinary medicine have been increasing and although single-protein concentration assessed by antibody-based affinity is still the gold standard of diagnostics for clinical practice, there is now a general agreement that a panel of independent disease-related proteins could substantially improve the diagnosis of animal diseases. In particular, farm animals~~'~~ proteomic approaches have been explored and recently reviewed [1,2]. Comparatively to farm animals, other companion animal species such as *Canis lupus familiaris* have not had their proteomes as well studied in a large scale, and most studies using proteomic approaches are focused on studies of infectious [3–7] and neoplasic disease [8–15]~~, in particular~~.

One of the reasons for the scarcity of proteomics research on dog diseases, when compared to farm animals, is certainly the importance of the latter as economic resources. Additionally, when compared to rodents and humans, the information on the genetics and proteomics of companion animals such as the dog~~,~~ is much less abundant, which makes the effective use of this information for diagnostics much more difficult. ~~However, b~~Better biomarkers are urgently needed in veterinary medicine of companion animals for diagnosis and prognosis of diseases [16].

Dogs are affected by a large number of diseases, some specific of *Canis lupus familiaris*, others affecting their human partners, ~~and~~ therefore dogs may serve as models of human conditions [17]. There are several examples which have been reviewed previously [17,18], most notably cancer [19–21], neurological [22] and cardiac diseases [23]. In fact, it is has been proposed that some human adaptations to the environmental shifts, that contribute (through antagonistic pleiotropy) to disease, such as highly reactive immune systems that protects from infectious disease, but predispose individuals to autoimmune disorders, might have evolved in parallel in dogs [24].

Additionally, it is known that different dog breeds are affected by different diseases [25] and knowledge of the risk of ~~breed~~ specific breed diseases is very useful in developing a differential diagnosis list~~,~~ compatible with clinical

signs presented by the patient. However, presently most of the knowledge is clinical and mainly not ~~largely~~ based on molecular data underlying the physiological and pathological changes present ~~in disease~~. The development of new diagnostic strategies and personalized therapeutic regimens depends on the generation and interpretation of molecular data which reflect the clinical changes associated with disease and treatment. The Omics sciences have a determinant role in ~~the~~ generating ~~on~~ ~~of~~ these data, as has been the case in human health. In fact, ~~the~~ a systems approach enabled by the use of Omics data, allows for the complete catalogue and a wider screening of molecules, which is more effective in biomarker discovery~~,~~ than studies directed at one or a few molecules. In the last years, some studies reporting molecular data relative to the different dog breeds ~~diseases~~ have been published [25], but information is largely dispersed and lacking a functional interpretation underpinned by the clinical and physiological data. The great amount of data generated by the Omics sciences, however, can only be explored and conjugated with other data using bioinformatics tools and databases.

There are few databases specifically dedicated to dogs. Examples are a database with the mitochondrial genome for *Canis lupus familiaris* (http://clf.mtdna.tree.cm.umk.pl/) [26] and the database DoGSD (http://dogsd.big.ac.cn), that focuses on whole genome SNP data from domesticated dogs and grey wolves [27]. T~~However, t~~here are also molecular databases which include genetic information for *Canis lupus* such as the Ensemble (http://www.ensembl.org/Canis_familiaris/Info/Annotation) and NCBI Entrez databases (http://www.ncbi.nlm.nih.gov/projects/mapview/static/dogsearch.html) where the dog genomes are available. Furthermore, because dogs are genetic model species, there are databases with specific SNP-STR/microsatellite compound markers in dog, along with other model species (http://www.sbg.bio.ic.ac.uk/~ino/SNPSTRdatabase.html) [28] and the IPD: the Immuno Polymorphism Database (http://www.ebi.ac.uk/ipd/) which is included in a set of specialist databases related to the study of polymorphic genes in the immune system of several animals, including dogs [29].

As far as proteomics databases, *Ca~~n~~nis lupus familiaris* reference proteome deposited in Uniprot is based on the genome and not experimentally derived.

In fact, the complete annotation of the protein-coding and non-protein-coding gene sets of the 2005 published genome is still on-going. The dog genome has 39 chromosomes containing 2.4 Gb and an estimate of 25,000 protein-coding genes of which only 812 have been reviewed so far (September 2015). http://www.uniprot.org/proteomes/UP000002254.

A database gathering all the information generated by proteomics analysis of *Canis lupus familiaris* samples and associated information, such as the ones existing for humans (Human protein reference database (http://www.hprd.org/index html) [30], Human Proteinpedia (http://www.humanproteinpedia.org/)[31] and OralCard (http://bioinformatics.ua.pt/OralCard/)[32,33]) is still lacking. The aim of this paper is to present such a resource which allows ~~for~~ the functional interpretation of the proteomic data. This study and the associated database, enables the integrated interpretation of proteomics data from various tissue and fluid samples of *Canis lupus familiaris*, and is a fundamental tool for biomarker identification and the development of innovative molecular diagnostic strategies.

2. MATERIALS & METHODS

2.1. Compilation and curation of CanisOme database

To create the CanisOme database the first step was to compile all the information produced by proteomics studies focused on finding specific proteins altered in ~~diseases of~~ different ~~breeds~~breed's diseases of *Canis lupus familiaris*. Bibliographic references of proteomic studies in which a complete list of proteins was provided were analysed and ~~thirty~~forty-~~seven~~ three articles published between 2005 and 2015 [3–15, 34–57] were included. These studies used samples from different sources which are annotated in Table 1.

In this database, each protein (identified by its UniProtKBAC) is annotated

with ~~its~~ name, the dog breed, the sample type and the technique used for protein identification. Further annotation includes the sample donor information such as healthy or diseased (in which case the disease is identified), age, gender and gonadectomy status. When the protein entry corresponds to a sample from an individual with an infectious disease, the type of microorganism (virus, bacteria or parasite) and its species are annotated in the column ~~Organism~~Microorganism. When available, the data on that protein's~~s~~' quantification is also registered, either as a fold change value or as an up or down regulated protein.~~, and i~~I If the protein has already been suggested as a biomarker that is also annotated. Finally, the source of information for that entry is inserted as a PMID, as well as the year of publication.

The curated list of proteins identified in this work was stored in the database CanisOme developed for this purpose (http://www.crb.ucp.pt/salivatec/canisome/).

2.2. *Canis lupus familiaris* proteome analysis

2.2.1. Gene Ontology (GO) analysis

For this work, ~~P~~proteins of CanisOme ~~are~~ were further annotated with the plug-ins ClueGo [58] and CluePedia [59] for Cytoscape [60]. The enrichment analysis of the biological processes in which *Canis lupus familiaris* proteins participate and which are compromised in all the diseases reflected ~~are~~ were identified.

All Results were created with ClueGO v2.2.3 + CluePedia V1.2.3, using the Ontology GO_BiologicalProcess-Custom_07.12.2015_15h48 selecting the options:

GO Fusion = true;

GO Group = true;

Kappa Score Threshold = 0.4;

Over View Term = SmallestPValue

; Group By Kappa Statistics = true;

Initial Group Size = 1 and

Sharing Group Percentage = 50.0.

The Statistical Test used was Enrichment/Depletion (Two-sided hypergeometric test) corrected with Bonferroni step down.

~~The ontology "GO Biological Process" dated 30.06.2015, was used. The option "Use GO Term Fusion" and an alpha value of 0.05.~~

2.2.2. Disease Association analysis

Canis lupus familiaris proteins were scrutinized for their involvement in diseases using Uniprot Retrieve Tool [61].

The prevalence of diseases for each breed followed the classification proposed by Dorn 2002 [25] in which each disease has an odds ratio defined as the risk (odds of diagnosis) of disease X in breed Y as compared to the risk (odds of diagnosis) of disease X in all other breeds of dogs combined.

The choice of canine cutaneous mast cell tumors, for and in depth analysis, was based on the fact that this is the disease in which a larger number of proteins (mainly exclusive ~~one~~) were identified and quantified.

The functional analysis of the proteins involved in this disease was done using STRING v10 - *Search Tool for the Retrieval of Interacting Genes/Proteins* [62], ~~using~~ in Action View to visualize the effect of each protein in a Protein-Protein Interaction (PPI) with high confidence (0.4 score). The STRING Enrichment Tool was used to catalogue each protein ~~about~~ relative to the GO Cellular Components.

2.3. Host-pathogen interactomics

The PPIs established between *Leishmania infantum* proteins and *Canis lupus familiaris* was performed using the OralInt V2.0 tool developed by our group [63] which allows for the prediction of interspecies PPIs. The input for this analysis were the 16 dog proteins associated to Leishmaniosis in CanisOme database and the *Leishmania infantum* proteome deposited in Uniprot as of May 2015. Only high confidence PPIs were considered (score≥0.9). A network of the predicted PPIs was generate and visualized using the Cytoscape software [60] ~~was used to visualize the PPI network~~.

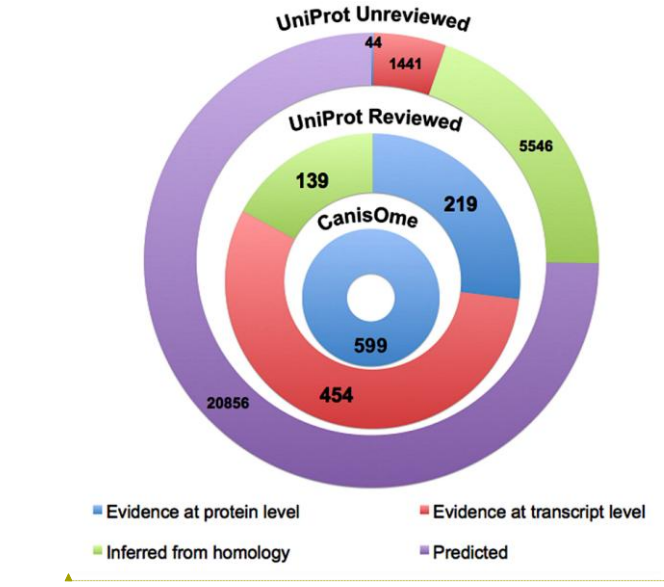
3. RESULTS AND DISCUSSION

The ~~congregation~~ joining of information generated by the Omics sciences in electronic platforms, which enable a simple and efficient search, provides useful tools for the identification of protein signatures and the discovery of the

role these proteins play in the molecular mechanisms underlying different diseases. The information provided by this analysis can be used to find new diagnostic and therapeutic solutions.

CanisOme is a database that integrates the information produced by proteomics or studies focused on finding specific proteins altered in diseases of different breeds of *Canis lupus familiaris* (http://www.crb.ucp.pt/salivatec/canisome/).

When the information included in CanisOme is compared with the other proteome database with information on *Canis lupus familiaris* (UNIPROT) it can be seen that it has manually reviewed information on ~~599~~ 549 proteins (Figure 1), whereas Uniprot has information on only ~~219~~ 222 proteins identified at the protein level and ~~44~~ 46 proteins with un-reviewed information (a total of ~~263~~ 268 proteins with evidence at the protein level). It can thus be concluded that CanisOme is the largest repository of information on proteins identified in *Canis lupus familiaris*~~-~~, not only because it has the largest number of proteins identified at the protein level, but also because each protein is annotated with detailed information.



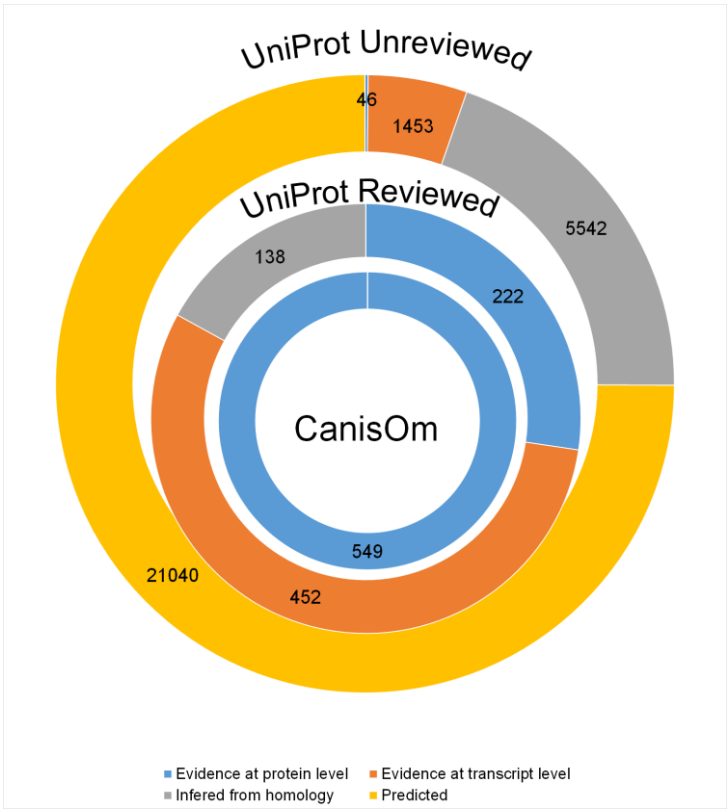


Figure 1. Comparison of the protein number and type of evidence for protein existence for the *Canis lupus familiaris* proteome deposited in the Uniprot and CanisOme databases (December 2015).

When the 5499 proteins of CanisOme are annotated with the applications ClueGo and CluePedia for Cytoscape, the different biological processes in which they participate are identified (Figure 2). Biological processes such as regulation of endopeptidase activity, symbiosis encompassing mutualism through parasitism, oxoacid metabolic process ~~the negative regulation of programmed cell death, platelet activation, the complement activation~~ and others are noted in figure 2 with (**) and are the most enriched.

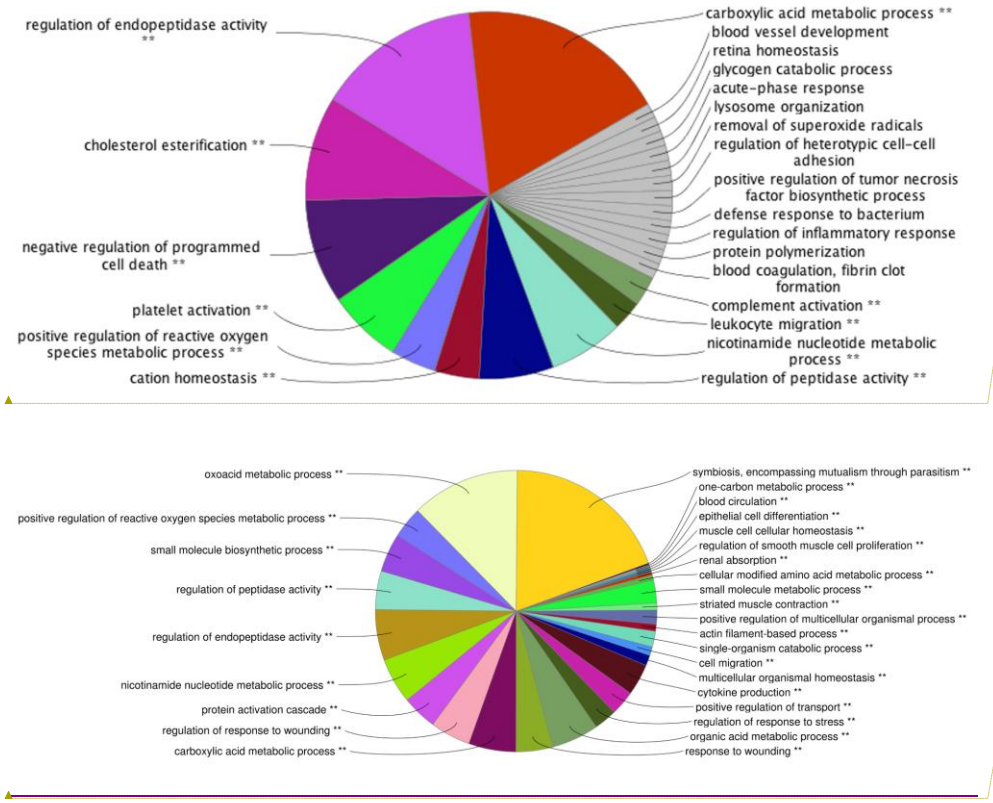


Figure 2. Enrichment analysis of the biological processes in which *Canis lupus familiaris* proteins participate. Analysis done using the CluePedia+ClueGo plugins for Cytoscape. Processes marked with ** denote a significant enrichment at the alpha=0.05 level.

Of the 5499 proteins listed in CanisOme, 11124 are identified only in health and ~~428~~ 388 are present in samples from healthy and diseased donors. However, there are ~~47~~ 50 proteins which were identified only in disease

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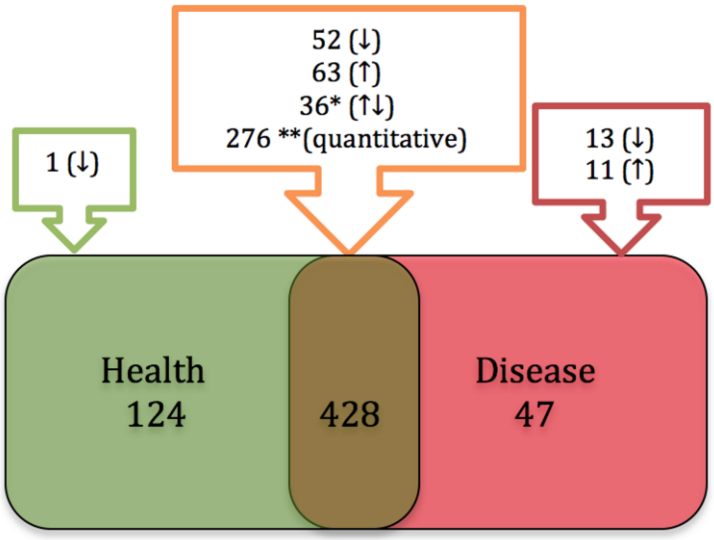
samples. Figure 3 shows the number of proteins with quantitative and qualitative data in health, disease and both ~~health and disease~~. ~~From~~ In figure 3 it is possible to verify that the larger number of proteins identified are present both in health and disease (~~42~~388) and that the most quantitative data are relative to the disease caused by the influenza virus (276). For rabies, 36 proteins were identified and these are counter regulated, meaning that are up or down regulated differently in the two stages of the disease (furious and paralytic).



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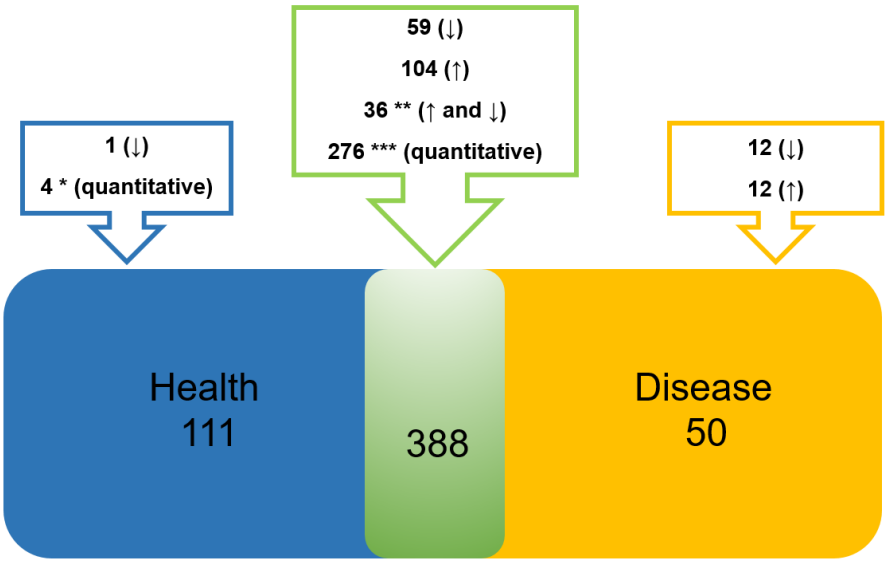

Figure 3. Proteins annotated in CanisOme regarding their involvement in health, disease or both. ↑ and ↓ represent up and downregulated proteins respectively. * denotes proteins up or down regulated more than threefold after exercise, ** represents counter regulated proteins all found in rabies (furious and paralytic stages) and ~~**~~* ~~represents~~ denotes proteins which are only referred as to being up or down regulated but haven´t been quantified in influenza virus infection.

The ~~599~~ 549 proteins of CanisOme represent ~~58~~ 63 diseases of which, the four with more altered proteins identified are, in addition to the canine influenza virus A (276), Duchenne muscular dystrophy (8~~3~~4), rabies (34~~6~~) and meningoencephalitis (34~~5~~).

Table 1 presents the main information present in CanisOme regarding disease distribution by breed. For each disease the number of associated proteins, as well as the biological sample used for diagnosis is presented. Also for each disease, the number of proteins which are identified exclusively in that disease, and therefore present the greatest potential for diagnostic purposes, are indicated. As an example it is possible to verify in the CanisOme database that for Duchenne muscular dystrophy 83 proteins were identified in Golden Retrievers and ~~2~~3 proteins (UniProt ID: Q863Z4/ Myotrophin, ~~and~~ UniProt ID: F6X7L07/ Tropomyosin 4, and UNIPROT ID: F1PV45/ Titin) were only identified in this disease.

The comparison of the information available in CanisOme with the information on the most prevalent diseases in each dog breed published by Dorn in 2002

[25] shows that there is scarce information on the most prevalent diseases as far as proteomics studies. There are proteomics data on two prevalent dog diseases: obesity in Beagles and lethal acrodermatitis in Bull terrier (Table1). ~~This~~ The analysis by breed is hampered by the fact that some authors of proteomics studies do not refer the dog breed used in the study or ~~refer to the~~ use mixed breed donors ~~as mixed breeds~~ (Table 2). Some other studies use pooled samples from different breeds [37,43]. That is the reason why in table 1 these diseases are annotated with the same information for all the breeds contributing to the sample pool.

Table 1 – Proteins present in CanisOme classified by dog breed, the tissue from which they originated and their association with disease. Proteins marked with an* have been classified by Dorn 2002 [25] as being more prevalent in the breed indicated, when compared to other breeds.

Breeds	**Disease name**	**Nº of proteins**	**Nº of exclusive proteins**	**Nº of proteins with regulation data**	**Sample source**
Beagles	Meningoencephalitis	34		1	Cerebrospinal fluid
	Obesity *	3		3	Serum
	Encephalitis	1		1	Cerebrospinal fluid
	Canine influenza virus A (H3N2)	276		276	Lung
Bull terrier	**Lethal acrodermatitis***	12	6	5	Liver
Golden retriever	Duchenne muscular dystrophy	83	2	83	Muscle
American eskimo	Transitional cell carcinoma	10		0	Urine
Australian shepherd	Lymphoma	5		5	Lymph nodes and plasma
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Boxer	Canine cutaneous mast cell tumors	13	6	13	Skin
	Multicentric lymphoma	20		0	Serum
	Various cancers	5	1	3	Tears
Brazilian fila	Various cancers	5	1	3	Tears
Cairn terrie	Prostatic carcinoma	2		2	Prostate
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Chihuahu as	Meningoencephalitis	34		1	Cerebrospinal fluid
Dachshund	Canine cutaneous mast cell tumours	13	6	13	Skin
Dalmatian	Lymphoma	5		5	Lymph nodes and plasma
Doberman pinscher	Various cancers	5	1	3	Tears

English cocker spaniel	Various cancers	5	1	3	Tears
German shepherd	Canine leishmaniasis	16		4	Serum and saliva
	Multicentric lymphoma	20		0	Serum
	Breast cancer	5		5	Serum
	Various cancers	5	1	3	Tears
Irish setter	Various cancers	5	1	3	Tears
Labrador retriever	Multicentric lymphoma	20		0	Serum
	Prostatic carcinoma	2		2	Prostate
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Malteses	Meningoencephalitis	34		1	Cerebrospinal fluid
Miniature pinscher	Various cancers	5	1	3	Tears
Newfoundland	Lymphoma	5		5	Lymph nodes and plasma
Pekingeses	Meningoencephalitis	34		1	Cerebrospinal fluid
Pembroke welsh corgi	Transitional cell carcinoma	10		0	Urine
	Lymphoma	5		5	Lymph nodes and plasma
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Poodle	Prostatic carcinoma	2		2	Prostate
	Various cancers	5	1	3	Tears
Pugs	Meningoencephalitis	34		1	Cerebrospinal fluid
Scottish terrier	Transitional cell carcinoma	10		0	Urine
Shar-pei	Canine cutaneous mast cell tumours	13	6	13	Skin
Shih-tzus	Meningoencephalitis	34		1	Cerebrospinal fluid
Staffordshire mixture	Canine cutaneous mast cell tumours	13	6	13	Skin
Viszla	Various cancers	5	1	3	Tears
Weimaraner	Canine cutaneous mast cell tumours	13	6	13	Skin
	Various cancers	5	1	3	Tears
Yorkshire terriers	Meningoencephalitis	34		1	Cerebrospinal fluid
Pit bull	Canine cutaneous mast cell tumours	13	6	13	Skin
	Various cancers	5	1	3	Tears
West Highland White Terrier	Idiopathic pulmonary fibrosis	7		1	Bronchoalveolar lavage fluid
English Springer Spaniel	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid

Table 2 lists the diseases for which exclusive proteins (proteins found only in that disease) were identified, as well as the ~~reduced~~ number of proposed biomarker proteins. Unfortunately, not only is the number of proposed

biomarkers low, but also, for the most prevalent diseases, there are no biomarkers proposed.

Table 2. Protein involvement in diseases for which the dog breed is not specified.

Disease name	Nº of proteins	Nº of exclusive proteins	Biomarker	Most prevalent diseases*
Allergies	10	6		
Alzheimer's disease	1	1		
Breast cancer	12	2		X
Canine babesiosis	15	1	X	
Canine cardiopulmonary dirofilariosis	7	1		
Canine inflammatory bowel disease	8	5		
Canine leishmaniasis	16			
Intervertebral disc herniation	1	1	X	
Lymphoma	5			
Osteoarthritis	15	8		X
Prostatic carcinoma	2			
Renal insufficiency	1	1	X	
Transitional cell carcinoma	10			
Various cancers	4	1		X
XLHN	14	4		
Duchenne muscular dystrophy	1		X	
Envenomation by European adder (Vipera berus berus)	5			
Joint osteoarthritis secondary to cranial cruciate ligament disease	17			

*According to Dorn 2002 [25].

Data in the CanisOme database shows that, up to the moment, protein biomarkers have been proposed only for renal insufficiency (UniProt ID: E2RCE0/ Cystatin C, up-regulated), intervertebral disc herniation (UniProt ID: Q6YKA4/ High-mobility group box 1, up-regulated), Duchenne muscular dystrophy (UNIPROT ID: F1PV45/ Titin) and canine babesiosis (UniProt ID: P51742/ TNF-α). However, because the studies leading to ~~this~~ these proposals were done on mixed breeds or in unmentioned breeds, it is not possible to associate this information with the breeds presented on table 1.

The analysis of the distribution of the 5499 proteins of CanisOme by biological sample (figure 4) shows that fluids such as saliva have led to the identification of 122 111 proteins, whereas 113 67 proteins were identified in serum, 87 94 in urine, 378 in cerebrospinal fluid and 74 in plasma. Figure 4 shows which salivary proteins can be found for each type of dog biological sample when the human homolog proteins are considered. Because a significant number of proteins is potentially found in saliva, we can propose this fluid, which can be, obtained in a noninvasive manner, as a good alternative to other fluids in future proteomics studies in dog.

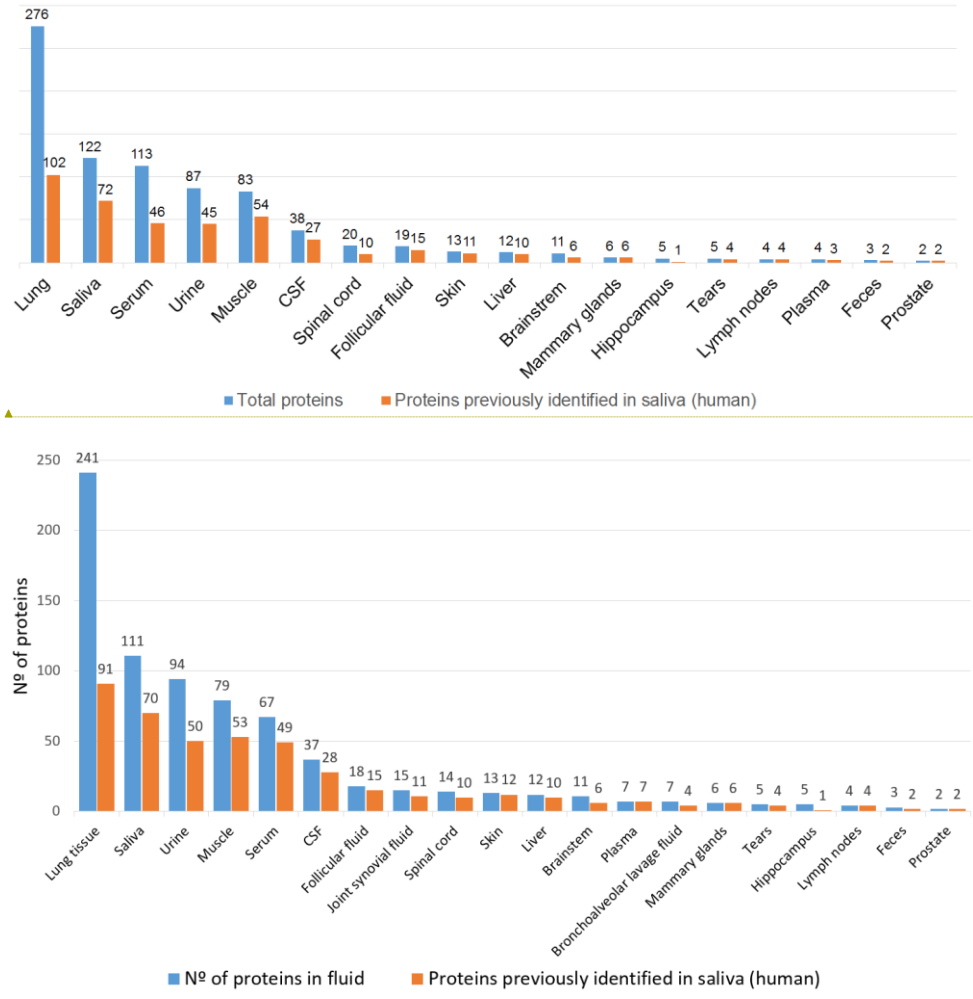

Figure 4. Total number of proteins in biological samples deposited in the database CanisOme and the homologous proteins previously identified in human saliva according to OralCard.

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One of the conditions disease biomarker candidate proteins must fulfill are to be altered only in that disease or to be up/down regulated relative to their expression in health. In table 1 we noted that the disease with the largest number of exclusive proteins is canine cutaneous mast cell tumors with 13 proteins identified and quantified of which 6 are exclusive. The names and the gene name used by STRING [62] of the 13 proteins referred are presented in Table 3. Figure 5 shows how the proteins identified as altered in canine cutaneous mast cell tumors diseaseome are organized in networks, establishing functional relationships.

Table 3. – Attributes and regulation details for proteins deposited in CanisOme associated with c~~C~~anine cutaneous mast cell tumors. ↑ and ↓ refer to up and down regulated proteins respectively.

Uniprot KB AC	Gene Name	Protein name	Regulation	Exclusive
E2R0L9	TCP1	T-complex protein 1, alpha subunit	↑	×
E2RB81	CCT5	T-complex protein 1, epsilon subunit	↑	×
F1P679	Arp3	Actin related protein 3	↑	×
F1P797	ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	↑	×
F1PR93	WDR1	WD repeat domain 1	↑	×
E2RAU5	HSPA9	Heat shock 70 kDa protein 9 (mortalin)	↑	
E2RCI8	ANXA6	Annexin A6	↑	
E2RD86	PDIA3	Protein ~~disulfide~~disulphide isomerase A3	↑	
P15944	ENSCAFG00000031939	Tryptase alpha/beta 1	↓	×
J9P430	TF	Transferrin	↓	
O18840	ACTB	Actin, beta	↓	
P49822	ALB	Serum albumin	↓	
Q6TEQ7	ANXA2	Annexin A2	↓	

Nine of the proteins form a larger network, two~~2~~ ~~other~~ proteins, in spite of interacting (evidence from text mining and co-expression, not shown), it is not known the functional nature of the interaction ~~is not known~~ and 2 other proteins do not interact with any other in the group (figure 5). Of the 13 proteins, 10 can be present in exosomes (Figure 5), and therefore contribute to extracellular communication. Of the exclusive proteins listed on Table 3, the tryptase (UniProt ID: P15944) is the major neutral protease present in mast cells and is secreted upon the coupled activation-degranulation response of

these cells. T, this protein is down-regulated, therefore it may be an interesting candidate as a marker for the role of immune response in tumoral processes.

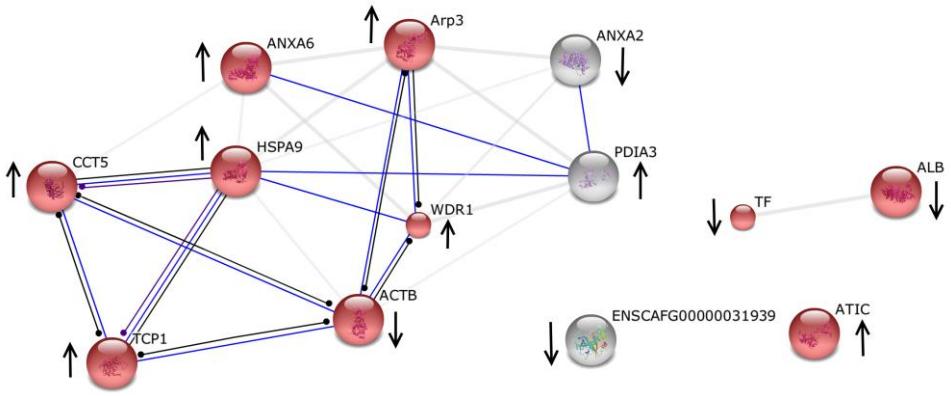

Figure 5. Protein-protein interactions determined by STRING V10 using the Action View and the Enrichment Tool for GO Cellular Components to find which of the 13 proteins associated with canine cutaneous mast cell tumors were identified in exosomes (proteins colored in red). Up regulated (↑) and down regulated (↓) proteins. Blue edges represent binding and black edges with circles undirected interactions.

Canine leishmaniosis caused by the parasite *Leishmania infantum,* is a systemic disease with variable clinical signs that is endemic in the Mediterranean countries and for which dogs are the main domestic reservoir of the parasite. The immune mechanisms underlying the lack of disease resolution are not completely known, however it is well established that the effective immune response to the parasite is cell mediated and depends on a Th1/ Th2 response [64]. Th1/IFNγ secreting cells that also produce IL2 activate the infected macrophages to kill *Leishmania*, whereas Th2/IL4 secreting cells divert the immune response to humoral immunity and down regulation of cellular immunity with Th1 cell anergy.

Although proteomics data for this pathology are scarce, with only 16 proteins identified as being altered and only 4 of them quantified, we can study the role of these 16 proteins present in CanisOme with OralInt [63] for the determination of interspecies interactome. This analysis allows for the identification of the predicted PPI interactions of dogs and *Leishmania infantum* proteins. Figure 6 represents the interaction with a combined score ≥

0.9 (very high confidence), and shows 176 interactions involving 10 dog proteins and 153 *Leishmania infantum* proteins.

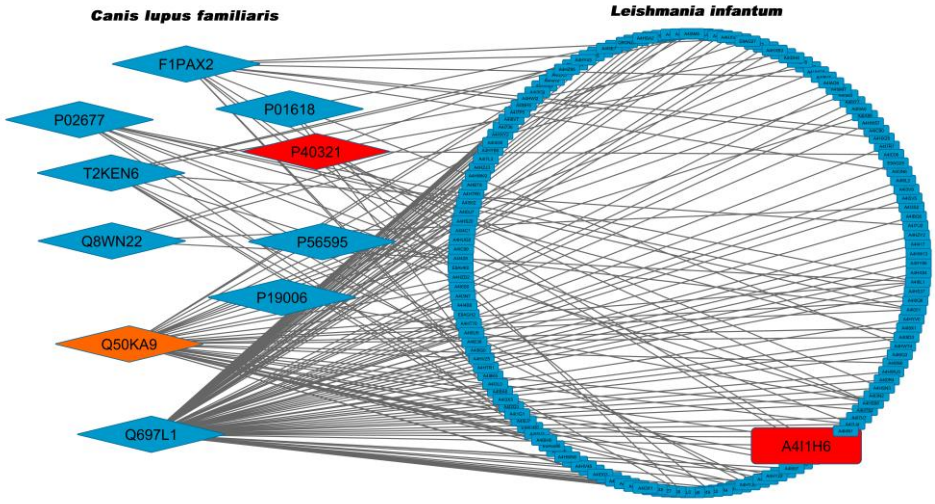

Figure 6. Partial network of the *Canis lupus familiaris*/*Leishmania infantum* interactome generated by the OralInt algorithm and visualized in Cytoscape. *Canis* proteins are represented by diamonds, *Leishmania* proteins are represented by rectangles.

Of the interactions in figure 6 the most interesting, due to its functional meaning, is the PPI between the Interleukin-2 receptor (UniProt ID: P40321), which is up regulated in the diseased animal, and the parasite's protein serine/threonine-protein phosphatase (UniProt ID: A4I1H6) (both proteins are colored red in the figure).

It is documented that *Leishmania* can activate several molecules that inhibit intracellular signaling cascades, including the activation of the host protein phosphatases (PTP) by the parasite's gp63 protease [65,66]. An important negative regulatory molecule is the PTP SHP-1, which is expressed mainly in hematopoietic host cells. The SHP-1 is able to bind to an evolutionarily conserved immune receptor tyrosine-based inhibitory motif (ITIM)-like motif found in the kinase domain of IL-1 receptor-associated kinase 1 (IRAK-1), causing its inactivation [67].

It was demonstrated that macrophage PTP activity is activated very rapidly, when exposed to *Leishmania donovani* promastigotes, which is correlated with a rapid, general tyrosine dephosphorylation of high-molecular-weight proteins [65]. Our analysis suggests that the intracellular dephosphorylation in macrophages may occur via the parasites serine/threonine-protein phosphatase (UniProt ID: A4I1H6), which should be tested and verified experimentally. As far as we know, this is the first time such a mechanism of macrophage parasite response inhibition is proposed. Furthermore, one of the dog's proteins with the most interactions (labeled in Orange in figure 6) is a nucleoside diphosphate kinase (Ndk) (UniProt ID: Q50KA9) with serine/threonine-specific protein kinase ~~activity~~ and histidine protein kinase activity, among others (Uniprot) [61]. Therefore, it may also be responsible for alterations of the phosphorylation mechanism occurring in the macrophages, if its functions are compromised by the unspecific binding/interaction/inhibition to many *L. infantum* proteins, as the results from the OralInt analysis suggests. Microbial Ndks have been related to virulence and subversion of immunity via purinergic signaling [68]. Furthermore, the disruption of the enzyme's ~~´~~ function in human hosts has been related to enhanced metastatic potential [69], to the endocytic caveolae stability and complex formation with G-proteins [70]. Dogs Ndk (UniProt ID: Q50KA9) may be affected by the *Leishmania infantum* proteins in a similar fashion, disrupting the host cells metabolism, contributing to the infection. A quantification of this protein will be fundamental to enlighten this aspect of the of dog ~~and~~ /parasite~~s´~~ interaction.

3. CONCLUSION

The gathering of information relative to the *Canis lupus familiaris* proteome, as demonstrated in the different examples and strategies presented, is a useful tool ~~useful~~ for the search of mechanisms associated to different dog diseases. Therefore, as more proteomics studies are published, CanisOme may develop into an important tool in biomarker identification for diagnostic purposes and disease mechanism proposal.

4. Acknowledgements

Work developed in SalivaTec laboratory financed by Mais Centro - Programa Operacional Regional do Centro under the National Strategic Reference Framework, and through the European Regional Development Fund (CENTRO-07-CT62-FEDER-005004).

References

[1] Almeida AM, Bassols A, Bendixen E, Bhide M, Ceciliani F, Cristobal S, et al. Animal board invited review: advances in proteomics for animal and food sciences. Anim Int J Anim Biosci 2015;9:1–17. doi:10.1017/S1751731114002602.

[2] Eckersall PD, de Almeida AM, Miller I. Proteomics, a new tool for farm animal science. J Proteomics 2012;75:4187–9. doi:10.1016/j.jprot.2012.05.014.

[3] Zygner W, Gójska-Zygner O, Bąska P, Długosz E. Increased concentration of serum TNF alpha and its correlations with arterial blood pressure and indices of renal damage in dogs infected with Babesia canis. Parasitol Res 2014;113:1499–503. doi:10.1007/s00436-014-3792-1.

[4] Thanomsridetchai N, Singhto N, Tepsumethanon V, Shuangshoti S, Wacharapluesadee S, Sinchaikul S, et al. Comprehensive proteome analysis of hippocampus, brainstem, and spinal cord from paralytic and furious dogs naturally infected with rabies. J Proteome Res 2011;10:4911–24. doi:10.1021/pr200276u.

[5] Kuleš J, Mrljak V, Barić Rafaj R, Selanec J, Burchmore R, Eckersall PD. Identification of serum biomarkers in dogs naturally infected with Babesia canis canis using a proteomic approach. BMC Vet Res 2014;10:111. doi:10.1186/1746-6148-10-111.

[6] Ceron JJ, Eckersall PD, Martýnez-Subiela S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet Clin Pathol Am Soc Vet Clin Pathol 2005;34:85–99.

[7] Britti D, Gaspari M, Massimini G, Casalinuovo F, Morittu VM, Cuda G. Proteomic analysis in canine leishmaniasis. Vet Res Commun 2010;34 Suppl 1:S91–6. doi:10.1007/s11259-010-9410-5.

[8] Zamani-Ahmadmahmudi M, Nassiri SM, Rahbarghazi R. Serological proteome analysis of dogs with breast cancer unveils common serum biomarkers with human counterparts. Electrophoresis 2014;35:901–10. doi:10.1002/elps.201300461.

[9] Zamani-Ahmadmahmudi M, Nassiri SM, Jahanzad I, Shirani D, Rahbarghazi R, Yazdani B. Isolation and characterization of a canine mammary cell line prepared for proteomics analysis. Tissue Cell 2013;45:183–90. doi:10.1016/j.tice.2012.11.002.

[10] McCaw DL, Chan AS, Stegner AL, Mooney B, Bryan JN, Turnquist SE, et al. Proteomics of canine lymphoma identifies potential cancer-specific protein markers. Clin Cancer Res Off J Am Assoc Cancer Res 2007;13:2496–503. doi:10.1158/1078-0432.CCR-06-2699.
[11] LeRoy B, Painter A, Sheppard H, Popiolek L, Samuel-Foo M, Andacht TM. Protein expression profiling of normal and neoplastic canine prostate and bladder tissue. Vet Comp Oncol 2007;5:119–30. doi:10.1111/j.1476-5829.2006.00121.x.
[12] de Freitas Campos C, Cole N, Van Dyk D, Walsh BJ, Diakos P, Almeida D, et al. Proteomic analysis of dog tears for potential cancer markers. Res Vet Sci 2008;85:349–52. doi:10.1016/j.rvsc.2007.11.006.
[13] Chu P-Y, Hsu NC, Liao AT, Shih N-Y, Hou M-F, Liu C-H. Overexpression of α-enolase correlates with poor survival in canine mammary carcinoma. BMC Vet Res 2011;7:62. doi:10.1186/1746-6148-7-62.
[14] Bracha S, McNamara M, Hilgart I, Milovancev M, Medlock J, Goodall C, et al. A multiplex biomarker approach for the diagnosis of transitional cell carcinoma from canine urine. Anal Biochem 2014;455:41–7. doi:10.1016/j.ab.2014.03.017.
[15] Atherton MJ, Braceland M, Fontaine S, Waterston MM, Burchmore RJ, Eadie S, et al. Changes in the serum proteome of canine lymphoma identified by electrophoresis and mass spectrometry. Vet J Lond Engl 1997 2013;196:320–4. doi:10.1016/j.tvjl.2012.12.010.
[16] Ceciliani F, Eckersall D, Burchmore R, Lecchi C. Proteomics in veterinary medicine: applications and trends in disease pathogenesis and diagnostics. Vet Pathol 2014;51:351–62. doi:10.1177/0300985813502819.
[17] Ostrander EA. Franklin H. Epstein Lecture. Both ends of the leash--the human links to good dogs with bad genes. N Engl J Med 2012;367:636–46. doi:10.1056/NEJMra1204453.
[18] Shearin AL, Ostrander EA. Leading the way: canine models of genomics and disease. Dis Model Mech 2010;3:27–34. doi:10.1242/dmm.004358.
[19] Ranieri G, Gadaleta CD, Patruno R, Zizzo N, Daidone MG, Hansson MG, et al. A model of study for human cancer: Spontaneous occurring tumors in dogs. Biological features and translation for new anticancer therapies. Crit Rev Oncol Hematol 2013;88:187–97. doi:10.1016/j.critrevonc.2013.03.005.
[20] Dobson JM. Breed-predispositions to cancer in pedigree dogs. ISRN Vet Sci 2013;2013:941275. doi:10.1155/2013/941275.
[21] Cadieu E, Ostrander EA. Canine genetics offers new mechanisms for the study of human cancer. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol 2007;16:2181–3. doi:10.1158/1055-9965.EPI-07-2667.
[22] Ostrander EA, Beale HC. Leading the way: finding genes for neurologic disease in dogs using genome-wide mRNA sequencing. BMC Genet 2012;13:56. doi:10.1186/1471-2156-13-56.
[23] Parker HG, Meurs KM, Ostrander EA. Finding cardiovascular disease genes in the dog. J Vet Cardiol Off J Eur Soc Vet Cardiol 2006;8:115–27. doi:10.1016/j.jvc.2006.04.002.
[24] Boyko AR. The domestic dog: man's best friend in the genomic era. Genome Biol 2011;12:216. doi:10.1186/gb-2011-12-2-216.

[25] Dorn CR. Canine breed-specific risks of frequently diagnosed diseases at veterinary teaching hospitals. AKC Canine Health Found 2002.
[26] Duleba A, Skonieczna K, Bogdanowicz W, Malyarchuk B, Grzybowski T. Complete mitochondrial genome database and standardized classification system for Canis lupus familiaris. Forensic Sci Int Genet 2015;19:123–9. doi:10.1016/j.fsigen.2015.06.014.
[27] Bai B, Zhao W-M, Tang B-X, Wang Y-Q, Wang L, Zhang Z, et al. DoGSD: the dog and wolf genome SNP database. Nucleic Acids Res 2015;43:D777–83. doi:10.1093/nar/gku1174.
[28] Agrafioti I, Stumpf MPH. SNPSTR: a database of compound microsatellite-SNP markers. Nucleic Acids Res 2007;35:D71–5. doi:10.1093/nar/gkl806.
[29] Robinson J, Halliwell JA, McWilliam H, Lopez R, Marsh SGE. IPD--the Immuno Polymorphism Database. Nucleic Acids Res 2013;41:D1234–40. doi:10.1093/nar/gks1140.
[30] Keshava Prasad TS, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, et al. Human Protein Reference Database--2009 update. Nucleic Acids Res 2009;37:D767–72. doi:10.1093/nar/gkn892.
[31] Kandasamy K, Keerthikumar S, Goel R, Mathivanan S, Patankar N, Shafreen B, et al. Human Proteinpedia: a unified discovery resource for proteomics research. Nucleic Acids Res 2009;37:D773–81. doi:10.1093/nar/gkn701.
[32] Rosa N, Correia MJ, Arrais JP, Lopes P, Melo J, Oliveira JL, et al. From the salivary proteome to the OralOme: comprehensive molecular oral biology. Arch Oral Biol 2012;57:853–64. doi:10.1016/j.archoralbio.2011.12.010.
[33] Arrais JP, Rosa N, Melo J, Coelho ED, Amaral D, Correia MJ, et al. OralCard: a bioinformatic tool for the study of oral proteome. Arch Oral Biol 2013;58:762–72. doi:10.1016/j.archoralbio.2012.12.012.
[34] Tvarijonaviciute A, Carrillo-Sanchez JD, García-Martinez JD, Tecles F, Martinez-Subiela S, German AJ, et al. Measurement of salivary adiponectin concentrations in dogs. Vet Clin Pathol Am Soc Vet Clin Pathol 2014;43:416–21. doi:10.1111/vcp.12169.
[35] Tvarijonaviciute A, Gutiérrez AM, Miller I, Razzazi-Fazeli E, Tecles F, Ceron JJ. A proteomic analysis of serum from dogs before and after a controlled weight-loss program. Domest Anim Endocrinol 2012;43:271–7. doi:10.1016/j.domaniend.2012.04.004.
[36] Su S, Tian J, Hong M, Zhou P, Lu G, Zhu H, et al. Global and quantitative proteomic analysis of dogs infected by avian-like H3N2 canine influenza virus. Front Microbiol 2015;6:228. doi:10.3389/fmicb.2015.00228.
[37] Schlieben P, Meyer A, Weise C, Bondzio A, Einspanier R, Gruber AD, et al. Differences in the proteome of high-grade versus low-grade canine cutaneous mast cell tumours. Vet J Lond Engl 1997 2012;194:210–4. doi:10.1016/j.tvjl.2012.04.002.
[38] Sarasa L, Allué JA, Pesini P, González-Martínez A, Sarasa M. Identification of β-amyloid species in canine cerebrospinal fluid by mass spectrometry. Neurobiol Aging 2013;34:2125–32. doi:10.1016/j.neurobiolaging.2013.03.009.
[39] Roerig A, Carlson R, Tipold A, Stein VM. Cerebrospinal fluid tau protein as a biomarker for severity of spinal cord injury in dogs with intervertebral disc herniation. Vet J Lond Engl 1997 2013;197:253–8. doi:10.1016/j.tvjl.2013.02.005.

579 [40] Polovic N, Wadén K, Binnmyr J, Hamsten C, Grönneberg R, Palmberg C, et al.
580 Dog saliva - an important source of dog allergens. Allergy 2013;68:585–92.
581 doi:10.1111/all.12130.
582 [41] Parra MD, Tecles F, Martínez-Subiela S, Cerón JJ. C-reactive protein
583 measurement in canine saliva. J Vet Diagn Investig Off Publ Am Assoc Vet
584 Lab Diagn Inc 2005;17:139–44.
585 [42] Nakamura M, Takahashi M, Ohno K, Koshino A, Nakashima K, Setoguchi A, et
586 al. C-reactive protein concentration in dogs with various diseases. J Vet Med
587 Sci Jpn Soc Vet Sci 2008;70:127–31.
588 [43] Nakamura K, Miyasho T, Nomura S, Yokota H, Nakade T. Proteome analysis
589 of cerebrospinal fluid in healthy beagles and canine encephalitis. J Vet Med
590 Sci Jpn Soc Vet Sci 2012;74:751–6.
591 [44] Nakajima M, Ohno K, Goto-Koshino Y, Fujino Y, Tsujimoto H. Plasma
592 transferrin concentration as a nutritional marker in malnourished dogs
593 with nutritional treatment. J Vet Med Sci Jpn Soc Vet Sci 2014;76:539–43.
594 [45] Miyasho T, Nakamura K, Nomura S, Kawasako K, Nakade T, Yamada S, et al.
595 High mobility group box 1 (HMGB1) protein is present in the cerebrospinal
596 fluid of dogs with encephalitis. J Vet Med Sci Jpn Soc Vet Sci 2011;73:917–22.
597 [46] Miller I, Preßlmayer-Hartler A, Wait R, Hummel K, Sensi C, Eberini I, et al. In
598 between - Proteomics of dog biological fluids. J Proteomics 2014;106:30–45.
599 doi:10.1016/j.jprot.2014.04.016.
600 [47] Hormaeche M, Carretón E, González-Miguel J, Gussoni S, Montoya-Alonso JA,
601 Simón F, et al. Proteomic analysis of the urine of Dirofilaria immitis infected
602 dogs. Vet Parasitol 2014;203:241–6. doi:10.1016/j.vetpar.2014.01.025.
603 [48] Guevel L, Lavoie JR, Perez-Iratxeta C, Rouger K, Dubreil L, Feron M, et al.
604 Quantitative proteomic analysis of dystrophic dog muscle. J Proteome Res
605 2011;10:2465–78. doi:10.1021/pr2001385.
606 [49] Grider A, Mouat MF, Mauldin EA, Casal ML. Analysis of the liver soluble
607 proteome from bull terriers affected with inherited lethal acrodermatitis.
608 Mol Genet Metab 2007;92:249–57. doi:10.1016/j.ymgme.2007.07.003.
609 [50] Gharbi M, Sanchez C, Mazzucchelli G, De Pauw E, Henrotin Y. Identification
610 of differential pattern of protein expression in canine osteoarthritis serum
611 after anterior cruciate ligament transection: a proteomic analysis. Vet J
612 Lond Engl 1997 2013;197:848–53. doi:10.1016/j.tvjl.2013.05.037.
613 [51] Fahiminiya S, Reynaud K, Labas V, Batard S, Chastant-Maillard S, Gérard N.
614 Steroid hormones content and proteomic analysis of canine follicular fluid
615 during the preovulatory period. Reprod Biol Endocrinol RBE 2010;8:132.
616 doi:10.1186/1477-7827-8-132.
617 [52] de Sousa-Pereira P, Cova M, Abrantes J, Ferreira R, Trindade F, Barros A, et
618 al. Cross-species comparison of mammalian saliva using an LC-MALDI based
619 proteomic approach. Proteomics 2015;15:1598–607.
620 doi:10.1002/pmic.201400083.
621 [53] de Sousa-Pereira P, Abrantes J, Colaço B, Castagnola M, Amado F, Esteves PJ,
622 et al. Characterization of thymosin β4 in mammals' saliva. Peptides
623 2013;40:1–7. doi:10.1016/j.peptides.2012.12.007.
624 [54] Collins MT. Canine inflammatory bowel disease: current and prospective
625 biomarkers for diagnosis and management. Compend Contin Educ Vet
626 2013;35:E5.

627 [55] Brandt LE, Ehrhart EJ, Scherman H, Olver CS, Bohn AA, Prenni JE.
628 Characterization of the canine urinary proteome. Vet Clin Pathol Am Soc Vet
629 Clin Pathol 2014;43:193–205. doi:10.1111/vcp.12147.
630 [56] Atherton MJ, Braceland M, Harvie J, Burchmore RJ, Eadie S, Eckersall PD, et
631 al. Characterisation of the normal canine serum proteome using a novel
632 electrophoretic technique combined with mass spectrometry. Vet J Lond
633 Engl 1997 2013;196:315–9. doi:10.1016/j.tvjl.2012.12.011.
634 [57] Antognoni MT, Siepi D, Porciello F, Fruganti G. Use of serum cistatin C
635 determination as a marker of renal function in the dog. Vet Res Commun
636 2005;29 Suppl 2:265–7. doi:10.1007/s11259-005-0058-5.
637 [58] Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al.
638 ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology
639 and pathway annotation networks. Bioinforma Oxf Engl 2009;25:1091–3.
640 doi:10.1093/bioinformatics/btp101.
641 [59] Bindea G, Galon J, Mlecnik B. CluePedia Cytoscape plugin: pathway insights
642 using integrated experimental and in silico data. Bioinforma Oxf Engl
643 2013;29:661–3. doi:10.1093/bioinformatics/btt019.
644 [60] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al.
645 Cytoscape: a software environment for integrated models of biomolecular
646 interaction networks. Genome Res 2003;13:2498–504.
647 doi:10.1101/gr.1239303.
648 [61] UniProt Consortium. UniProt: a hub for protein information. Nucleic Acids
649 Res 2015;43:D204–12. doi:10.1093/nar/gku989.
650 [62] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J,
651 et al. STRING v10: protein-protein interaction networks, integrated over the
652 tree of life. Nucleic Acids Res 2015;43:D447–52. doi:10.1093/nar/gku1003.
653 [63] Coelho ED, Arrais JP, Matos S, Pereira C, Rosa N, Correia MJ, et al.
654 Computational prediction of the human-microbial oral interactome. BMC
655 Syst Biol 2014;8:24. doi:10.1186/1752-0509-8-24.
656 [64] Papadogiannakis EI, Koutinas AF. Cutaneous immune mechanisms in canine
657 leishmaniosis due to Leishmania infantum. Vet Immunol Immunopathol
658 2015;163:94–102. doi:10.1016/j.vetimm.2014.11.011.
659 [65] Olivier M, Gregory DJ, Forget G. Subversion mechanisms by which
660 Leishmania parasites can escape the host immune response: a signaling
661 point of view. Clin Microbiol Rev 2005;18:293–305.
662 doi:10.1128/CMR.18.2.293-305.2005.
663 [66] Gupta G, Oghumu S, Satoskar AR. Mechanisms of immune evasion in
664 leishmaniasis. Adv Appl Microbiol 2013;82:155–84. doi:10.1016/B978-0-
665 12-407679-2.00005-3.
666 [67] Shio MT, Hassani K, Isnard A, Ralph B, Contreras I, Gomez MA, et al. Host cell
667 signalling and leishmania mechanisms of evasion. J Trop Med
668 2012;2012:819512. doi:10.1155/2012/819512.
669 [68] Spooner R, Yilmaz Ö. Nucleoside-diphosphate-kinase: a pleiotropic effector
670 in microbial colonization under interdisciplinary characterization. Microbes
671 Infect Inst Pasteur 2012;14:228–37. doi:10.1016/j.micinf.2011.10.002.
672 [69] Saha A, Robertson ES. Functional modulation of the metastatic suppressor
673 Nm23-H1 by oncogenic viruses. FEBS Lett 2011;585:3174–84.
674 doi:10.1016/j.febslet.2011.08.007.

675 [70] Hippe H-J, Wolf NM, Abu-Taha HI, Lutz S, Le Lay S, Just S, et al. Nucleoside
676 diphosphate kinase B is required for the formation of heterotrimeric G
677 protein containing caveolae. Naunyn Schmiedebergs Arch Pharmacol
678 2011;384:461–72. doi:10.1007/s00210-011-0618-x.
679

Table 1

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Breeds	Disease name	Nº of proteins	Nº of exclusive proteins	Nº of proteins with regulation data	Sample source
Beagles	Meningoencephalitis	34		1	Cerebrospinal fluid
	Obesity *	3		3	Serum
	Encephalitis	1		1	Cerebrospinal fluid
	Canine influenza virus A (H3N2)	276		276	Lung
Bull terrier	**Lethal acrodermatitis***	12	6	5	Liver
Golden retriever	Duchenne muscular dystrophy	83	2	83	Muscle
American eskimo	Transitional cell carcinoma	10		0	Urine
Australian shepherd	Lymphoma	5		5	Lymph nodes and plasma
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Boxer	Canine cutaneous mast cell tumors	13	6	13	Skin
	Multicentric lymphoma	20		0	Serum
	Various cancers	5	1	3	Tears
Brazilian fila	Various cancers	5	1	3	Tears
Cairn terrie	Prostatic carcinoma	2		2	Prostate
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Chihuahu as	Meningoencephalitis	34		1	Cerebrospinal fluid
Dachshund	Canine cutaneous mast cell tumours	13	6	13	Skin
Dalmatian	Lymphoma	5		5	Lymph nodes and plasma
Doberman pinscher	Various cancers	5	1	3	Tears
English cocker spaniel	Various cancers	5	1	3	Tears
German shepherd	Canine leishmaniasis	16		4	Serum and saliva
	Multicentric lymphoma	20		0	Serum
	Breast cancer	5		5	Serum
	Various cancers	5	1	3	Tears
Irish setter	Various cancers	5	1	3	Tears
Labrador retriever	Multicentric lymphoma	20		0	Serum
	Prostatic carcinoma	2		2	Prostate
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Malteses	Meningoencephalitis	34		1	Cerebrospinal fluid
Miniature pinscher	Various cancers	5	1	3	Tears
Newfoundland	Lymphoma	5		5	Lymph nodes and plasma
Pekingeses	Meningoencephalitis	34		1	Cerebrospinal fluid
Pembroke welsh corgi	Transitional cell carcinoma	10		0	Urine
	Lymphoma	5		5	Lymph nodes and plasma
	Chronic bronchitis	7		6	Bronchoalveolar lavage fluid
Poodle	Prostatic carcinoma	2		2	Prostate

Table 2

Click here to download Table: Table2.xlsx

Disease name	Nº of proteins	Nº of exclusive proteins	Biomarker	Most prevalent diseases*
Allergies	10	6		
Alzheimer's disease	1	1		
Breast cancer	12	2		X
Canine babesiosis	15	1	X	
Canine cardiopulmonary dirofilariosis	7	1		
Canine inflammatory bowel disease	8	5		
Canine leishmaniasis	16			
Intervertebral disc herniation	1	1	X	
Lymphoma	5			
Osteoarthritis	15	8		X
Prostatic carcinoma	2			
Renal insufficiency	1	1	X	
Transitional cell carcinoma	10			
Various cancers	4	1		X
XLHN	14			
Duchenne muscular dystrophy	1		X	
Envenomation by European adder (Vipera berus berus)	5			
Joint osteoarthritis secondary to cranial cruciate ligament disease	17			

*According to Dorn 2002 [25].

Table 3

Click here to download Table: Table3.xlsx

Uniprot KB AC	String Code	Protein name	Regulation	Exclusive
E2R0L9	TCP1	T-complex protein 1, alpha subunit	↑	×
E2RB81	CCT5	T-complex protein 1, epsilon subunit	↑	×
F1P679	Arp3	Actin related protein 3	↑	×
F1P797	ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	↑	×
F1PR93	WDR1	WD repeat domain 1	↑	×
E2RAU5	HSPA9	Heat shock 70 kDa protein 9 (mortalin)	↑	
E2RCI8	ANXA6	Annexin A6	↑	
E2RD86	PDIA3	Protein disulfide isomerase A3	↑	
P15944	ENSCAFG00000031939	Tryptase alpha/beta 1	↓	×
J9P430	TF	Transferrin	↓	
O18840	ACTB	Actin, beta	↓	
P49822	ALB	Serum albumin	↓	
Q6TEQ7	ANXA2	Annexin A2	↓	

Figure 1

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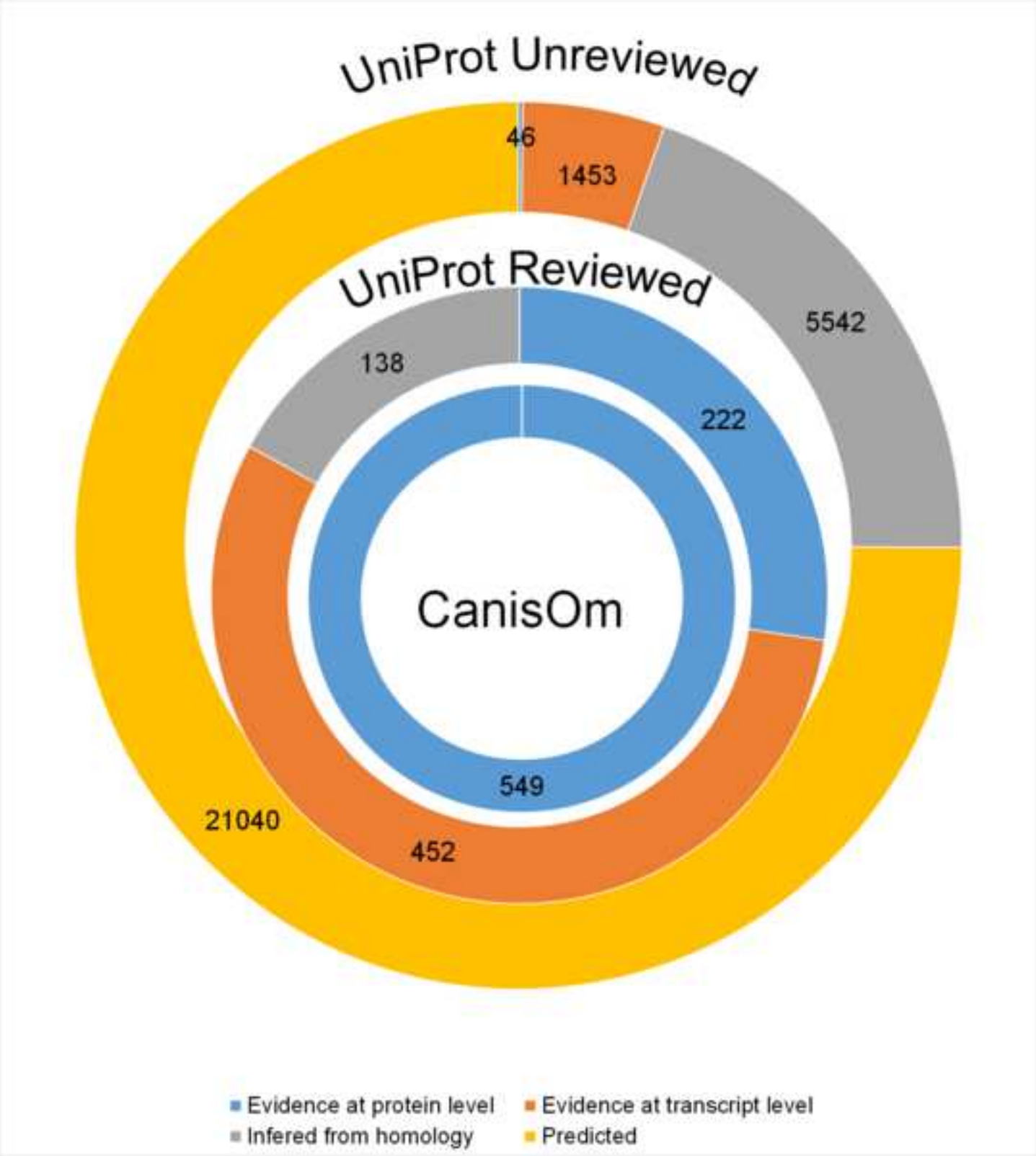

Figure 2

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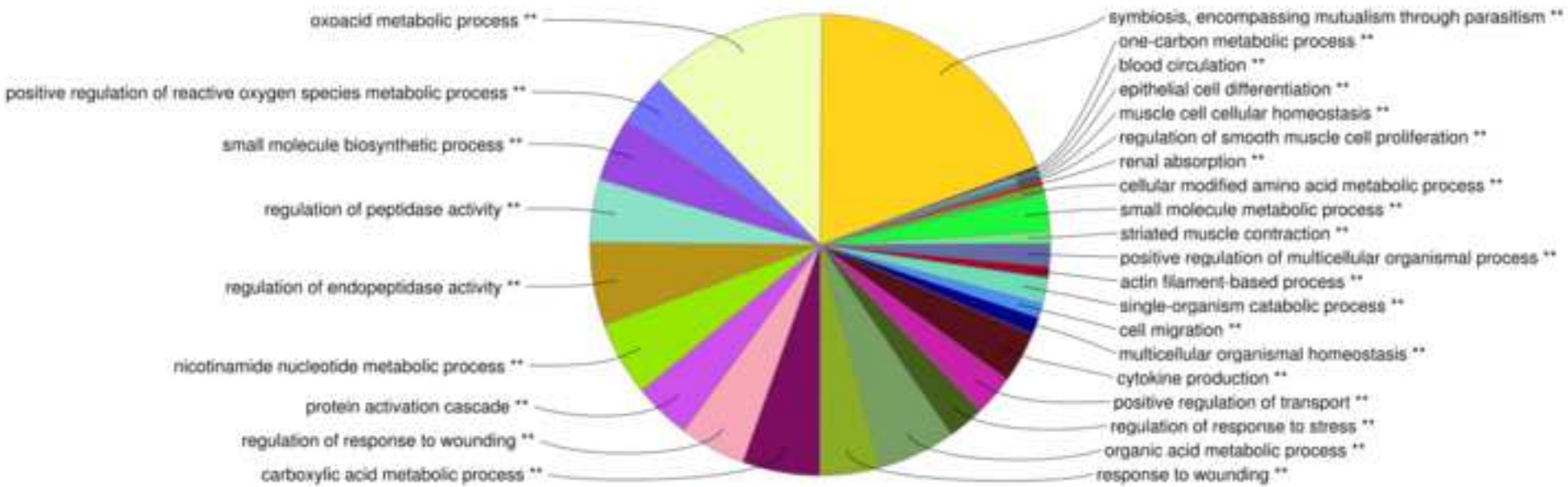

Figure 3

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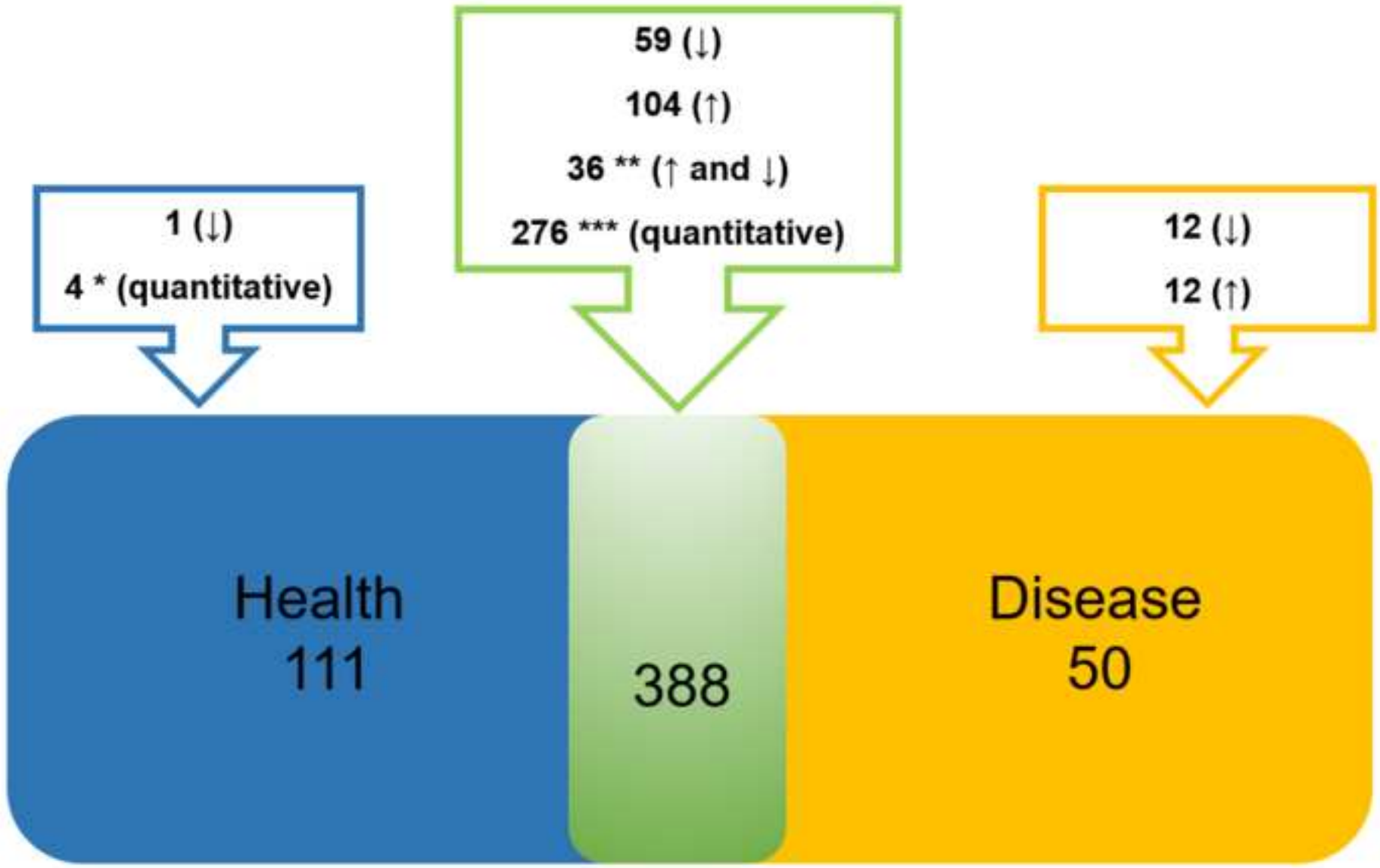

Figure 4

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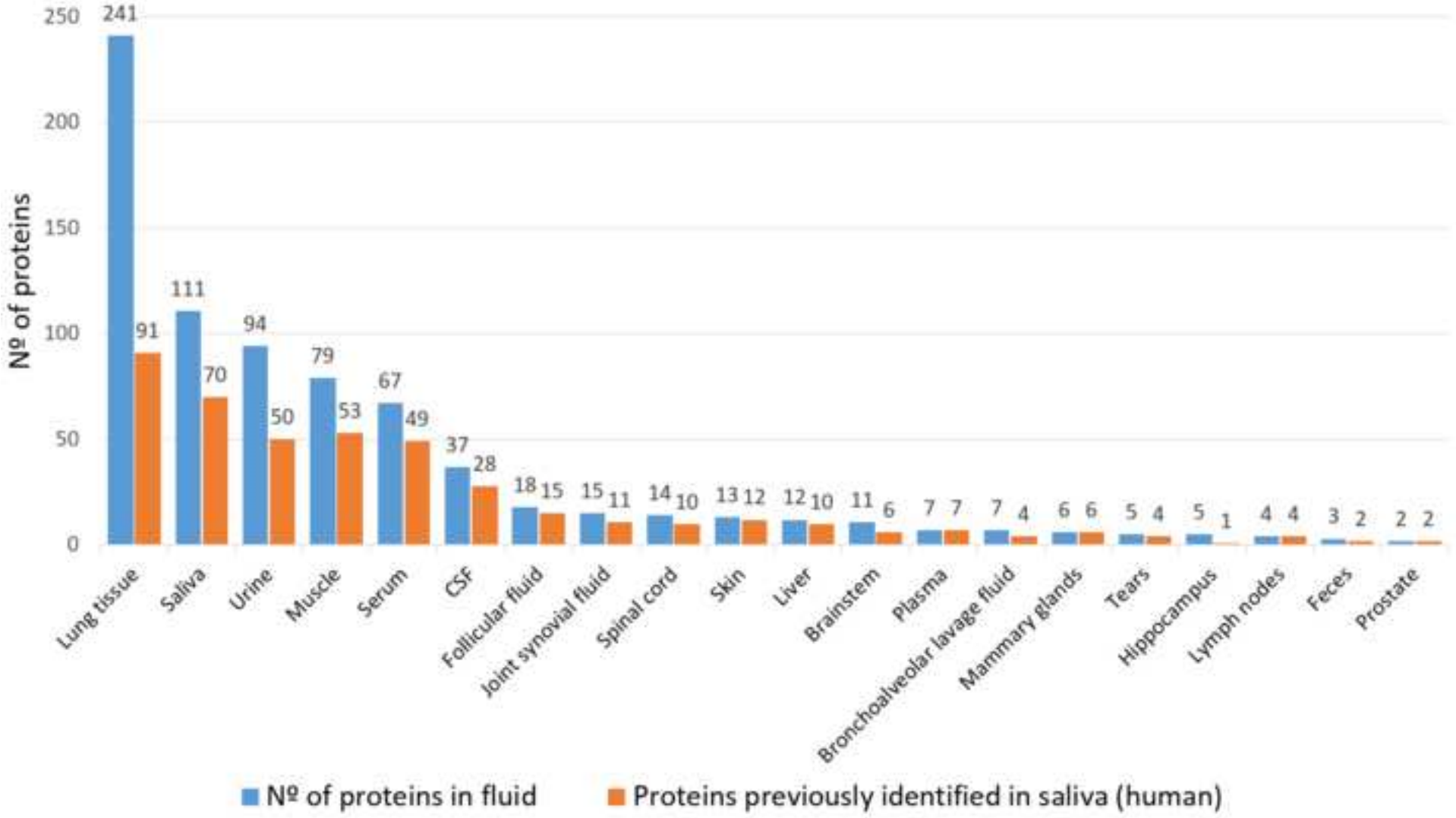

Figure 5

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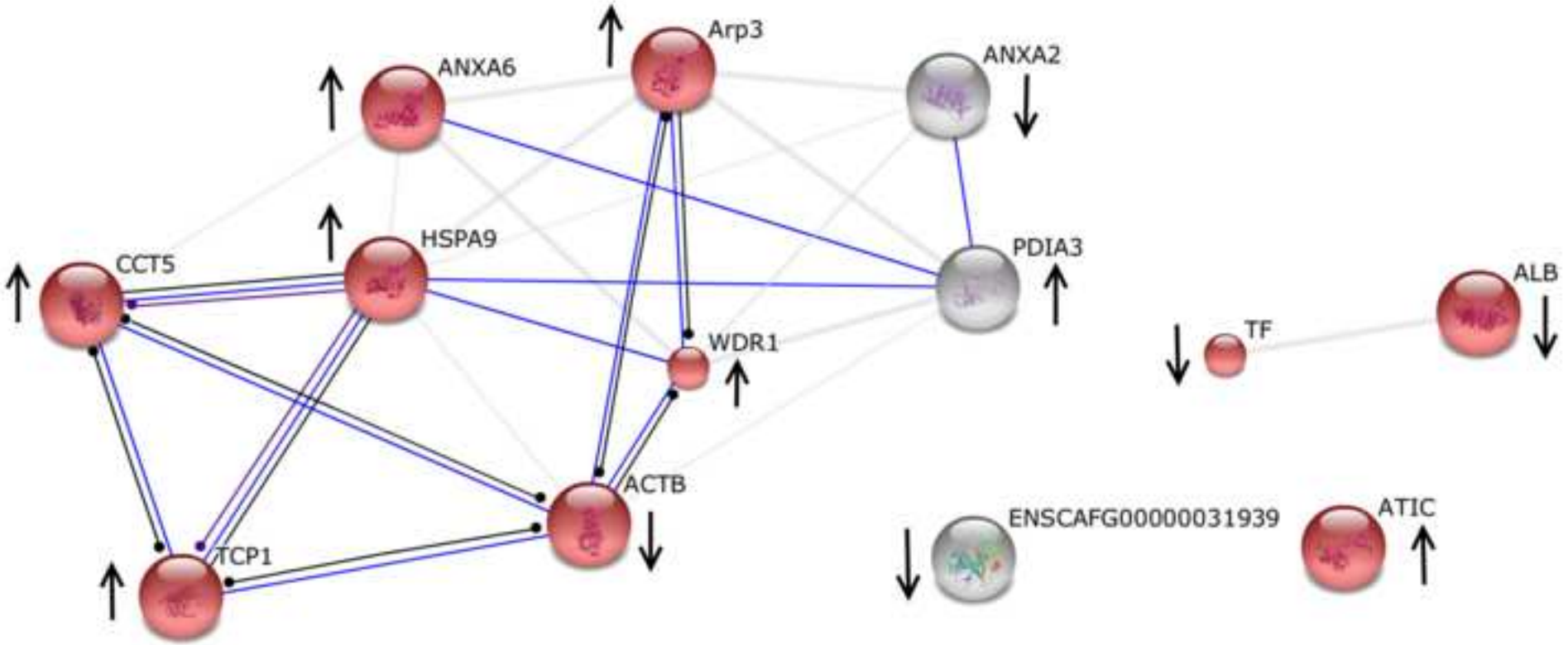

Figure 6

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