DOI: 10.21005/AAPZ2023.66.2.4

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2023, 367(66)2, 35–48

Received 6 Feb 2023
Revised 27 Mar 2023
Accepted 31 Mar 2023

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MOLECULAR DIAGNOSTIC TESTS IDENTIFYING CARRIERS OF *SLC3A1* AND *SLC7A9* GENE MUTATIONS CAUSING CYSTINURIA IN DOMESTIC CAT-*IN SILICO* ANALYSIS

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Abstract. Cystinuria is an inherited genetic disease associated with mutations in the *SLC3A1* and *SLC7A9* genes. The results of studies in cats indicate heterogeneity of the disease. So far 1 mutation in the *SLC3A1* gene and 5 mutations in the *SLC7A9* gene have been discovered. Cystinuria is often detected too late (later in life), when adults have already had offspring. Quick and easy diagnosis of the disease is therefore important, even before the symptoms appear. The aim of this study was to design simple diagnostic tests to detect 6 mutations that can cause feline cystinuria. Bioinformatic analysis of the linkage of *SLC3A1* and *SLC7A9* with other proteins was performed and attempts were made to evaluate the structural and functional changes that occur due to mutations. The following programs and databases were used in the bioinformatics analysis: Ensembl, NCBI, Primer-BLAST, NEBcutter, STRING, SWISS-MODEL, Variant Effect Predictor (VEP). The proposed primers and enzymes for PCR-RFLP and AS-PCR assays enable the identification of mutation carriers. Identified proteins interactions suggest possible links between cystinuria and the development of other diseases. This may indirectly explain the heterogeneous and complex symptoms accompanying cystinuria. A mutation in exon 5 of the *SLC7A9* gene (p.Asp236Asn) causes a change in the predicted spatial conformation of the protein. Using the VEP program, the effect of all mutations on protein functionality was assessed as deleterious. It is imperative that cat breeders first perform a molecular test for the mutation of the *SLC7A9* gene (exon 7 at position c.881), as previous studies have shown that this is the most common mutation.

Key words: cystinuria, urolithiasis, *SLC31A1*, *SLC7A9*, mutations.

INTRODUCTION

Cystinuria is a genetically determined condition primarily associated with abnormal resorption of the amino acids cystine and the divalent amino acids ornithine, lysine and arginine (COLA) in the proximal tubules of the kidney, which can lead to the precipitation of cystine stones in the

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urinary tract (Mattoo and Goldfarb 2008). Feline cystinuria was first described in 1991 as a single case diagnosed in a male Siamese individual (DiBartola et al. 1991).

Cystinuria also occurs in other animal species, including humans (Palacín et al. 2013) and domestic dogs (Brons et al. 2013; Kovaříková et al. 2021). In all affected animal species, the disease is characterized by a wide variation in the symptoms present. Typical symptoms include dysuria, hematuria and frequent urination (Osborne et al. 1999).

In humans and domestic dogs, the disease is inherited in an autosomal dominant or recessive manner (Brons et al. 2013). In the domestic cat, the pathways of inheritance of the disease are not clear. Cystinuria in the domestic dog and human is caused by DNA point or frameshift mutations occurring in the *SLC3A1* or *SLC7A9* genes (Brons et al. 2013; Palacín et al. 2013; Mizukami et al. 2015, 2016; Kovaříková et al. 2021). These genes encode, respectively, the heavy (rBAT protein) and light (b0,+ AT protein) subunits of the b0,+ transporter, through which COLA amino acid transport takes place.

To date, 6 DNA point mutations causing cystinuria in the domestic cat have been discovered: 1 mutation is located in the *SLC3A1* gene and 5 mutations in the *SLC7A9* gene (Mizukami et al. 2015, 2016). In the *SLC3A1* gene, the mutation causing cystinuria was detected in exon 8 and it is a missense mutation. The cytosine at position c.1342 is replaced by thymine (C>T), which at the amino acid level results in the insertion of an aromatic, hydrophobic tryptophan in place of the highly conserved, hydrophilic arginine at position 448 (p.Arg448Trp) (Mizukami et al. 2015).

In the *SLC7A9* gene, a mutation was found in exon 7 at position c.881. This is a transversion of thymine into adenine, which results in the conversion of the hydrophobic amino acid valine (Val) into hydrophilic glutamic acid (Glu) at position p.294. Another mutation was detected, in exon 5 of the *SLC7A9* gene; this is a transversion of nucleotide position 706 – guanine is replaced by adenine. As a result of the mutation in the amino acid sequence of the protein, instead of the highly conserved aspartic acid (item p.236), the amino acid asparagine is formed. A point mutation is also identified in exon 10; thymine replaces cytosine at position c.1175, leading to the synthesis of a protein that has hydrophobic methionine instead of the highly conserved hydrophilic threonine at position p.392.

In addition, two point mutations were detected in the intron nucleotide sequence of the *SLC7A9* gene, which may affect the formation of cystinuria. The first change is in intron 10. At position c.1233+1, adenine was incorporated instead of guanine. On the other hand, in another cystinuric cat, an adenine to guanine transition occurred in intron 11 at position c.1409-30 (Mizukami et al. 2016). These changes involve highly conserved nucleotides (Lim and Burge 2001), so it is highly likely that they adversely affect the proper formation of mRNA during transcription, thereby preventing the proper translation of the COLA transporter subunit (Mizukami et al. 2016).

Nowadays, the diagnosis of cystinuria is usually lengthy and complex process. It requires several tests and the collation of various results: crystallographic analysis of stones present in the urine, microscopic examination of the urine, cyanide-nitroprusside test or quantitative evaluation of amino acid COLA in the excreted urine. What's more, the methods commonly used allow the diagnosis to be made only after the appearance of urinary stones; the animal is therefore doomed to unpleasant discomfort. Cystinuria is troublesome for both the affected animal and its owner, the breeder. It often requires invasive solutions for the body, such as surgical correction of urinary tract obstruction, which is the eventual solution for sick animals. The disease also requires a lifetime of special recommendations. Most often, a proper diet, with reduced protein and sodium content, is recommended, as well as special preparations to enhance urine alkalinization and arginine supplementation. It is important to consume plenty of fluids, in order to dilute the urine. Potassium citrate can also be administered to increase urine

pH. That's why it's so important to take steps to diagnose the disease quickly and easily, even before symptoms appear.

The aim of the study was to design simple PCR-RFLP or AS-PCR diagnostic tests to identify 6 DNA mutations that cause cystinuria in the domestic cat. An analysis of the known and predicted interactions of SLC3A1 and SLC7A9 proteins with other proteins in the domestic cat was also performed, and an attempt was made to evaluate structural and functional changes in the proteins that occur due to mutations in the nucleotide sequence.

MATERIAL AND METHODS

The nucleotide sequences of the domestic cat *SLC3A1* (ENSFCAG00000000611.6) and *SLC7A9* (ENSFCAG00000014914.6) genes were obtained from the Ensembl v.105 database (Howe et al. 2021, https://www.ensembl.org/index.html).

Available protein isoforms that are products of the *SLC3A1* gene for the domestic cat were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/genbank/): isoform X1 – XP_003983986.3, isoform X2 – XP_019682692.3, and two protein isoforms that are products of the *SLC7A9* gene: isoform X1 – XP_019675188.3, isoform X3 – XP_019675192.1.

Creation of mutant nucleotide and amino acid sequences

Based on literature data (Mizukami et al. 2015, 2016), mutant sequences of individual exons and introns of *SLC3A1* and *SLC7A9* genes were created, as well as mutant amino acid sequences altered due to point mutations in exons of nucleotide sequences. For this purpose, the nucleotides in the obtained *SLC3A1* (ENSFCAG00000000611.6) and *SLC7A9* (ENSFCAG00000014914.6) gene sequences were altered, including all 6 DNA mutations causing cystinuria in the domestic cat: in the *SLC3A1* gene in exon 8, the cytosine at position c.1342 was changed to thymine. The changes that were made to the nucleotide sequence of the *SLC7A9* gene were: in exon 5, guanine at position c.706 was converted to adenine; in exon 7, thymine at position c.881 was converted to adenine; in exon 10, cytosine at position 1175 was converted to thymine; in intron 10, guanine at position 1233+1 was converted to adenine; in intron 11, adenine at position c.1409-30 was converted to guanine. The obtained amino acid sequences were processed similarly, including exon mutations. In the sequences of the X1 (XP_003983986.3) and X2 (9 XP_019682692.3) isoforms of the SLC3A1 protein, arginine at position p.448 was replaced with tryptophan. In the sequences of the X1 and X3 isoforms of the SLC7A9 protein, aspartic acid at position p.236 was converted to asparagine, valine at position p.294 was converted to glutamic acid, and threonine at position p.392 was converted to methionine.

Designing PCR-RFLP assays to detect 4 of 6 DNA mutations that cause cystinuria in the domestic cat

The primers for each reaction were designed using Primer-BLAST software (Ye et al. 2012; https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Four pairs of primers were obtained for amplification of DNA regions (Table 1).

Table 1. Localization of mutations responsible for cystinuria in the domestic cat.

Gene	Exon/intron	Position of mutation site
SLC3A1	exon 8	c.1342
SLC7A9	exon 5	c.706
SLC7A9	exon 7	c.881
SLC7A9	intron 11	c.1409-30

Restriction enzymes for the RFLP reaction were selected using NEBcutter v.3.0.15 (Vincze et al. 2003; https://nc3.neb.com/NEBcutter/). Four enzymes were selected, suitable for detecting the presence or absence of mutations at adequat position (Table 1).

Sequences, which are the products of the PCR reaction carried out with the primers discussed above, were used as a template.

Designing a PCR-RFLP diagnostic test for the two remaining mutations: at position c.1175 of exon 10 of the *SLC7A9* gene and at position c. 1233+1 of intron 10 of the *SLC7A9* gene, was prevented by the lack of suitable restriction enzymes capable of identifying mutation sites in the mutant or wild-type nucleotide sequence. Therefore, the AS-PCR assay was chosen to identify the mentioned mutations.

Designing AS-PCR assays to detect 2 of 6 DNA mutations that cause cystinuria in the domestic cat

For each reaction, 2 pairs of primers were selected: a universal one, for both mutant and normal sequence, and an "allele-specific" one for mutation detection. When designing pairs of the first type of primers – uniwersal (surrounding the mutation, enabling amplification of both wild and mutant sequences), it was important for the primers to be at unequal distances from the mutation site.

Specific forward primers were designed by the author of the study using the wild-type sequence as a template, so that the nucleotide at the 3' end covered the mutation site.

Specific reverse primers were designed by the author of the study using the mutant sequence as a template, so that the nucleotide at the 3' end covers the mutation site.

The quality of the designed primers was then checked with Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The results including the average melting temperature of the primers (Tm), percentage of guanine-cytosine pairs and self 3' complementarity are shown in Table 2.

Analysis of the interaction of SLC3A1 and SLC7A9 proteins with other proteins

Known and predicted interactions between the proteins SLC3A1, SLC7A9 and other proteins of the domestic cat were examined using the STRING v.11.5 database (https://string-db.org/). This program allows analysis of direct (physical) and indirect (functional) associations.

The attempt to assess structural changes due to mutations

To identify possible differences between the structure of proteins encoded by the mutant and wild-type nucleotide sequence, the SWISS-MODEL program (https://swissmodel.expasy.org/), which predicts the 3D structure of proteins based on amino acid sequence, was used. Spatial structure predictions were carried out sequentially for the amino acid sequences of the SLC3A1 protein isoforms, followed by the SLC7A9 protein isoforms encoded by the wild-type and mutant nucleotide sequence. The study was conducted while maintaining the program's initial settings.

The attempt to assess the functional changes occurring due to mutations

The effect of mutations within the exons and introns of the *SLC3A1* and *SLC7A9* genes on the normal function of the resulting proteins was predicted using the VEP v.105 program (https://www.ensembl.org/info/docs/tools/vep/index.html). The study was conducted using nucleotide sequences of *SLC3A1* and *SLC7A9* genes containing intron and exon mutations as data, with the following program settings: species – cat, transcript database to use – Ensembl transcripts, additional configurations – initial settings.

Table 2. Proposed primers for amplification of *SLC3A1* and *SLC7A9* gene fragments of the domestic cat

No	Gene Exon/intron	Flanked position	Primer	Sequence (5'–3')	PCR product length (bp)	Tm (°C)	GC%	Self 3' complementarity
1	*SLC3A1* Exon 8	c.1342	forward	GCCAGGCTTGCTGATACCAT	225	60.0	55.0	2.00
			reverse	GGGTTCCAGGGAGTGTGAAA			55.0	1.00
2	*SLC7A9* Exon 5	c.706	forward	GGCACAAAACTGTCTGTGGG	198	59.7	55.0	3.00
			reverse	GCTGAACCAAATGGAGACGTG			52.4	2.00
3	*SLC7A9* Exon 7	c.881	forward	CTGGTGACAGGCTGCTACAT	231	59.4	55.0	2.00
			reverse	ATGGCATTGGGTCTTTGCTG			50.0	1.00
4	*SLC7A9* Exon 10	c.1175	forward	GGACAGGGACCCCGTTTTTA	334	58.1	55.0	2.00
			reverse	AATCAGGAGCAAACCCCACT			50.0	1.00
5		–	forward – specific to the wild allele	TGGCTGTTTTATGGCCTGAC	203		50.0	1.00
6			reverse –specific to the mutant allele	GACAACCAATCCCAGAATCA	170		45.0	1.00
7	*SLC7A9* Intron 10	c.1233+1	forward	GGACAGGGACCCCGTTTTTA	334	59.4	55.0	2.00
			reverse	AATCAGGAGCAAACCCCACT			50.0	1.00
8		–	forward – specific to the wild allele	GACCTGGAAAGGCCTATCAAGG	146		54.5	12.00
9			reverse – specific to the mutant allele	GACATTTGAGGCAACGAAACTTAT	233		37.5	2.00
10	*SLC7A9* Intron 11	c.1409-30	forward	CCCTGTAGAGGACGGTGTTTG	293	60.0	57.1	0.00
			reverse	GGCTTGCTACTCTGGAGCTT			55.0	2.00

RESULTS AND DISCUSSION

PCR-RFLP tests

The primers for the PCR-RFLP reaction, which will amplify DNA fragments that may have cystinuria-causing mutations, are shown in Table 2 (primers #1, 2, 3, 10).

The selected restriction enzymes for the PCR-RFLP reaction are shown in Table 3. The products of the reaction downstream should be subjected to electrophoretic separation in a 10% polyacrylamide gel. The number of DNA striations visualized in the gel will distinguish individuals homozygous for the wild allele, homozygous for the mutant allele and heterozygous. The schematic location of the striations in the gel depending on the result of the RFLP reaction with a given restriction enzyme is shown in Fig. 1.

Table 3. Restriction enzymes selected for PCR-RFLP assay

Name of the restriction enzyme	Recognized sequence including the cut site	Enzyme cut sequence – number of cut sites	Localization of sequences in the DNA of the domestic cat	Size of products after enzyme cutting [bp]
*Nci*I	5'…CC▾SGG…3' 3'…GGS▴CC…5'	wild – 1	*SLC3A1* gene exon 8	73; 152
*BsiW*I	5'…C▾GTACG…3' 3'…GCATG▴C…5'	wild – 1	*SLC7A9* gene exon 5	56; 142
*Btg*I	5'…C▾CRYGG…3' 3'…GGYRC▴C…5'	wild – 1	*SLC7A9* gene exon 7	86; 145
*Hpy188*I	5'…TCN▾GA…3' 3'…AG▴NCT…5'	wild – 3	*SLC7A9* gene intron 11	49; 53; 86; 105
		mutant – 2		49; 105; 139

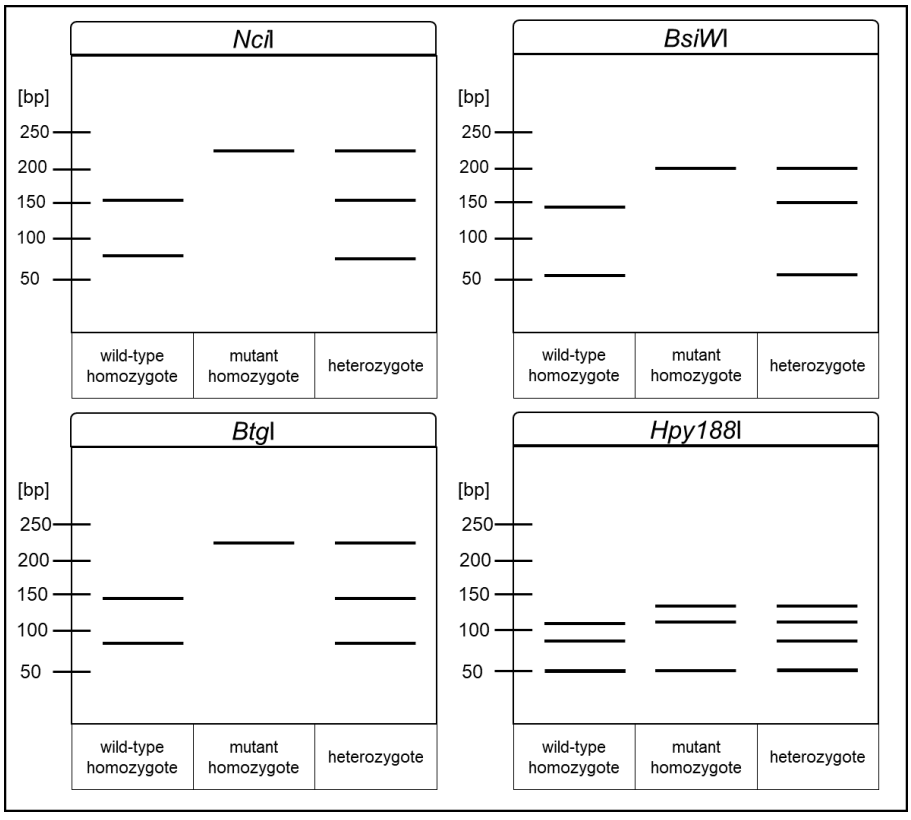

Fig. 1. Schematic representation of possible results of PCR-RFLP diagnostic tests using individual enzymes

AS-PCR tests

The primers for the two AS-PCR reactions, are shown in Table 2 (primers #4, 5, 6, 7, 8, 9). The products of the AS-PCR reactions are identified by performing electrophoresis in a 10% polyacrylamide gel and analyzing the visualized DNA bands. A schematic of the possible results is shown in Fig. 2.

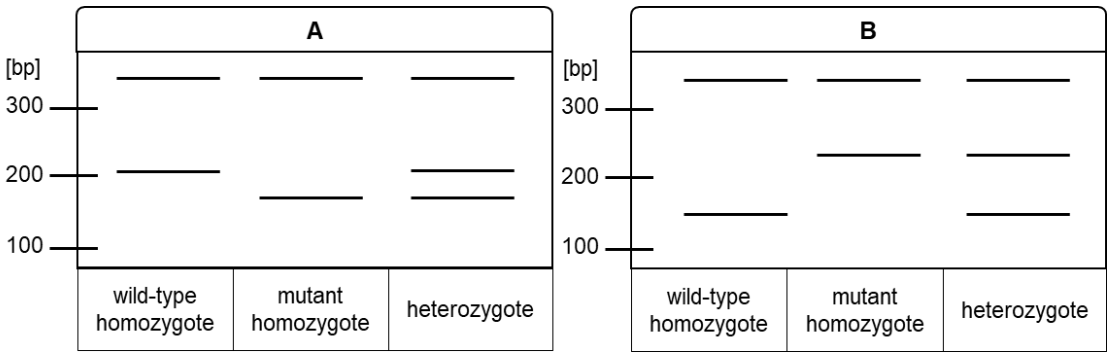

Fig. 2. Scheme of possible AS-PCR diagnostic test results; 10% polyacryloamide gel separation; A – *SLC7A9,* exon 10 c.1175, B – *SLC7A9,* intron 10 c.1233+1

Breeders should first carry out a test to detect the *SLC7A9* gene mutation of exon 7 at position c.881, as it appears to be the most prevalent mutation in the domestic cat population. It has been detected in individuals of different breeds of domestic cat – Maine coon, Sphynx, domestic semi-longhair cat (DMH), Siamese cat, from different geographical areas – North America and Europe (Mizukami et al. 2016; Hilton et al. 2017).

Interactions of SLC3A1 and SLC7A9 proteins with other proteins

The SLC3A1 and SLC7A9 proteins are most strongly related to each other. This is understandable, as together they form and are responsible for the proper function of the amino acid transporter COLA.

Other results show interactions of SLC3A1 and SLC7A9 proteins primarily with other proteins belonging to a group of membrane transport proteins called solute channel (Fig. 3 and 4).

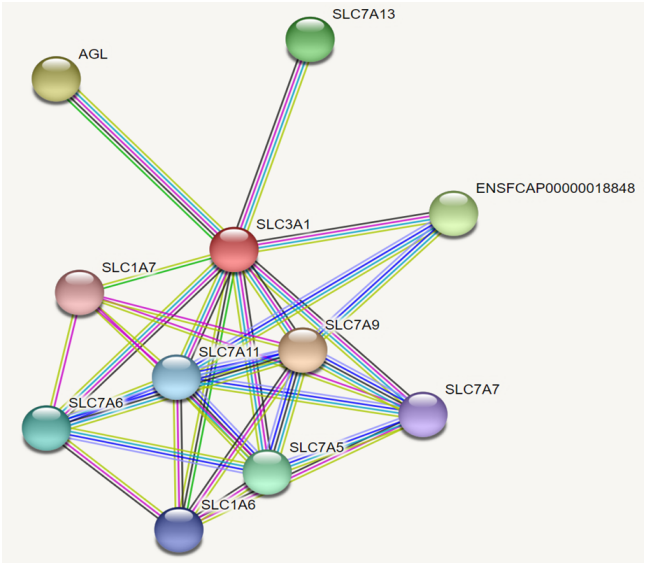

Fig. 3. Predicted SLC3A1 protein interactions (STRING V.11.5 – Szklarczyk et al. 2019, https://string-db.org/)

Among other things, the SLC3A1 protein is associated with the enzyme protein 4-alpha-glucanotransferase (AGL). The probability of interaction is estimated at 0.903. The enzyme is involved in the processes of glycogen biosynthesis and catabolism (Gaudet et al. 2011). Mutations in the nucleotide sequence encoding the AGL protein result in the development of glycogen storage disease type III (Cheng et al. 2009). Another protein with which domestic cat SLC3A1 interacts is the solute channel protein belonging to family 7 – SLC7A13. The probability of interaction is estimated at 0.846. SLC7A13 exhibits transmembrane L-amino acid transporter activity (Gaudet et al. 2011). In humans, SLC7A13 dysfunction is associated with the development of retinopathy pigmentosa type 68 (RP-68), which is a degeneration of the retina (Yahyaoui and Pérez-Frías 2019). Additionally, according to the MalaCards v.5.7.0.531 database (https://www.malacards.org/), RP-68, of all diseases, is most closely associated with cystinuria. Thus, it is likely that cystinuria in the domestic cat may also be accompanied by retinal disorders and deterioration of the animal's vision but this hypothesis needs to be confirmed in further studies. SLC7A5 is a protein that is related to SLC3A1 with a probability of 0.839. Recently, elevated expression of the gene encoding the SLC7A5 protein has been linked to the development of breast cancer (Törnroos et al. 2021). The SLC3A1 protein is also known to interact with the SLC7A7 protein, which exhibits the activity of a transmembrane basic amino acid transporter. The probability of the interaction is estimated at 0.771. Mutations within the gene encoding the SLC7A7 protein cause the development of lysinuric intolerance of the protein, associated with impaired metabolism of amino acids, particularly ornithine, lysine and arginine (MalaCards database v.5.7.0.531).

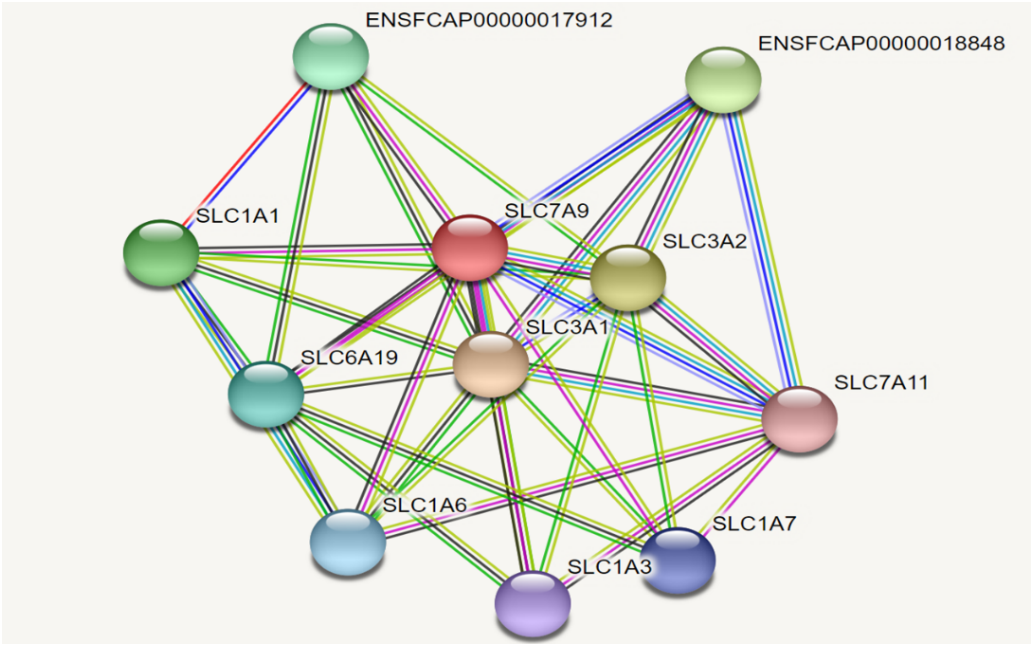

Fig. 4. Predicted SLC7A9 protein interactions (STRING V.11.05 – https://string-db.org/)

The SLC7A9 protein is related to the solute channel protein of family 3 – SLC3A2 with a probability of 0.886. Overexpression of the SLC3A2 protein has been linked in humans to the development of the malignant tumor osteosarcoma (Zhu et al. 2017). It is the most common cancer in cats (Attar et al. 2016). An interaction between SLC7A9 and the solute channel protein of family 1, SLC1A1, has also been detected. The probability of linkage is estimated at 0.701. The SLC3A1 protein is an amino acid transporter with many different molecular functions. Its dysfunction results in the development of carboxylic aminoaciduria, manifested by elevated concentrations of aspartic acid and glutamic acid in excreted urine (Lai 2013). Abnormal SLC1A1

function can also lead to the onset of obsessive-compulsive disorder in human (Hanna et al. 2002). Another protein related to SLC7A9 is the solute channel protein of family 6, SLC6A19. The probability of interaction is estimated to be 0.647. The SLC6A19 protein is a transmembrane neutral amino acid transporter that acts by symport (UniProtBETA database v.2021_04). Mutations in the gene encoding SLC6A19 result, as in cystinuria, in impaired transport of amino acids in the intestine and renal tubules. In humans, this leads to the development of Hartnup disease (Seow et al. 2004).

Proteins that are products of the *SLC3A1* and *SLC7A9* genes, mutations of which cause cystinuria, have been shown to be associated with many proteins that are implicated in the development of various ailments. Therefore, impairment of SLC3A1 and SLC7A9 proteins may promote the appearance of other diseases. This may indirectly explain the heterogeneous, complex symptoms accompanying cystinuria.

In the domestic cat, no coexpression of the *SLC3A1* gene or *SLC7A9* gene is observed with any gene encoding a related protein. In contrast, coexpression of the *SLC3A1* gene and *SLC7A9* with most genes encoding related proteins has been detected in humans. This indicates that gene coexpression may exist in the domestic cat, but further studies should confirm this.

Structural changes occurring due to mutations

The SWISS-MODEL program (Waterhouse et al. 2018, https://swissmodel.expasy.org/) predicts the spatial structure of the X1 isoform of the domestic cat rBAT protein, encoded by both wild-type and mutant nucleotide sequence, using the 6li9.1.A template with the greatest similarity. This is the conformation of the rBAT protein, which is part of the b0,+ transporter of basic and neutral amino acids in humans. The predicted structure is in the form of a monomer containing a single ligand, the calcium ion Ca2+ (Fig. 5). There are no significant differences in the spatial conformation of the normal and mutation-altered forms. The predicted 3D structure of the X2 isoform of the domestic cat rBAT protein is also based on the 6li9.1.A template. The normal and mutation-altered proteins are ligand-free monomers (Fig. 5). In this case, too, no significant changes are observed between the spatial conformations of the resulting proteins.

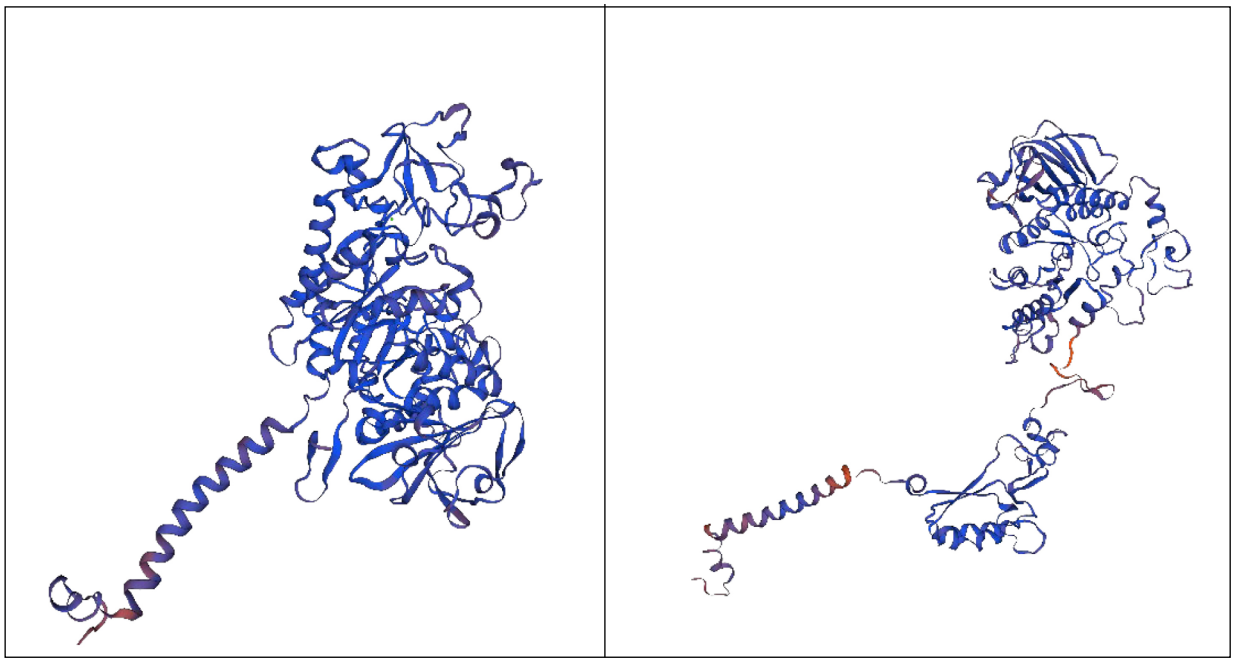

Fig. 5. Predicted spatial conformation of isoform X1 (left) and isoform X2 (right) of normal and mutant SLC3A1 protein (https://swissmodel.expasy.org/)

Three exon mutations were detected in the *SLC7A9* gene, which were included in the study.

The prediction of the spatial structure of the X3 isoform of the domestic cat SLC7A9 protein is based on the 6li9.1.B template available in the SWISS-MODEL program (https://swissmodel.expasy.org/). This is the conformation of the b0,+ AT protein, which is a light subunit of the b0,+ transporter of basic and neutral amino acids characterized in humans. The predicted structure of the X3 isoform is in the form of a monomer having a single ligand, arginine (Fig. 6).

In the X3 isoform of the SLC7A9 protein, asparagine is incorporated instead of aspartic acid at position p.236 due to the first exon mutation in the nucleotide sequence (item c.706, exon 5). The spatial structure model of this type of protein differs from the normal form in the absence of a ligand in the form of arginine. Apart from this aspect, the conformation of the protein was identical. No structural changes are expected between the conformation of the X3 isoform of the normal protein and that resulting from two other mutations in the nucleotide sequence, exon 7 at position c.881 and exon 10 at position c.1175, resulting respectively in the conversion of valine to glutamic acid at position p.294 and threonine to methionine at position p.392 of the X3 isoform of the domestic cat SLC7A9 protein (Fig. 6).

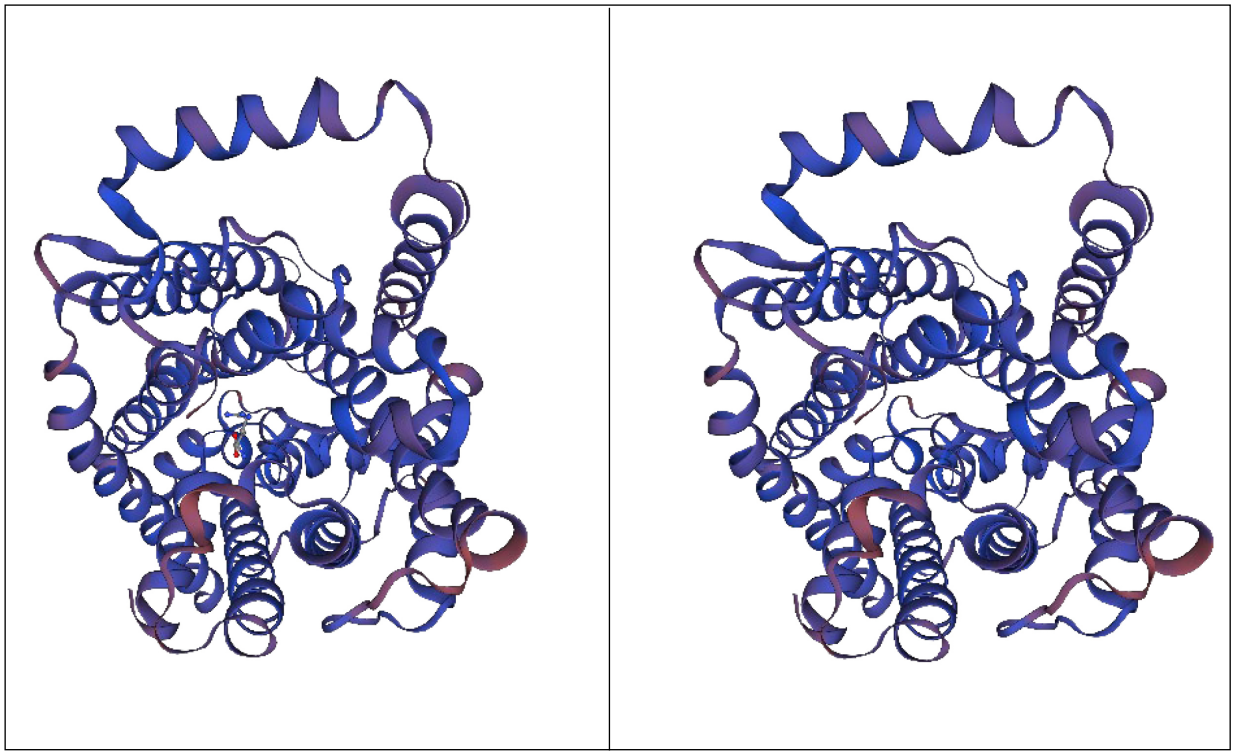

Fig. 6. Predicted spatial conformation of SLC7A9 protein based on the correct amino acid sequence of the X3 isoform and mutated with respect to p.Val294Glu, p.Thr392Met (left), p.Asp236Asn (right) mutations (https://swissmodel.expasy.org/)

A study of the spatial structure of the domestic cat SLC7A9 protein carried out using the X1 isoform yielded very similar results to the X3 isoform study. The predicted protein conformation is based on the template 6li9.1.B. Only a mutation at position c.706 of exon 5 of the *SLC7A9* gene, resulting in an amino acid substitution in the amino acid sequence to aspartic acid at position p.236, causes a difference in the 3D structure of the resulting protein. The product is then devoid of the ligand arginine. The other two mutations occurring in the nucleotide sequence do not affect the predicted structure of the protein (Fig. 7).

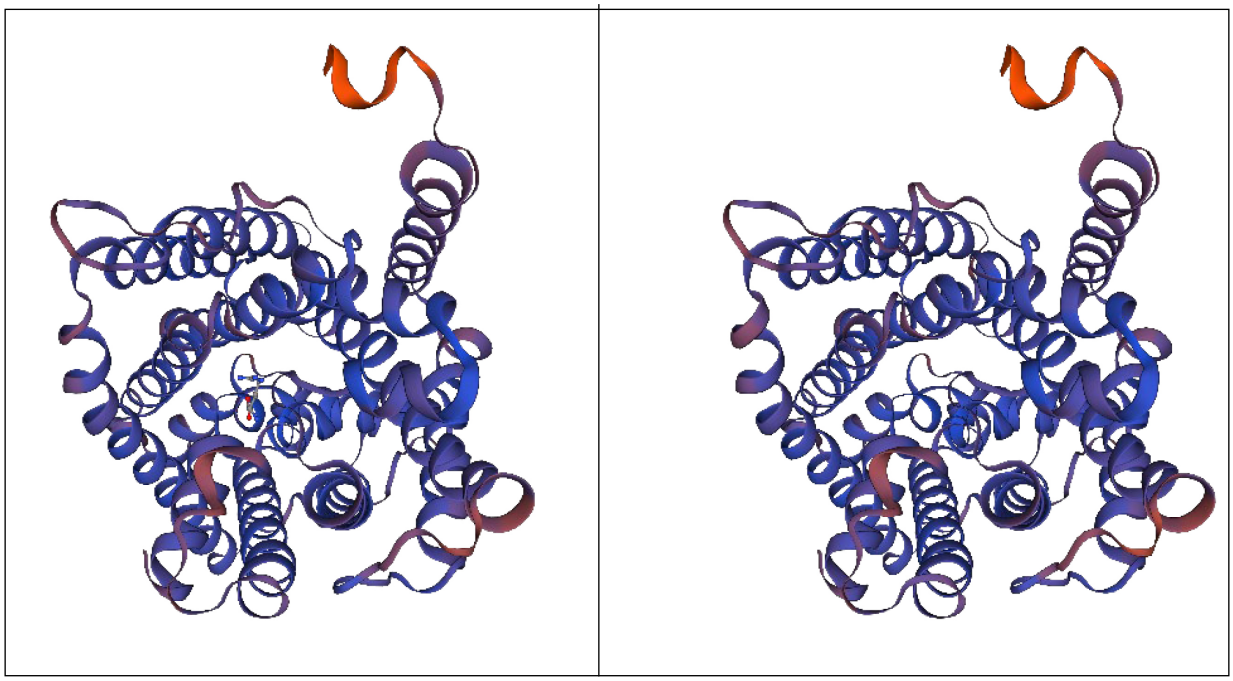

Fig. 7. Predicted spatial conformation of SLC7A9 protein based on correct amino acid sequence of X1 isoform and mutated with respect to p.Val294Glu, p.Thr392Met (left), p.Asp236Asn (right) mutations (https://swissmodel.expasy.org/)

Deleterious effect of changes due to mutations

The deleterious effects of the six mutations under study were confirmed in a program predicting the effects of VEP nucleotide variants (Table 4).

Table 4. Results from the VEP program. From left: position of mutation, deleterious allele, mutation type, impact of mutation, gene symbol and sequence number, SIFT value = 0; 0.01; 0.02 indicates deleterious effect of mutation on protein functionality. (source: https://www.ensembl.org/info/docs/tools/vep/index.html)

Position of mutation site	Allele	Consequence	Impact	Symbol of gene	No of gene	SIFT
c.1342C>T	T	Missense variant	moderate	SLC3A1	ENSECAG00000000611	0 ▲
c.706G>A	A	Missense variant	moderate	SLC7A9	ENSECAG00000014914	0.02 ▼ 0.01 ▼
c.1175C>T	T	Missense variant	moderate	SLC7A9	ENSECAG00000014914	0 ▼
c.881T>A	A	Missense variant Splice region variant	moderate	SLC7A9	ENSECAG00000014914	0
c.1409-30A>G	G	Intron variant	Modifier	SLC7A9	ENSECAG00000014914	–
		Downstream gene variant	Modifier	TDRD12	ENSECAG00000014914	–
		Downstream gene variant	Modifier	SLC7A9	ENSECAG00000014914	–
c.1233+1G>A	A	Splice donor variant	High	SLC7A9	ENSECAG00000014914	–

Three exon mutations, at position c.1342 of exon 8 of the *SLC3A1* gene, c.706 of exon 5 of the *SLC7A9* gene, and c.1175 of exon 10 of the *SLC7A9* gene, are missense mutations, which have been determined to be deleterious in the context of protein function. The program predicts

the mutation at position c.881 of exon 7 of the *SLC7A9* gene to be a 50% sense change mutation and a 50% mutation affecting the RNA folding site, since the change affects one of the last three nucleotides of the exon. Its effect is detrimental to the function of the resulting protein. The mutation at position c.1233+1 of intron 10 of the *SLC7A9* gene is 100% a change affecting the splicing site donor, which can affect the correct excision of non-coding sequences. Its impact is assessed as high. The impact of the mutation at position c.1409-30 of intron 11 of the *SLC7A9* gene was classified as modifying by the VEP program.

CONCLUSIONS

The analyses performed confirmed the complex, heterogeneous nature of cystinuria. The proposed tools, such as primers and enzymes, can be used to PCR-RFLP and AS-PCR testing protocols identifying six mutations in the DNA of the domestic cat, identified as causative for cystinuria. The simple diagnostic tests will make it possible to screen large numbers of domestic cats. This will make it possible to eliminate mutations from the domestic cat population by not allowing individuals carrying harmful mutations to breed. Diagnostic tests should be directed primarily at animals that are related to individuals with cystinuria, but have not yet developed symptoms of the disease. This will allow the possible detection of cystinuria before the appearance of symptoms, the rapid implementation of treatment, and this will increase the chance of preventing the development of unpleasant consequences of the disease. In addition, it will be possible to determine the impact of heterozygous mutations on the course of the disease. Certainly, there is a need for further studies that will allow an accurate description of the changes that occur in the structure of proteins due to mutations and the function they perform. So far, the evaluation of changes has been based on predictions, made possible by information on other species of animals affected by cystinuria.

Funding: Research financed by the project “Student scientific circles create innovations” financed by the Ministry of Education and Science.

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MOLEKULARNE TESTY DIAGNOSTYCZNE IDENTYFIKUJĄCE NOSICIELI MUTACJI GENÓW *SLC3A1* I *SLC7A9* POWODUJĄCYCH CYSTYNURIĘ U KOTA DOMOWEGO – ANALIZA *IN SILICO*

Streszczenie. Cystynuria to dziedziczna choroba o podłożu genetycznym, związana z mutacjami w obrębie genów *SLC3A1* i *SLC7A9*. Wyniki badań u kotów wskazują na niejednorodność tej choroby. Do tej pory odkryto 1 mutację genu *SLC3A1* oraz 5 mutacji genu *SLC7A9*. Cystynuria jest chorobą nierzadko wykrywaną zbyt późno, gdy osobniki dorosłe mają już potomstwo. Dlatego tak ważne jest podejmowanie działań, które pozwolą na szybką i łatwą diagnozę choroby, jeszcze przed pojawieniem się objawów. Celem badań było zaprojektowanie prostych testów diagnostycznych umożliwiających identyfikację nosicieli zmutowanych alleli, które mogą powodować cystynurię u kota domowego. Przeprowadzono analizę bioinformatyczną powiązań białek SLC3A1 i SLC7A9 z innymi białkami i próbowano ocenić zmiany strukturalne i funkcjonalne zachodzące na skutek mutacji. W analizach bioinformatycznych wykorzystano następujące programy i bazy danych: Ensembl, NCBI, Primer-BLAST, NEBcutter, STRING, SWISS-MODEL, Variant Effect Predictor (VEP). Proponowane startery i enzymy do testów PCR-RFLP i AS-PCR umożliwiają identyfikację osobników będących nosicielami mutacji. Interakcje białek SLC3A1 i SLC7A9 z innymi białkami sugerują możliwe powiązania cystynurii z rozwojem innych chorób. Może to pośrednio tłumaczyć niejednorodne, złożone objawy towarzyszące cystynurii. Mutacja w egzonie 5 genu *SLC7A9* (p.Asp236Asn) powoduje zmianę w przewidywanej konformacji przestrzennej białka. Z wykorzystaniem programu bioinformatycznego VEP oceniono wpływ wszystkich mutacji na funkcjonalność białka jako szkodliwy. Uważa się za konieczne, aby hodowcy w pierwszej kolejności przeprowadzali test molekularny wykrywający mutację genu *SLC7A9* egzonu 7 w pozycji c.881, ponieważ wydaje się, że jest to najbardziej rozpowszechniona mutacja w populacji kota domowego.

Słowa kluczowe: cystynuria, kamica moczowa, *SCL3A1*, *SLC7A9*, mutacje