Submitted to CRATER - Conference on Recent Advances in Translational Eye Research 2025

X-linked hereditary nephropathy in Canis lupus familiaris as a translational model for Alport syndrome in humans

Barbara Kominek*[1], Joanna Gruszczyńska[2], Beata Grzegrzółka[1,2]
[1]*Scientific Association of Experimental and Laboratory Animals, Warsaw University of Life Sciences, Poland*
[2]*Department of Animal Genetics and Conservation, Institute of Animal Sciences, Warsaw University of Life Sciences, Poland*
[#]*corresponding author email: s212840@sggw.edu.pl*

1. Main Text

Alport syndrome is an inherited disorder in humans characterized by progressive renal dysfunction, accompanied by visual impairments. Chronic kidney disease represents a significant clinical challenge in both human and veterinary medicine. Although understanding of the genetic basis of hereditary nephropathies is expanding, the underlying molecular mechanisms vary depending on the mode of inheritance, complicating the development of universal diagnostic and therapeutic strategies. The domestic dog, with its well-characterized genome and spontaneously occurring pathogenic variants, serves as a valuable translational model. Comparative analysis of *COL4A5* gene variants in dogs and humans not only advances our understanding of disease pathogenesis but also supports the development of targeted treatment strategies for both veterinary and human medicine.

The aim of this study was to analyze the current knowledge on hereditary kidney failure in the domestic dog, with particular emphasis on the X-linked variant caused by a single mutation in the COL4A5 gene. The objectives included a bioinformatic comparison of the molecular basis of the canine X-linked variant and its relevance to the pathogenesis of Alport syndrome in humans.

Hereditary nephritis in the domestic dog, associated with dysfunction of the glomerular basement membrane resulting from an abnormal structure of type IV collagen, is characterized by three different mechanisms of inheritance (Tab.1). In the autosomal recessive form, occurring among others in English cocker spaniel dogs, and autosomal dominant, described among others in bull terrier dogs, the causative mutations concern the *COL4A4* gene, encoding one of the six alpha chains of type IV Collagen (Fig. 1). In the case of X-linked inheritance, characteristic of Samoyed dogs, the causative mutation is located in the *COL4A5* gene, crucial for the synthesis of the Collagen α6 (IV) chain. The high degree of homology of the *COL4A5* gene sequence between dogs affected with hereditary nephropathy and patients with Alport syndrome indicates the possibility of using animal models in translational studies, enabling simultaneous analysis of molecular and clinical aspects of the disease in both species.

Tab. 1. The gene group responsible for encoding the α chains of type IV collagen in the domestic dog.

α chain	Gene	Chromosome	Mutation characteristics
α1	*COL4A1*	22	Lethal mutations at the embryonic developmental stage
α2	*COL4A2*	22	
α3	*COL4A3*	25	Mutations associated with autosomal inheritance
α4	*COL4A4*	25	
α5	*COL4A5*	X	Mutations associated with X-linked inheritance
α6	*COL4A6*	X	

Fig. 1. Six type IV collagen genes (*COL4A1–COL4A6*), organized in pairs on three chromosomes, encode six α chains with a C-terminal non-collagenous domain. These chains assemble into triple-helical molecules (Tryggvason, 2009).

2. Methods and results

For bioinformatic analyses, nucleotide and amino acid sequences available in the GenBank database under accession number NM_001002979.1 were used. Primers defining amplification sites, selected based on literature data, were designed with the Primer3 software. Sequence analysis and the characterization of the encoded proteins were performed using the BLAST tool. DNA sequences were further analyzed for restriction enzyme recognition sites with NEBcutter, which allows the identification of restriction sites. The STRING v.12.0 platform was applied to evaluate the strength of COL4A5 protein interactions with other proteins, as well as the co-expression level of COL4A5 with other genes at the mRNA level in the domestic dog.

Fig. 2. Protein interaction network of COL4A5 in domestic dog (STRING v12. - https://string-db.org/).

Tab. 2. Predicted interaction strength of proteins with COL4A5 in the domestic dog and in humans.

Protein		Domestic dog	Human
COL4A2	Collagen α2 (IV) chain	0.934	0.826
COL4A6	Collagen α6 (IV) chain	0.912	0.982
GP6	Platelet glycoprotein VI	0.907	0.904
ITGA3	Integrin α3 subunit	0.854	0.789
PELO	Pelota homolog	0.851	-
ITGB	Integrin β subunit	0.823	0.818
ITGA2	Integrin α2 subunit	0.768	0.744
ITGA8	Integrin α8 subunit	0.752	0.734
ITGA5	Integrin α5 subunit	0.742	0.718
ITGA6	Integrin α6 subunit	0.741	0.825

Gene *COL4A5* forms protein interaction primarily with type IV collagen chains and integrins, which mediate cell adhesion to the extracellular matrix and intercellular signaling (Fig.2). In the domestic dog, gene co-expression analysis revealed moderate values, particularly between *COL4A5* and *COL4A2/COL4A6*, confirming their shared role in type IV collagen structure (Tab.2). In humans, co-expression values were notably higher. The most important biological process associated with *COL4A5* is cell adhesion to the extracellular matrix, highlighting its role in maintaining the integrity and proper function of the glomerular basement membrane.

Analysis of interaction networks and co-expression confirmed the central role of *COL4A5* in the function of the glomerular basement membrane and in the pathogenesis of hereditary nephropathies in dogs and Alport syndrome in humans. Differences in co-expression levels between species suggest regulatory divergences that may underlie variations in clinical manifestations. The high structural and functional similarity of *COL4A5* in dogs and humans supports the use of the domestic dog as an animal model for *COL4A5*-related diseases.

3. References

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Bioinformatic tools:

1. BLAST - https://blast.ncbi.nlm.nih.gov/

2. GenBank - https://www.ncbi.nlm.nih.gov/genbank/

3. NEBcutter - https://nc3.neb.com/NEBcutter/

4. Primer 3 - https://primer3.ut.ee/

5. STRING v. 12.0 - https://string-db.org