ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Zootechnica 21(3) 2022, 13–18

www.asp.zut.edu.pl pISSN 1644-0714 eISSN 2300-6145 DOI:10.21005/asp.2022.21.3.02

REVIEW ARTICLE

Received: 10.08.2022
Accepted: 12.10.2022

HYPERURICOSURIA IN THE DOMESTIC DOG (*CANIS LUPUS FAMILIARIS*)

Joanna Gruszczyńska[1], Kamila Szydłowska[1], Magdalena Łopieńska[1], Katarzyna Siewruk[2], Paulina Jundziłł-Bogusiewicz[1], Beata Grzegrzółka[1]

[1]Department of Animal Genetics and Conservation, Institute of Animal Sciences, Warsaw University of Life Sciences – SGGW, Ciszewskiego 8, 02-786 Warszawa, Poland
[2]Department of Large Animal Diseases and Clinic, Institute of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Nowoursynowska 100, 02-797 Warszawa, Poland

ABSTRACT

Elevated plasma urate levels are associated with metabolic diseases such as hyperuricosuria. Long-term deposition of urea in the renal tubules can cause gout. Due to proper transport activity of GLUT9 (glucose transporter 9) protein, urate homeostasis in the body is maintained. GLUT9 is encoded by the *SLC2A9* gene, which is expressed in the kidney and liver. The *SLC2A9* c.616GG> T mutation results in a change in the amino acid sequences of the GLUT9 protein (p.Cys188Phe, which in turn leads to hyperuricosuria. Molecular methods were used to identify the mutation in *SLC2A9* gene. The occurrence of this mutation was found in many breeds of domestic dog (over 20), but the highest number of recessive homozygotes was recorded in the breeds: Dalmatian, Russian Black Terrier and English Bulldog.

Key words: hyperuricosuria, *SLC2A9*, GLUT9, domestic dog

Clinical picture

In a healthy organism allantoin and uric acid are the final products of the purines metabolic pathway. Allantoin, which is excreted in high quantities in the urine, is highly soluble, whereas uric acid, resorbed in the renal proximal tubule, is not. In case of hyperuricosuria the proportions of these final metabolic substances are reverted and uric acid levels significantly exceed the allantoin concentration. Prolonged, increased concentration of uric acid in the urine creates favourable conditions for the precipitation of crystals and the formation of aggregates. However, they do not appear in all individuals with a mutation in the *SLC2A9* gene. Uric acid crystals are found in the bladder or ureters and eventually form so-called "stones" – urite uroliths. Their presence is difficult to notice for dog owners and the condition is usually found by accident at ultrasonography or computer tomography, also with individuals diagnosed earlier with different kidney diseases [Syme 2012] and in cases of urethral atresia.

Urite uroliths (urinary stones) are formed from the precipitated crystals and from different organic compounds, e.g. proteins, lipids and polysaccharides. Their formation begins with nucleation, when free ions begin to form molecules. The precipitation of crystals can occur at various places, e.g. in the nephron, on the surface of cells, on the walls of the bladder or in the kidney tubules. The second stage is aggregation – here comes the agglomeration of the resulting crystals or the formation of new crystals on the surface of the previously formed ones that were created during nucleation (the so-called 2nd degree nucleation). The third stage is the growth of crystals, leading to the formation of stones of clinically relevant sizes. At this stage, free ions join the aggregates of crystals; also the smaller crystals that can be attached are of great importance in this process. Second degree nucleation may also occur [Ratkalkar and Kleinman 2011].

Both promoters and inhibitors of the formation of urinary stones are present in the urine. The promoters include, among others, uric acid or acidic pH [Ratkalkar

joanna_gruszczynska@sggw.edu.pl

and Kleinman 2011]. Disturbing symptoms suggesting the presence of urinary stones in a dog may be frequent urination in small amounts (polakisuria), pain response accompanying urination (dysuria) and hematuria [McCue et al. 2009]. Urinary stones are more frequently diagnosed in males than in females and this may be caused by sex-related differences in the structure of the urinary tract [Bannasch et al. 2004]. The presence of the penile bone in males makes their ureter narrower and longer than the ureter of females. It is therefore possible that the stones will be removed from the female body during urination and their presence will not be noticed by the owner. In males urethral atresia and intensification of clinical symptoms may occur [Bannasch et al. 2004, Albasan et al. 2005].

Discovery of the cause of hyperuricosuria

The first studies aimed at understanding the causes of hyperuricosuria consisted of performing a kidney transplant between two Dalmatian dogs showing disease symptoms and two healthy crossbreds [Simkin 2005 after Appleman et al. 1966].

It was shown that the transplanted organs began to work according to the host's original physiology and functioned long enough to obtain data from five 24-hour secretion of uric acid [Simkin 2005 after Appleman et al. 1966]. In the next stage of the research liver transplants were performed between two Dalmatians, two mutts and in the scheme: three mutts as donors for three Dalmatians and four Dalmatians for four mutts.

Analysis of data from 24-hour uric acid secretion showed that the transplanted organs functioned properly. It was found that in Dalmatian dogs it was the liver that determined changes in the amount of uric acid released. Thus it was assumed that the liver in the tested dogs of this breed produces an inhibitor of uric acid reabsorption or the liver of mutts generates a promoter that allows its resorption. In order to check the above theories, mutt's hepatocytes were transplanted into a Dalmatian. In a result a marked decrease in urine acid excretion into urine was observed, together with its increased blood concentration, suggesting that a promoter of the urine acid reabsorption was present in the mutt's liver [Simkin 2005 after Kuster et al. 1972].

Studies of the uricase enzyme in Dalmatians, which catalyses transformation of uric acid to allantoin, began many years ago. In 1918, H. G. Wells reported an increased in vitro activity of uricase in dogs of this breed in homogenized liver cells. The activity of uricase in Dalmatians was at a similar or higher level than in mixed breeds [Wells 1918]. Despite the increased activity of uricase in the homogenised liver of Dalmatians, it was shown that in the hepatic lobes, where the cells were not damaged, the enzyme activity was significantly lower. Based on these observations, it was found that differences in uricase activity were caused by abnormalities in the transport of uric acid through cell membranes [Giesecke and Tiemeyer 1984].

In further studies on the cause of hyperuricosuria nucleotide sequences of the uricase gene in the Dalmatian and some other disease-free breeds were compared. Neither any difference nor a deletion in the coding sequences was found and thus the possible relation between the enzyme and disease was excluded [Safra et al. 2005].

In 2008, research was conducted to detect the mutation responsible for the occurrence of hyperuricosuria in various dog breeds (the largest share in the research sample was held by Dalmatians) [Bannasch et al. 2008]. Using the microsatellite sequences, 4 genes were detected (*KIAA1729*, *WDR1*, *SLC2A9*, *MIST*), which could be responsible for the occurrence of this disease in a domestic dog. The expression of these genes in the kidney and liver of this species was confirmed by RT-PCR. Sequencing of cDNA and genomic DNA was performed and UTR regions were determined using the 5′ and 3′ RACE-PCR method. As the 5′UTR region and exons 1–7 of the *MIST* gene were outside the region of non-equivalent linkage, they were not further analysed. In a single exon of the *KIAAA1729* gene, three silent mutations and deletions / insertions were detected in both affected and healthy individuals, and therefore this gene was excluded from further studies. One mutation has been detected in the intron of the *WDR1* gene, which has no effect on the cleavage site.

Sequencing of the *SLC2A9* gene allowed the detection of six mutations, two of which were in exons (5 and 11), two in introns (1 and 10) and the other two in the distance of 99 bp and 101 bp 5′ from the start codon. Mutations identified in introns were not found in the cleavage region. Mutations located at the 5′ end were also found in healthy individuals of other breeds of dogs, whereas mutations in exon 5 and 11 were found in all tested, non-related Dalmatians.

Two independent mutations in *SLC2A9* gene were evaluated in the PANTHER and SIFT programs. The PANTHER program evaluated the mutation in exon 11 (Val135Ile) at 4.047 points (on a scale of 0–10, where 10 defines the most unfavourable mutation) with an error probability of 0.74. The SIFT program evaluated the mutation in exon 5 (Cys188Phe) as harmful with an error probability of 0.01. The point mutation in exon 5 (Cys188Phe) was then examined in 247 Dalmatian dogs and 387 dogs from 58 other breeds [Bannasch et al. 2008]. All examined Dalmatians were diagnosed as homozygotes for the occurrence of the above-mentioned mutation. Researchers suggest that this mutation was fixed within the Dalmatian breed during the 'preservation' of the characteristic ticking pattern. During the study, there were also many cases of homozygosity in

Black Russian Terriers and English Bulldogs [Bannasch et al. 2008, Farrell et al. 2015].

Metabolism of purines

The *SLC2A9* gene in the domestic dog (*Canis lupus familiaris*) is located on chromosome 3 and codes for the glucose transporter 9 (GLUT9) – a protein mainly in the kidneys and liver [Le et al. 2008, Bannasch and Henthorn 2009, McCue et al. 2009]. It takes part in the reabsorption of nutrients to the blood and in secretion of unnecessary amounts of these substances into the urine. In the proximal renal tubule this protein is responsible for the transport of uric acid, a product of reactions in the body [Le et al. 2008]. The uric acid in the blood acts as an antioxidant, but in excessive amounts it may have toxic properties. Therefore, it is removed from the body along with urine. Depending on needs, the GLUT9 protein is involved in the reabsorption of uric acid into the blood or into the urine. In a properly functioning organism, the vast majority of uric acid produced in metabolic processes is reabsorbed into the bloodstream [Frédéric et al. 2009].

Uric acid is one of the products of metabolic transformations of purines – compounds that are components of nucleotides [Ware et al. 2015]. Their synthesis can take place in two ways – the first is recovering of purines from food or dead cells, the second is de novo synthesis from smaller available chemical compounds, mainly these of carbon and nitrogen [Foschi 2008].

In a properly functioning organism, exo- and endogenous purines are metabolized to hypoxanthine and adenosine monophosphate (AMP) is involved in this reaction. With the help of guanosine monophosphate (GMP) and under the influence of xanthine oxidase, hypoxanthine turns into xanthine and then into uric acid. In the majority of mammalian species, uric acid is transformed into allantoin, and this reaction is catalysed by uricase, the enzyme produced in the liver [Bannasch and Henthorn 2009, McCue et al. 2009]. Allantoin is far more soluble in urine than uric acid [Bannasch et al. 2004, Bibert et al. 2009].

Genetic background of hyperuricosuria

The mutation of the *SLC2A9* gene leads to disruption of metabolic processes associated with the transport of uric acid by the GLUT9 protein [Terkeltaub 2008]. This is a missense point mutation [Karmi et al. 2010, Mellersh 2012, Cosgrove et al. 2015] and it is inherited according to Mendel's 1st law in an autosomal recessive way, which means that the individual must receive a recessive allele from both parents to develop the disease. This mutation is the underlying cause of a condition called hyperuricosuria involving increased uric acid secretion into urine [Bannasch and Henthorn 2009, Syme 2012]. Being poorly soluble, it may form crystals there, which increases the probability of urinary stones formation [Bannasch et al. 2004].

Using the BLAST program, the nucleotide sequences of the *SLC2A9* gene (Fig. 1) and the amino acid sequences of the GLUT9 protein (Fig. 2) of the healthy and affected individual were compared. According to information in the literature [Donner et al. 2016], the affected individual has thymine instead of guanine at position 616 of the *SLC2A9* gene sequence and phenylalanine instead of cysteine at position 188 of the GLUT9 protein amino acid sequence. This confirms that this is a missense point mutation [Cosgrove et al. 2015].

In molecular tests, the HpyCH4V restriction enzyme is used to identify individuals with the mutation. It recognizes the specific sequence (TG | CA / AC | GT) and finds the cleavage site at position 616 of exon 5 of the *SLC2A9* gene only in individuals without the above mutation [Bannasch et al. 2008].

The molecular test described above allows the detection of only one mutation in the *SLC2A9* gene, while the simultaneous detection of many mutations is possible through the use of new sequencing technologies allowing for faster, more accurate identification of mutations [Karmi et al. 2030].

Treatment of hyperuricosuria and prognosis

In general, for dogs with hyperuricosuria that are fed an appropriate low-purine diet (reduced meat protein intake

```
healthy  ...CTGCCATCTTCATCTGCATCGGTGTGTTCACCGGGCAGCTGCTGGGCCTGCCTGAGCTGC...
                |||||||||||||| ||||||||||||||||||||||||||||||||||||||||
affected ...CTGCCATCTTCATCTTCATCGGTGTGTTCACCGGGCAGCTGCTGGGCCTGCCTGAGCTGC...
```

Fig. 1. Comparison of the fragment of the nucleotide sequence of the *SLC2A9* gene of a healthy and affected individual

```
healthy  ...QVTAIFICIGVFTGQLLGLPELLGKESTWPYLFGVIAVPALVQLVSLPFLPESPRFLLFE...
             QVTAIFI IGVFTGQLLGLPELLGKESTWPYLFGVIAVPALVQLVSLPFLPESPRFLLFE
affected ...QVTAIFIFIGVFTGQLLGLPELLGKESTWPYLFGVIAVPALVQLVSLPFLPESPRFLLFE...
```

Fig. 2. Comparison of the fragment of the amino acid sequence of the GLUT9 protein of a healthy and affected individual

can also lower serum uric acid levels) the prognosis is good [Bende and Németh 2004, Westropp et al. 2016]. Low-purine diets are sometimes used by drugs (e.g. allopurinol) [Brown et al. 2003, Larsen et al. 2016]. Such dogs must consume large amounts of fluids – dilution of urine inhibits the supersaturation of lithogenic products, and thus prevents the formation of stones. Care should be taken to increase the alkalinity of the pH of the urine (protective effect). Using medical dissolution therapy to alkalize the urine at least once a day (bringing the pH of the urine to about 6.5) is sufficient to dissolve the urinary stone. Ultrasound can be used to track the progress of therapy. Continuing treatment with alkalizing agents is the recommended maintenance therapy for most uric acid-forming stones.

It is often necessary to surgically remove residual stones. However, individuals with a mutation in the *SLC2A9* gene, i.e. those with hyperuricosuria, will still have a predisposition to urinary stones formation due to increased uric acid levels [McCue et al. 2009].

REFERENCES

Albasan, H., Lulich, J.P., Osborne, C.A., Lekcharoensuk, Ch. (2005). Evaluation of the association between sex and risk of forming urate uroliths in Dalmatians. J. Am. Vet. Med. Assoc., 227(4), 565–569. DOI: 10.2460/javma.2005.227.565.

Bannasch, D.L., Henthorn, P. (2009). Changing Paradigms In Diagnosis of Inherited Defects Associated with Urolithiasis. Vet. Clin. North Am.: Small Anim. Pract., 39(1), 111–125. DOI: 10.1016/j.cvsm.2008.09.006.

Bannasch, D.L., Ling, G.V., Bea, J., Famula, T.R. (2004). Inheritance of Urinary Calculi in the Dalmatian. J. Vet. Intern. Med., 18, 483–487. DOI: 10.1111/j.1939-1676.2004.tb02571.x.

Bannasch, D.L., Safra, N., Young, A., Karmi, N., Schaible, R.S., Ling, G.V. (2008). Mutations in the *SLC2A9* Gene Cause Hyperuricosuria and Hyperuricemia in the Dog. Publ. Libr. Sci. Gen., 4(11):e1000246. DOI: 10.1371/journal.pgen.1000246.

Bende, B., Németh, T. (2004). High prevalence of urate urolithiasis in the Russian black terrier. Vet. Rec., 155, 239–240. DOI: 10.1136/vr.155.8.239.

Bibert, S., Hess, S.K., Firsov, D., Thorens, B., Geering, K., Horisberger, J.D., Bonny, O. (2009). Mouse GLUT9: evidences for urate uniporter. Am. J. Physiol. – Renal Physiol., 297: F612-F619. DOI: 10.1152/ajprenal.00139.2009.

Brown, W.Y., Vanselow, B.A., Walkden-Brown, S.W. (2003). One dog's meat is another dog's poison – nutrition in the Dalmatian dog. Rec. Adv. Anim. Nutr. Austr., 13, 123–131.

Cosgrove, L., Hammond, G., Mclauchlan, G. (2015). Promary portal vein hypoplasia and *SLC2A9* mutation associated with urolithiasis in a Spanish water dog. Can. Vet. J., 56, 1153–1157. PMID: 26538670.

Donner, J., Kaukonen, M., Anderson, H., Moeller, F., Kyoestilae, K., Sankari, S., Hytoenen, M., Giger, U., Lohi, H. (2016). Genetic Panel Screening of Nearly 100 Mutations Reveals New Insights into the Breed Distribuion of Risk Variants for Canine Heredity Disorders. Publ. Libr. Sci. One, 11(8): e0161005, 1–18. DOI: 10.1371/journal.pone.0161005.

Farrell, L.L., Schoenebeck, J.J., Wiener, P., Clements, D.N., Summers, K.M. (2015). The challenges of pedigree dog health: approaches to combating inherited disease. Canine Gen. Epidemiol., 2:3. DOI: 10.1186/s40575-015-0014-9.

Foschi, G. (2008). Nucleotide metabolism and Urate excretion in the Dalmatian dog breed. Animal Science – Batchelor Degree Project 15hp, Literature study, SLU, Uppsala.

Frédéric, P., Bonny, O., Laverriére, A, Rotman, S., Firsov, D., Da Costa, A., Metref, S., Thorens, B. (2009). Glut9 is a major regulator of urate homeostasis and its genetic inactivation induces hyperuricosuria and urate nephropathy. Proc. Nat. Acad. Sci., 106(36), 15501–15506. DOI: 10.1073/pnas.0904411106.

Giesecke, D., Tiemeyer, W. (1984). Defect of uric acid uptake in Dalmatian dog liver. Experientia, 40, 1415–1416. DOI: 10.1007/BF01951919.

Karmi, N., Brown, E.A., Hughes, S.S., McLaughlin, B., Mellersh, C.S., Biourge, V., Bannasch, D.L. (2010). Estimated frequency of the canine hyperuricosuria mutation in different dog breeds. J. Vet. Intern. Med., 24(6), 1337–1342. DOI: 10.1111/j.1939-1676.2010.0631.x.

Karmi, N., Safra, N., Young, A., Bannasch, D.L. (2030). Validation of a urine test and characterization of the putative genetic mutation for hyperuricosuria in Bulldogs and Black Russian Terriers. Am. J. Vet. Res., 71(8), 909–914. DOI: 10.2460/ajvr.71.8.909.

Larsen, J.A., Johnson, E.G. Bannasch, D., Fascetti, A.J., Biourge, V., Queau, Y. (2016). Evaluation of dogs with genetic hyperuricosuria and urate urolithiasis consuming a purine restricted diet: a pilot study. BMC Vet. Res., 13, 45. DOI: 10.1186/s12917-017-0958-y.

Le, M.T., Shafiu, M., Mu, W., Johnson, R.J. (2008). *SLC2A9* – a fructose transporter identified as a novel uric acid transporter. Nephr. Dial. Transpl., 23, 2746–2749. DOI: 10.1093/ndt/gfn349.

McCue, J., Langston, C., Palma, D., Gisselman, K. (2009). Urate Urolithiasis., Compendium: Continiuing Education for Veterinarians, 468–474.

Mellersh, C. (2012). DNA testing and domestic dogs. Mamm. Genome, 23, 109–123. DOI: 10.1007/s00335-011-9365-z.

Ratkalkar, V.N., Kleinman, J.G. (2011). Mechanizm of Stone Formation. Clin. Rev. Bone and Min. Met., 9(3-4), 187–197. DOI: 10.1007/s12018-011-9104-8.

Safra, N., Ling, G.V., Schaible, R.H., Bannasch, D.L. (2005). Exclusion of Urate Oxidase as a Candidate Gene for Hyperuricosuria in the Dalmatian Dog Using an Interbreed Backcross, J. Hered., 96(7), 750–754. DOI: 10.1093/jhered/esi078.

Simkin, P.A. (2005). The Dalmatian Defect: A hepatic Endocrinopathy of Urate Transport. Am. Coll. Rheumatol., 52(8), 2257–2262. DOI: 10.1002/art.21241.

Syme, H.M. (2012). Stones in cats and dogs: What can be learnt from them? Arab J. Urol., 10, 230–239. DOI: 10.1016/j.aju.2012.06.006.

Terkeltaub, R.A. (2008). A Sugar Transporter Regulates Serum Urate Levels: Implications for Prevention and Management of Hyperuricemia in Gout. Curr. Rheumatol. Rep., 11, 83. DOI: 10.1007/s11926-009-0012-6.

Ware, E.B., Riehle, E., Smith, J.A., Zhao, W., Turner, S.T., Kardia, S.L.R., Lieske, J.C. (2015). *SLC2A9* Genotype Is Associated with *SLC2A9* Gene Expression and Urinary Acid Concentration. Publ. Libr. Sci. One, 10(7): e0128593, 1–15. DOI: 10.1371/journal.pone.0128593.

Wells, H.G. (1918). The purine metabolism of the Dalmatian coach hound. J. Biol. Chem., 125, 445–449. DOI: 10.1016/S0021-9258(18)86453-5.

Westropp, J.L., Johnson, E.G., Fuller, M.C., Safra, N., Bannasch, D.L., 2014, Urate urolithiasis and hyperuricosuria in a Weimaraner, secondary to the SLC2A9 transporter defect. Vet. Rec. Case Rep., 2:e000016, 1–5.

HIPERURYKOZURIA U PSA DOMOWEGO (*CANIS LUPUS FAMILIARIS*)

STRESZCZENIE

Podwyższony poziom moczanów w osoczu jest związany występowaniem chorób metabolicznych takich jak hiperurykozuria. Długotrwałe odkładanie się mocznika w kanalikach nerkowych może wywołać dnę moczową. Dzięki prawidłowej aktywności transportowej białka GLUT9 (transporter glukozy 9) zostaje zachowana homeostaza moczanów w organizmie. GLUT9 jest kodowane przez gen *SLC2A9*, który ulega ekspresji w nerkach i wątrobie. W wyniku mutacji *SLC2A9* c.616G> T dochodzi do zmiany sekwencji aminokwasowej białka GLUT9 (p.Cys188Phe), co w efekcie prowadzi do wystąpienia hiperurykozurii. Do identyfikacji mutacji w genie *SLC2A9* wykorzystano metody molekularne. Występowanie tej mutacji stwierdzono u wielu ras psa domowego (ponad 20), jednak największą liczbę homozygot recesywnych odnotowano u ras: dalmatyńczyk, czarny terier rosyjski i buldog angielski.

Słowa kluczowe: hiperurykozuria, *SLC2A9*, GLUT9, pies domowy

Joanna Gruszczyńska https://orcid.org/0000-0001-6062-6353
Katarzyna Siewruk https://orcid.org/0000-0003-1609-0816
Paulina Jundziłł-Bogusiewicz https://orcid.org/0000-0002-3077-3042
Beata Grzegrzółka https://orcid.org/0000-0002-0619-8383