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Review article

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ALPORT SYNDROME – A RARE KIDNEY DISEASE OF DOMESTIC DOG *CANIS LUPUS FAMILIARIS*

ZESPÓŁ ALPORTA – RZADKA CHOROBA NEREK PSA DOMOWEGO *CANIS LUPUS FAMILIARIS*

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Streszczenie. Zespół Alporta jest rzadką dziedziczną chorobą nerek spowodowaną brakiem jednego z łańcuchów kolagenu typu IV (α3, α4, α5). Kolagen typu IV jest podstawowym elementem budującym błonę podstawną kłębuszka nerkowego. Zespół Alporta występuje zarówno u człowieka, myszy, jak i psa domowego. Mutacje w obrębie genów COL4A3 i COL4A4 mają charakter zarówno recesywny, jak i dominujący. Jednakże najliczniejsza grupa mutacji występująca w obrębie genu COL4A5 jest sprzężona z chromosomem płci X. Poprzez mutacje w tych genach synteza łańcuchów: α3, α4, α5 jest niemożliwa, w następstwie czego kolagen typu IV nie jest poprawnie zbudowany, nie może w pełni następować filtracja osocza. Konsekwencją zaburzeń w filtracji jest postępująca niewydolność nerek i w efekcie zaprzestanie pracy nerek. Przy obecnej wiedzy medycznej jedynym sposobem na opóźnienie postępu choroby – zespółu Alporta są dializy i przeszczep nerki. W niniejszym artykule przedstawiono informacje na temat objawów wyżej wspomnianej choroby, opisano podstawy genetyczne oraz molekularne skutki mutacji powodującej zespół Alporta. Na podstawie dostępnej literatury przedstawiono również rady dla hodowców psów, głównie w celu ułatwienia podejmowania właściwych decyzji hodowlanych.

Key words: Alport syndrome, type IV collagen, *Canis lupus familiaris.*
Słowa kluczowe: zespół Alporta, kolagen typu IV, *Canis lupus familiaris.*

INTRODUCTION

Alport syndrome is a heterogenic disorder resulting from mutation of the genes encoding the synthesis of several (α3, α4, α5 and/or α6) chains of type IV collagen, the basic structural element of the glomerular basement membrane (Myers et al. 1990; Gunwar et al. 1998; Tryggvason and Martin 2001; Cox et al. 2003). In humans, 80% of all Alport syndrome cases are caused by mutations in chromosome X – located COL4A5 gene, whereas the remaining ones are either autosomal recessive (15%) or dominant (5%) mutations in COL4A3 and COL4A4 genes (Kashtan 2002; Cox and Murphy 2004).

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The proper structure of type IV collagen enables glomerular basement membrane its efficient functioning, i.e. plasma filtration. In case of any structural abnormalities (lack of any of type IV collagen chains) this filtration is impaired and leads to severe renal disease and insufficiency that can be only treated with dialysis and, eventually, transplantation (Gunwar et al. 1998).

Alport syndrome's symptoms are closely related to defective functioning of kidneys and generally include hematuria, progressive renal insufficiency and eventual failure. Some other symptoms are seemingly not kidney-related, and these include impaired vision and hearing.

Symptoms are variable, depending on the actual mutation.

Alport syndrome is a serious problem in breeding kennels, mainly in the Samoyed breed. The onsets of the first symptoms is quite late, ranging from 4 months up to, as late as, 4 years of life, thus the affected, though asymptomatic, dogs may be used in breeding and pass their mutated genes to their offsprings. Therefore, an early diagnosis based on clinical symptoms is of the utmost importance, as it enables breeders to exclude affected animals from their breeding programs.

THE STRUCTURE OF THE GLOMERULUS AND THE ROLE OF THE BASEMENT MEMBRANE

The glomerular basement membrane (GBM) is especially thick, laminar, homogenous basement membrane that stabilizes the whole structure of the glomerulus and contributes importantly to the kidney's filtration barrier. Its thickness depends on age and sex and consists of three layers: *lamina rara externa*, *lamina densa* and *lamina rara interna*; together they form an unbroken filtration barrier generated by two cell types – podocytes and, to a lesser degree, mesangiocytes. GBM is built of glycoproteins and polyanionic proteoglycans (Hałon 2011).

The main component of GBM is type IV collagen, which consists of α3, α4, α5 and α6 protein chains. In case of Alport syndrome the synthesis of one of those chains (depending on the actual mutation) is impaired, resulting in pronounced irregularities in GBM structure and thus severe impairment of plasma filtration and detoxification, leading in turn to progressive renal insufficiency and failure, and the inevitable death due to intoxication with waste metabolic products.

PHENOTYPIC CONSEQUENCES AND SYMPTOMS OF ALPORT SYNDROME IN DOMESTIC DOGS

Studies were carried out on dogs suffering from acute renal insufficiency diagnosed at the mean age of 18 months (ranging from 8 months up to 7 years). However, contrary to reports in humans, none of the dogs showed impaired vision and/or hearing. Therefore, depending on the type of mutation, clinical signs in dog may vary, as reported in humans (Gunwar et al. 1998; Hood et al. 2002; Kashtan 2002), with the exception of hearing loss and progressive blindness (Kashtan 2002; Lees 2013).

GENETIC BACKGROUND

Alport syndrome is a consequence of α3, α4 α5, α6 type IV collagen chains deficiency; as type IV collagen plays a key role in proper structure formation of GBM, which is essential for its function (Tryggvason and Martin 2001).

THE INHERITANCE OF THE COL4A5 MUTATION – XLAS (X-LINKED ALPORT SYNDROME)

With the proper structure of type IV collagen, GBM provides efficient filtration. However, when this structure is altered due to any deficiency of any of the components, filtration is impaired and leads to kidney disease and renal insufficiency that must be treated with dialysis and kidney transplantation. Mutations in collagen, type IV, alpha 5 gene (COL4A5), which is located in the longer q part of X chromosome, remain the most common cause of Alport syndrome, regardless of the actual type of the mutation, which may result either from a small deletion or insertion of one or more nucleotides and lead to changes in translation and finally to production of an altered protein product (Tryggvason and Martin 2001).

Mutations in COL4A5 gene are spontaneous and lead to the change of one base pair in DNA composition. Another mutation that causes Alport syndrome is the so-called nonsense mutation, when point mutations lead to that change of codon that was coding for amino acid and formation of the STOP codon. As a result, the synthetized protein is shorter and dysfunctional (Gross et al. 2002).

Additionally, large deletions may spread the mutation on chromosome X to the closely located COL4A6 gene. Such massive deletions lead to Alport syndrome together with leiomyomatosis (Tryggvason and Martin 2001).

According to Kashtan (2002), mutation in the sex-linked COL4A5 (NM_001002979.1) gene (The National Center..., http://ncbi.nlm.nih.gov) is the most frequent cause of Alport syndrome (XLAS- X-linked Alport syndrome). This gene encodes the α5 chain of type IV collagen and its mutation prevents the proper protein synthesis. The first symptoms of the disorder appear in humans at the age of about 10 years, in dogs – about 4 months (Kashtan 2002) and can be classified as otolaryngological and ophthalmologic in humans, and nephrological in both species.

Jansen et al. (1984) and Zheng et al. (1994) demonstrated substitution of G to T in exon 35 of COL4A5 gene in the Samoyed breed, which changed the glycine codon into the stop codon. Other study reports that deletion of 10 bz in exon 9 of COL4A5 gene, found in mixed breed dogs from Navasota, Texas, causes frame shift and presence of the premature stop codon in exon 10 (Cox et al. 2003). Hematuria as the first symptom of Alport syndrome occurs around 4 months of age in Samoyeds and 3–4 months in Navasota dogs, whereas the end-stage renal disease develops at the age of 8–10 months, and 10–15 months, respectively (Jansen et al. 1984; Zheng et al. 1994; Cox et al. 2003). Rao et al. (2003, 2005) demonstraited that in canine X-linked Alport syndrome (XLAS) is observed elevated expression of MMP-2, MMP-9, and MT1-MMP in fibrotic renal cortex from X-linked Alport syndrome dogs. examinated the evolution of renal damage and the expression of selected molecules potentially involved in the pathogenesis of XLHN. They indentificated that transforming

growth factor b, connective tissue growth factor, and platelet-derived growth factor a should be considered as key players in the initial events of XHLN. Clusterin and TIMP1 appear to be more associated with the progression rather than initiation of tubulointerstitial damage in chronic renal disease (Greer et al. 2006; Benali et al. 2016). In addition, Greer et al. (2006) found altered expression of numerous major histocompatibility complex (MHC) molecules suggests that the immune system plays a significant role in XLAS.

Among nephrological symptoms, hematuria remains the most common one, followed by proteinuria. Progressive renal insufficiency is both a symptom and a consequence of Alport syndrome and leads to end-stage disease that is treated with dialysis and renal transplantation. Other symptoms are increased blood creatinine levels and hypertension (Gubler et al. 1981).

Hematuria occurs more frequently in males, together with early progressive renal insufficiency and consequent failure. However, in females, Alport syndrome can develop without any noticeable symptoms. The first diagnostic symptom is hematuria and its presence should draw one's attention to other possibly existing symptoms. Patients usually develop progressive hearing loss (Genetic home…, http://ghr.nlm.nih.gov/gene/COL4A3, Genetic home…, http://ghr.nlm.nih.gov/gene/COL4A4).

The most frequent ophthalmologic symptoms are corneal opacity (cataract) and retinopathy; however, they are rarely observed and occur in about 10% of cases, mostly in the form of clouding of the lens and damage to the ophthalmic nerve. Visual impairment is progressive and irreversible, and its treatment can only delay the disease progress (Genetic home…, http://ghr.nlm.nih.gov/gene/COL4A3; Genetic home…, http://ghr.nlm.nih.gov/gene/COL4A4). Scott et al. (2001) proposed that in Alport syndrome, the loss of the α3/α4/α5 network eventually weakens the interaction of these cells with their extracellular matrix, resulting in reduced tension on the basilar membrane and the inability to respond to high frequency sounds. Occasionally, some abnormalities in gastrointestinal functions can be also observed (Gubler et al. 1981).

THE RECESSIVE AUTOSOMAL MUTATION

This type of mutation is responsible for approximately 15% of all Alport syndrome cases in dogs. It is located in COL4A3 (XM_005635768.1) and COL4A4 (AY263363.1) genes of chromosome number 25, which are responsible for α3 and α4 chain synthesis, respectively (Lee et al. 1998; The National Center..., http://ncbi.nlm.nih.gov). Kashtan (2002) reports identification of mutations in these genes in English cocker spaniels. The first symptoms (proteinuria) were apparent at the age of 5–8 months and the end-stage chronic kidney disease developed between 12–18 months. Similarly to the previous case, this type of mutation prevents the synthesis of proper α3 and α4 chains of type IV collagen and results in abnormalities in the GBM structure.

Sugahara et al. (2015) demonstrated proteinuria and kidney function abnormalities in a 9 month old Pyrenean mountain dog. Symptoms caused by mutations described above are identical to those caused by the sex-linked one, with the exception of hearing loss and progressive blindness (Kashtan 2002; Lees 2013). The only difference is the mode of inheritance and equal distribution of symptoms in both sexes (Gubler et al. 1981; Lees et al. 1998).

THE DOMINANT AUTOSOMAL MUTATION

This is by far the rarest of all Alport syndrome types and occurs in a very small percentage of affected specimens. Similarly to the previously described, it is caused by the mutation in COL4A3 or COL4A4 genes, yet ultrastructural analysis of glomeruli reveals the presence of both α3 and α5 chains of type IV collagen. In humans, hearing loss commonly occurs with this mutation, yet no visual impairment is observed (Jefferson et al. 1997; Hood et al. 2002).

Hood et al. (1995, 2000) were able to identify nephropathy in bull terrier breed; at the age of about one year it developed into the end-stage chronic kidney disease. Later studies confirmed this type of Alport syndrome in Dalmatians (Hood et al. 2002).

DIAGNOSTIC METHODS

Typical symptoms of Alport disease, i.e. hematuria, increased creatinine levels, hypertension, progressive renal insufficiency, impaired vision due to cataract or retinopathy, and hearing loss, are common among many other diseases, and thus, they are not particulary helpful for diagnosis. Alport syndrome can be more precisely diagnosed by kidney or skin biopsy, the latter showing improper structure of type IV collagen (Grenda 2010).

Kidney biopsy is to be performed three times and the obtained samples are treated immunohistochemically or with anti-GBM antibodies against some specific antigens present in the altered GBM. However, the result is still ambiguous, as it may also indicate some other diseases, e.g. Goodpasture syndrome, and only specific PCR reaction, that identifies the actual mutation, can prove the diagnosis definitely. In case of dogs this mutation is located in COL4A5 (Labor fur Klinische…, http://www.laboklin.de). Although this method is extremely precise, it is also rather expensive and time-consuming therefore, it is used in case the other methods are insufficient.

IMMUNOHISTOCHEMISTRY

This method of examining renal tissue in order to diagnose alterations of type IV collagen uses specific monoclonal antibodies and detects all α chains of the type IV collagen. It is based on the finding that different chains of type IV collagen show different expression levels, depending on the area of the biopsy and on the presence or the absence of the mutation that causes Alport syndrome. Comparing the biopsy with an analogical one from a healthy specimen, one is able to determine, whether the COL4A5 gene mutation occurs. Similar comparison method was used by Gubler et al. (1995) and Kashtan (2002). However, in this case samples were obtained from skin and analysis of α chains expression levels was performed on epidermal basement membranes.Biopsies are first taken from skin basement membranes. This technique is both simple and non-invasive and does not require anesthesia. In case of any abnormalities in α5 chain expression , the next biopsy comes from the kidney.

SUGGESTIONS FOR BREEDERS AND OWNERS

Alport syndrome is extremely difficult to diagnose, for its initial symptoms are discrete and similar to those of other diseases when more advanced. It is also quite dangerous due to its hereditary nature, as it may be passed to the next generations if remains undiagnosed before the sick animal is qualified for breeding. Considering the high costs of diagnosis, breeders do not routinely test their puppies, as there are many other higher priority hereditary diseases which should be tested for. As the disease is quite rare and thus unknown, it is important to educate breeders about its presence. In case any typical symptoms occur, there is a need for further, more advanced diagnosis. The danger of Alport syndrome lies in the fact that it is incurable and hereditary, so it is of extreme importance to study its symptoms thoroughly and to establish its mode of inheritance. When it happens, it will be possible to develop new, more efficient diagnostic methods and treatment. Such studies will be beneficial not only for the dogs, but first and foremost to the humans, as the disease seems to be of similar etiology in both species.

Raghu Kalluri and his collaborators from Harvard University, USA, were able to demonstrate that stem cells transplantation may be used in Alport syndrome's treatment. An infusion of bone marrow results in a marked withdrawal of symptoms in mice. Nevertheless, such therapy remains a matter of the distant future (Harvard Medical School, http://kalluri.med.harvard.edu/).

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Abstract. Alport syndrome is a rare, hereditary renal disease which is the result of a lack of one chain of type IV collagen (α3, α4, and α5). Type IV collagen is a basic structural component of the glomerular basement membrane. Alport syndrome has been reported in humans, mice and domestic dogs. Mutations in COL4A3 and COL4A4 genes are both of recessive and dominant type; however, the most common mutations in COLL4A5 gene are linked to X sex chromosome. These mutations render the synthesis of (α3, α4, α5) chain impossible, thus the resulting type IV

collagen does not have its proper structure and filtration of plasma is impaired, leading to progressive renal insufficiency and failure. With the current state of medical knowledge the only therapy, delaying the pathological processes, is limited to dialysis and kidney transplantation. This paper presents information on symptoms of Alport syndrome, as well as genetic basis and molecular effects of mutations causing the disease. It also offers dog breeders some advice, based on the available literature, in order to facilitate making the right breeding decisions.