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THE CAUDAL VERTEBRAE FUSION AND DYSMORPHOLOGY IN WHITE SWISS SHEPHERD DOG – A CASE STUDY

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Abstract. The *T-box* gene belongs to a very large family of genes with the T motif. It encodes transcription factors that control the course of embryogenesis process. It plays a major role in the formation of the mesoderm – the layer from which the axial structures of the body develop. In dogs, its mutation causes a short tail in heterozygotes, often with a kink, while in recessive homozygotes it is lethal. Dominant homozygotes have phenotypically normal, long tails. The aim of this study was to identify carriers of the mutated allele of the *T-box* gene and to check whether it determines the occurrence of a lack of a tail and a kink in White Swiss Shepherds. Hair, blood, soft tissue and epithelium samples from the inner side of the cheek were collected from 23 individuals of the White Swiss Shepherd breed. Twenty-two individuals had phenotypically long tails and one was tailless. DNA was isolated, amplified using polymerase chain reaction (PCR), the product was subjected to electrophoretic separation in agarose gel, and then the obtained product was cut with the restriction enzyme *Eco91*I (*BstE*II) to be able to visualize the samples in a polyacrylamide gel. All dogs of the White Swiss Shepherd breed included in the study, whose DNA was analysed for carrying the 259C>G mutation of the *T-box* gene, turned out to be homozygous recessive with a long tail, which is consistent with the breed standard. The reason for the lack of a tail in one of the examined individuals must have been different mutation of the *T-box* gene or a mutation in another gene.

Key words: *T-box* gene, kinked tail, White Swiss Shepherd Dog.

INTRODUCTION

More and more dogs have tail defects. In times when it was possible to crop their ears and dock their tails, this was not such a problem, but when the ban was introduced, especially

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in dog breeds where a straight tail was not one of the selection criteria, the problem began to be visible. This fact is a good reason to take a closer look at the problem of a kinked tail, because despite its seemingly low harmfulness, this problem can be really serious on a larger scale. A kinked tail is caused by deformation, most often of the penultimate caudal vertebrae. In many cases, it remains unnoticeable. Only palpation examination by running your fingers along the tail gives this possibility. During embryonic development, all organs of the body are formed from three germ layers (endoderm, ectoderm and mesoderm) (Farkas and Chapman 2009). The entire skeleton, blood vessels, heart and urinary and reproductive system are formed from the mesoderm. Correct formation of the axis in the developing embryo is crucial for the correct arrangement of organs. A spontaneous mutation, called the "kinked tail", has been identified in mice, which in heterozygotes causes a kinked tail due to fusion and dysmorphology of the caudal vertebrae, and in homozygotes causes death in early embryonic development. The cause of death is underdevelopment and distortion of the axial structures and the failure of the notochord to develop (Farkas and Chapman 2009).

Kinky tail is common in Syrian hamsters. It has been observed that this defect occurs in dark grey coat lines. The described mutation is probably located in the same locus in the chromosome as the genes responsible for the dark grey coat (*dg*). The genotype of an individual with a kink is designated as dgkdgk. This mutation can be observed in homozygous individuals. The kink is palpable during examination. This defect has not been found in heterozygotes (Jesariew et al. 2016). Domestic cats also have tails of varying lengths and kinks. This anomaly has been shown to occur in at least five modern cat breeds in the world, including the Japanese Bobtail, Manx, American Bobtail, Pixie-Bob, and Kuril Bobtail. This defect is caused by mutations in the *HES7* and/or *T-box* genes. However, research evidence suggest that at least three different episodes resulted in short tailed phenotypes in domestic cats (Xu et al. 2016).

The *T-box* gene belongs to a large family of genes with a T motif. It encodes transcription factors that control the course of embryogenesis process. It plays a major role in the formation of mesoderm, from which axial structures of the organism develop. Mutations in the *T-box* gene result in drastic defects in the development of organisms. In dogs, a mutation of this gene causes a short tail in heterozygotes, often with a kink, while in the dominant homozygotes it is lethal. This mutation has been identified in eighteen dog breeds (Haworth et al. 2001; Indrebø et al. 2008; Hytönen et al. 2009; Gruszczyńska and Czapla 2011; Gruszczyńska et al. 2013). A short tail with many kinks is very common in King Charles Spaniels. The natural bobtail tail is common among many breeds, but only eighteen dog breeds are affected by a mutation in the *T* gene (Haworth et al. 2001).

The gene mutation causing the tail kink may not be phenotypically visible for many generations, and then suddenly become apparent. By breeding an animal with the kink, we can obtain an individual with serious defects of the skeletal system and spinal cord in the next generation. For this reason, animals with this defect should not be bred (Jesariew et al. 2016).

T-box genes belong to the group of genes responsible for coding transcription factors involved in the process of embryogenesis. The proper synthesis of transcription factors is crucial in the development of cells in the animal body. Transcription factors encoded by genes belonging to the T-box family are considered to be among the most important during the embryonic development of vertebrates (Sebé-Pedrós et al. 2013).

Transcription factors are proteins responsible for the level of gene transcription. They bind to DNA and often have a characteristic motif. Transcription factors can modulate the transcription process in a positive or negative way, they can enhance or silence the transcription process of specific genes.

The *T-box* gene family is characterized by the presence of a conserved domain coding about 180–200 amino acids. This site is called the T-box domain. This domain plays a very important role during the process of embryogenesis. Genes with the T motif are expressed during organogenesis and gastrulation. They are also expressed in adult tissues (Papaioannou and Silver 1998; Smith 1999). The first gene with the T motif identified in mice was the *brachyury* gene (Herrmann 1991). Expression of the *brachyury* gene is essential for the proper development of the notochord and posterior body structures in mice (Halpern et al. 1993). This gene affects the development of the posterior mesoderm during gastrulation (Herrmann 1995). The binding domain of the *brachyury* gene consists of 229 amino acids (Kispert and Herrmann 1993). This gene is the most characteristic member of the T-box family. Its role in the formation of the mesoderm and notochord has been thoroughly investigated (Scholz and Technau 2003). Studies by Papaioannou and Silver (1998) indicate that this gene is responsible for the short tail in mice and that it is a gene encoding a protein that is a transcription factor (Herrmann 1995).

In subsequent years, the presence of genes related to the *brachyury* gene were demonstrated in other vertebrate species, including the frog *Xenopus laevis*, zebrafish, domestic chicken, domestic dog, and fruit fly (Smith et al. 1991; Schulte-Merker et al. 1992; Haworth et al. 2001). Agulnik et al. (1995) identified a group of four genes with a T motif in the genome of *Caenorhabditis elegans.* Genes with a T motif have also been discovered in humans. Mutation of the *TBX3* gene causes the Holt-Oram syndrome. It manifests itself with a developmental abnormality of the heart and upper limbs (Bamshad et al. 1997). Mutation in the *TBX1* gene results in the DiGeorge syndrome, which causes fetal death due to congenital heart defects (Packham and Brook 2003). *Brachyury* gene expression is essential for the proper development of the notochord and posterior structures in mice and zebrafish (Halpern et al. 1993).

Until recently, it was thought that *T-box* genes were found only in the animal kingdom. Current studies indicate that these genes were already present in early fungi and unicellular organisms from the *Holozoa* group (sponges, collared flagellates, platypods) (Sebé-Pedrós et al. 2013; Sebé-Pedrós and Ruiz-Trillo 2017). In the early 1990s, a gene called *omb* was discovered in *Drosophila*, which had a similar sequence to the *T* gene in mice (Pflugfelder et al. 1992). The expression of *T* genes in humans, chickens, and mice is very similar to the expression of *T* genes in fruit flies. During the larval stage, expression of the *Drosophila omb* gene in fruit flies affects the development of the brain and wings. Similarly, in domestic chickens and mice, expression of *T-box* genes affects the development of the retina, limbs, and – to a small extent – the brain. The greatest similarity in gene expression in humans, fruit flies, chickens, and mice is observed in developing tissues in both foetuses and adults (Chapman et al. 1996).

Sebé-Pedrós et al. (2011) studies report the presence of *T-box* genes in the unicellular amoeba *Capsaspora owczarzaki*, closely related to animals, and in the fungus *Spizellomyces punctatus*. The gene identified in *C. owczarzaki* is a homolog (has a similar motif) of the *brachyury* gene in mice. The degree of similarity between them and the occurrence of the *T* gene in other unicellular organisms still remains unclear. The above studies confirm that the *brachyury* gene is the oldest member of the *T-box* gene family with a very high diversity. *T-box* genes have different classes, including the following classes: *Tbx4/5*, *Tbx6*, *Tbx2/3*, *Eomes*, *Tbx1/15/20*. All *T-box* classes are distributed among animals with bilateral body symmetry and in organisms lacking bilateral body symmetry such as cnidarians, comb jellyfish or sponges (Martinelli and Spring, 2005). Phylogenetic studies have also allowed the identification of those genes that are likely to be orthologs of the same gene in different

species. Orthologous genes are defined as direct descendants of a common ancestral gene that was present in the genome of the common ancestor of the two species analysed (protein or DNA/RNA fragment with a common evolutionary origin, the origin of which occurred as a result of speciation). Often, but not always, they perform the same function. *Brachyury* gene orthologs are identified in many vertebrates and invertebrates (Papaioannou and Silver 1998).

In zebrafish, all three genes (*tbx16*, *tbx6*, and *ntl*) are involved in the formation of the transitional structures that give rise to vertebrae, skeletal muscle, and dermis. The *ntl* gene is expressed in the notochord region, the *tbx6* gene is expressed in blood precursor cells, and the *tbx16* gene is expressed in a colony of cells located at the tip of the notochord and in some interneurons of the spinal cord. As a result, the expression patterns of these three genes are very dynamic, with different functions, but it is possible that they are interdependent to ensure correct expression (Ruvinsky et al. 1998). Studies (Smith et al. 1991) have shown that the *Xbra* gene in the frog *Xenopus laevi* is an ortholog of the mouse *brachyury* gene in both sequence and expression patterns. The highest expression levels are present during gastrulation, mainly in the notochord cells. The expression pattern of the *Xbra* gene clearly resembles the expression of the *brachyury* gene in the mouse embryo.

Mutation of the *T-box* gene

The *T* gene determines brachycephaly and short tail. The mutation of the *T* gene in the domestic dog consists of substituting the nitrogen base cytosine (C) for guanine (G) in the 189th nucleotide of the gene (189C>G). This substitution leads to the formation of the amino acid isoleucine (ATC codon) instead of methionine (ATG codon) in the 63rd amino acid of the given protein. This is a missense mutation (Haworth et al. 2001; Hytönen et al. 2009). Heterozygotes have short tails without identified abnormalities. Dominant homozygotes die at an early embryonic stage with severe spinal defects, missing some caudal vertebrae or demonstrating incomplete bone mineralization, while recessive homozygotes have normal long tails (Indrebø et al. 2008).

In domestic dogs, the *T-box* gene is located on the first chromosome (1q23) (Haworth et al. 2001). Sequence analysis of the *T-box* gene of different dog breeds has revealed various types of polymorphism and unique missense mutations in bob-tailed dogs and their offspring. The *T-box* gene mutation is located in a highly conserved T domain, resulting in a reduced ability of the T protein to bind to the appropriate site on the DNA strand. Substitution of isoleucine for methionine may change the binding properties of the mutant protein. It binds to DNA less efficiently. Molecular analysis of the *T-box* gene in offspring of bob-tailed dogs indicates that the homozygous system is lethal at the embryonic level. Complete sequence analysis of the *T-box* gene has revealed a single 259C>G mutation in exon 1 in Pembroke Welsh Corgis. It is caused by the substitution of methionine for isoleucine at amino acid 63, which creates a cleavage site for the restriction enzyme *BstE*II. This mutation is responsible for the occurrence of a shortened tail in short-tailed dogs (Pembroke Welsh Corgis). All offspring of bob-tail × bob-tail were heterozygotes (carriers of the mutation). Offspring with a long tail were not carriers of the mutation. No homozygous dominant individuals were found, suggesting that in homozygotes this mutation is lethal. Studies indicate that the *T* gene is dominant (Haworth et al. 2001).

Lack of tail and brachycephaly due to mutations in the *T-box* gene occur in many dog breeds, including Beagle, Cocker Spaniel, and Pembroke Welsh Corgi (Haworth et al. 2001). The 259C>G mutation has been found in 18 brachycephalic dog breeds. Dogs with no tail, a shortened tail, or a kinked tail are to be found (Hytönen et al. 2009).

Detection of *T-box* gene mutations

To detect *T* gene mutation, the amplified *T* gene fragment is to be digested with the restriction enzyme *Eco91*I (*BstE*II). As a result of the polymerase chain reaction (PCR), a product of 702 bp is obtained. The *Eco91*I enzyme cuts the unmuted sequence at position 191 nt. Two fragments of 191 and 511 bp are created. If a mutation is detected, the enzyme cuts the sequence at an additional place at position 160 nt. Three fragments of 511 bp, 160 bp, and 31 bp are created. In the heterozygous system, four restriction fragments are obtained: 511 bp, 191 bp, 160 bp and 31 bp. The 31 bp fragment is not visible in the stained polyacrylamide gel (Gruszczyńska et al. 2013).

The White Swiss Shepherd – history of the breed

According to the FCI, the White Swiss Shepherd belongs to Group I – Sheepdogs and Cattledogs, section I – Sheepdogs, standard number 347. This breed is not subjected to working trials. For many years it was considered of little value (Räber 2001). During the breeding work of the German Shepherd in Germany, white-coated individuals were selectively eliminated. They were undesirable, considered completely worthless, among other things because the occurrence of various defects associated with the white coat in dogs. In 1933, the white coat was eliminated from the German Shepherd standard. From that time on, white German Shepherds began to vanish in Europe. In the United States, however, shepherds with both white and "traditional" coats were entered in stud books. In 1991, the Swiss Kennel Club was one of the first members of the FCI to recognize the white shepherd as a separate breed and developed provisional breed standard. A new breed was recognized under the name "White Shepherd". Within a few years, many other European countries registered this breed in their studbooks. In total, about 5,000 dogs were registered. Switzerland decided to take over the patronage of white shepherds. An application was submitted to the FCI for recognition of the breed and the creation of a standard. The breed standard was officially published only in 2002 and was given the name: "Berger Blanc Suisse" – White Swiss Shepherd. Since then, Switzerland has become the official patron of this breed. White Swiss Shepherds are lively, alert dogs, very easy to train. They have a strong, well-muscled silhouette, and their ears are always erect. The coat is either long or short. The tail is well coated, tapering downwards. One of the major faults that can lead to disqualification from breeding is a deformed, hooked or kinked tail (Räber 2001; FCI 2011). To date, neither the gene nor any mutation responsible for this fault have been identified.

The aim of the study was to identify carriers of a mutation in exon 1 of the *T-box* gene, which determines the presence of a short or kinked tail in White Swiss Shepherd dogs.

MATERIALS AND METHODS

Research material

In order to identify the occurrence of the *T-box* gene mutation in 23 White Swiss Shepherd dogs, genetic material was isolated from blood (n = 8), hair bulbs (n = 9) and epithelial tissue collected from the inner side of the muzzle (n = 5). Twenty-two dogs had phenotypically long tails. One of the examined individuals was a female dog (Fig. 1) born as one of eight offspring from a pair of parents with phenotypically long tails. The female dog was born without a tail. Shortly after birth, she had problems standing on her pelvic limbs, which caused problems with access to the mother's nipples. This puppy was euthanized by a veterinarian. In this case, the material for further tests was a tongue's fragment.

Fig. 1. White Swiss Shepherd female without any tail (phot. J. Gruszczyńska)

Methods

DNA was isolated depending on the type of biological material using the following commercial kits: peripheral blood – QIAmp DNA Mini Kit (Qiagen), inner wing epithelium – Swab-Extract DNA Purification Kit (EURx), hair roots and soft tissue – GeneMatrixTssueu&Bacterial DNA Purification Kit (EURx). Polymerase chain reaction (PCR) was performed by preparing the following composition of the reaction mixture: Ready Mix (EURx, colour OptiFaqPCR Master Mix) 20 µl, Forward Primer 5'GAAGAGCCTGCAGTACCGAGT 3' – 0.3 µl, Reverse Primer 5'CACTCTCCGTTCACGTACTTCC 3' – 0.3 µl, deionized water, RNAse and DNAse free (EURx) 6 µl (Gruszczyńska et al. 2013), in case of DNA isolate from blood and soft tissue, 4 µl was added, and in case of isolate from hair bulbs and swabs from the inner side of the cheek, 10 µl, and no water was added to the mixture. The final volume of the reaction mixture was 26.6 µl. PCR was performed in a T3 thermocycler (Biometra). The PCR reaction was carried out according to the thermal profile (Gruszczyńska et al. 2013). Then, the PCR products were electrophoretically separated in a 1.5% gel (1:1, standard: HR) and eventually cut with the *Eco91*I *(BstE*II*)* restriction enzyme (Thermo Scientific™). The composition of the reaction mixture: 10 × Buffer O – 2 µl, deionized water 18 µl, 1 µl of the *Eco91*I restriction enzyme. The whole was incubated at 37°C for an hour and at 65°C for 20 minutes. After cleavage with the *Eco91*I restriction enzyme the resulted productwas subjected to electrophoretic separation in a 12% polyacrylamide gel. 5 µl of the mass standard (EUROGENTEC, SmartLadder SF) was used in the electrophoretic separation. The duration of electrophoresis was 15 minutes under the following conditions: 300 V, 5 kWh, flow rate 0.6. The polyacrylamide gel was stained using the silver nitrate method.

RESULTS AND DISCUSSION

As a result of electrophoretic separation of PCR products in 1.5% agarose gel, a clearly visible PCR product of 702 bp in length was obtained (Fig. 2).

Then, PCR products were cut with the restriction enzyme *Eco91*I *(BstE*II*)* and subjected to electrophoretic separation in a 12% polyacrylamide gel (Fig. 3). All the tested White Swiss Shepherd dogs, whose DNA was analysed for carrying the 259C>G mutation of the *T-box* gene, turned out to be homozygous recessive with a long tail, which is consistent with the

breed standard. In the polyacrylamide gel, two bands are visible for each individual with a length of 511 bp and 191 bp, which means that they are homozygous recessive.

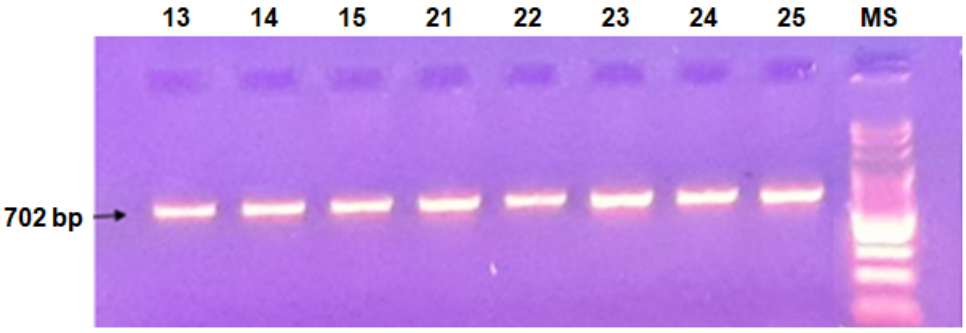

Fig. 2. Electrophoretic separation of PCR products of exon 1 of the *T-box* gene in 1.5% agarose gel. Probes 13–25 – PCR product sample/individual number, MS – SmartLadder SF mass standard (EUROGENTEC)

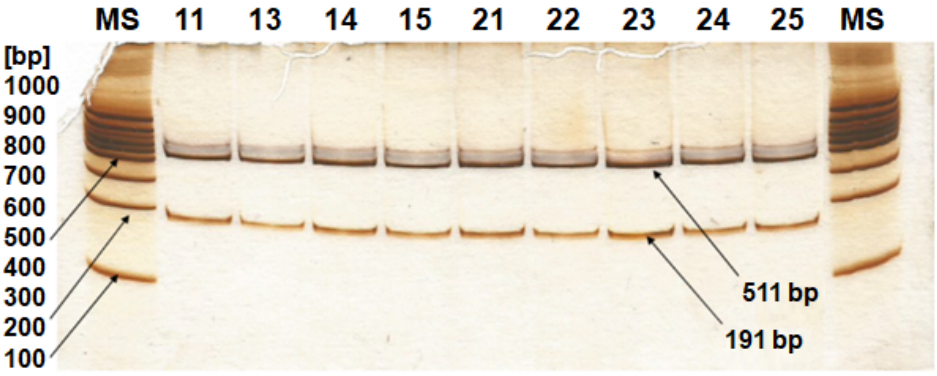

Fig. 3. Electrophoretic separation of the PCR product of the *T-box* gene after digestion with the restriction enzyme *Eco91*I (*BstE*II) in native 12% polyacrylamide gel. Probes 11–25 – PCR product digested with the restriction enzyme. MS – SmartLadder SF mass standard (EUROGENTEC)

One of the examined individuals was a female born tailless, euthanized a few days after birth, which, after molecular analysis, turned out to be homozygous recessive for the 259C>G mutation of the *T* gene – sample no. 25 (Fig. 2). She came from the parents (samples 23 and 24) with a phenotypically visible tail, and after molecular analysis it was found that they were also homozygous recessive for the 259C>G mutation of the *T* gene. Therefore, the reason for the lack of tail and problems with moving pelvic limbs in the examined individual no. 25 has not been explained.

Studies by Silva et al. (2018) aimed to examine a group of Labrador retrievers for the occurrence of the C189G mutation. For this purpose, a female with shortened tail was mated with a male with normal-length tail. After the birth of the puppies, the female from this litter with shortened tail was mated with a male with a normal, long tail from a completely different litter. Their offspring were analysed for the presence of the mutation. The results of the study showed that the C189G mutation was detected only in dogs with tail deformity. Some dogs had a shortened tail, others had no tail at all. In *in silico* analyses, a change of the amino acid isoleucine to methionine was observed at amino acid 63. The

result was identical to the results presented in the study by Haworth et al. (2001). The conducted studies show that this is an autosomal dominant mutation, lethal in homozygous dominant system and individuals with the dominant nature of the mutation die at the early stages of their development, which confirms the important role of the *T-box* gene in the process of embryogenesis. Gruszczyńska et al. (2013) came to the same conclusions in their earlier studies. These authors examined samples from dogs of the Polish Lowland Sheepdog breed for the occurrence of the 259C>G mutation of the *T* gene determining brachycephaly and taillessness. The results confirmed the occurrence of the mutation in this breed and its lethal nature, leading to the smaller number of puppies in litters from the mating of parents with short tails, compared to the size of litters from the mating of dogs that were not carriers of the mutation (Gruszczyńska et al. 2013). The observed reduction in litter size after heterozygous parents was similar to that in the study by Hytönen et al. (2009). These authors found a highly significant, 29% decrease in litter size from the mating of short-tailed Swedish Vallhund dogs. In this breed, the 259C>G mutation of the *T* gene is also responsible for shortening or absence of the tail. The study by Silva et al. (2018) confirms the occurrence of the *T-box* gene mutation also in Labrador Retrievers with abnormalities in tail development. Similar results were obtained in the study by Gruszczyńska and Czapla (2011), where thirty individuals of the Pembroke Welsh Corgi breed were examined. Twenty recessive homozygotes with long tails and ten heterozygotes carrying the 259C>G mutation were identified. Six individuals had a shortened tail and four had no tail. No dominant homozygotes were identified.

Indrebø et al. (2008) examined the offspring of the Pembroke Welsh Corgi breed from two different litters, the result of mating a short-tailed individual with a long-tailed individual, to determine whether they were heterozygous for a mutation in the *T* gene. The parents were also subjected to molecular analysis for this purpose. A total of 10 puppies were born to the same parents. Two puppies had long tails, six had short tails, and two had no tails at all. The analysis of the genetic material for the occurrence of a mutation in the *T* gene yielded the following results: both the parents and the short-tailed puppies were heterozygous for the dominant mutation in the *T* gene. Two long-tailed puppies were homozygous recessive. Two tailless puppies were homozygous dominant, which is the first report of the birth of puppies with this genotype. However, those puppies had to be euthanized immediately after birth. Since these reports, there have been no other reports in the literature of live births of dominant homozygotes. This is similar to what was observed in mice with a mutation in the *brachyury* gene. Homozygous mutants died at about day 10 of gestation (Showell et al. 2004).

The phenotypic outcome of *T-box* mutations in dogs is very similar to those of *T-box* mutations in mice. Heterozygous mice have short tails, whereas homozygous embryos die in utero at day 10 after fertilization, showing severe defects in tissues developing from the posterior mesoderm: notochord or allantois (Wilson et al. 1993). Mutation of the *Pax1* gene in mice causes severely shortened tails and the appearance of so-called kinks or crevices (Wilm et al. 1998), and mutation of the *Wnt-3* gene is responsible for the appearance of a rudimentary tail in mice (Greco et al. 1996). Conducting research at the molecular level is of great importance for breeding, because heterozygous individuals carrying the mutation may have a normal tail length with caudal vertebral recesses identifiable by palpation (Hytönen et al. 2009). As indicated by the studies presented above, animals with the *T* gene mutation should not be mated due to the reduced number of puppies born in the litter and the likelihood of giving birth to individuals with serious developmental defects.

CONCLUSIONS

As a result of the conducted studies, it was found that all the examined individuals are homozygous recessive and would be expected to have phenotypically long tails.

After conducting appropriate molecular analyses, it was found that the female of the White Swiss Shepherd breed (euthanized a few days after birth) born tailless and originating, as shown in these studies, from parents who were homozygous recessive, was also homozygous recessive. It is likely that the genetic mutation that caused the absence of a tail may be in this case located in a different place in the *T-box* gene sequence or in a different gene, therefore the genetic material should be subjected to further molecular tests.

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REFERENCES

Agulnik S.I., Bollag R.J., Silver L.M. 1995. Conservation of the *T-box* gene from *Mus musculus* to *Ceanorhabditis elegans*. Genomics 25(1), 214–219. DOI: 10.1016/0888-7543(95)80128-9.

Bamshad M., Lin R.C., Law D.J., Watkins W.C., Krakowiak P.A., Moore M.E., Franceschini P., Lala R., Holmes L.B., Gebuhr T.C., Bruneau B.G., Schinzel A., Seidman J.G., Seidman C.E., Jorde L.B. 1997. Mutations in human *TBX3* alter limb, apocrine and genital development in ulnar-mammary syndrome. Nat. Genet. 16(3), 311–315. DOI: 10.1038/ng0797-311.

Chapman D.L., Gravey N., Hancock S., Alexiou M., Agulnik S.I., Gibson-Brown J.J., Cebra-Thomas J., Bollag R.J., Silver L.M., Papaioannou V.E. 1996. Expression of *T-box* family genes, *Tbx-1–Tbx5*, during early mouse development. Dev. Dyn. 206(4), 279–390. DOI: 10.1002/(SICI)1097-0177(199608)206:4<379::AID-AJA4>3.0.CO;2-F.

Farkas D.R., Chapman D.L. 2009. *Kinked tail* mutation results in notochord defects in heterozygotes and distal visceral endoderm defects in homozygotes. Dev. Dyn. 238(12), 3237–3247. DOI: 10.1002/dvdy.22141.

FCI. 2011. Berger Blanc Suisse. White Swiss Shepherd Dog. St-FCI n°347/12.08.2011, http://www.fci.be/Nomenclature/Standards/347g01-en.pdf, access: 8.10.2024.

Greco T.L., Takada S., Newhouse M.M., McMahon J.A., McMahon A.P., Camper S.S. 1996. Analysis of the vestigial tail mutation demonstrates that *Wnt-3a* gene dosage regulates mouse axial development. Genes Dev. 10(3), 313–324. DOI: 10.1101/gad.10.3.313/.

Gruszczyńska J., Czapla A. 2011. A molecular test for the detection of the *C295G* mutation in the *T* gene responsible for shortened tail and taillessness in the Pembroke Welsh Corgi. Ann. Warsaw Univ. of Life Sci. SGGW, Anim. Sci. 49(49), 35–43.

Gruszczyńska J., Haska A., Grzegrzółka B. 2013. Identyfikacja mutacji C295G genu *T* u psów rasy polski owczarek nizinny [Detection of C295G mutation *T* gene in Polish Lowland Sheepdog]. Rocz. Nauk. Pol. Tow. Zootech. 9(4), 9–16 [in Polish].

Halpern M.E., Ho R.K., Walker C., Kimmel C.B. 1993. Induction of muscle pioneers and floor plate is distinguished by the zebrafish *no tail* mutation. Cell 75(1), 99–111. DOI: 10.1016/S0092-8674(05)80087-X.

Haworth K., Putt W., Cattanach B., Breen M., Binns M., Lingaas F., Edwards Y.H. 2001. Canine homolog of the *T-box* transcription factor *T*; failure of the protein to bind to its DNA target leads to a short-tail phenotype. Mamm. Genome 12(3), 212–218. DOI: 10.1007/s003350010253.

Herrmann B.G. 1991. Expression pattern of the *Brachyury* gene in whole-mount T^{WIS}/T^{WIS} mutant embryos. Development 113(3), 913–917. DOI: 10.1242/dev.113.3.913.

Herrmann B.G. 1995. The mouse *Brachyury* (*T*) gene. Semin. Cell Dev. Biol. 6(6), 385–394. DOI: 10.1016/S1044-5781(06)80002-2.

Hytönen M.K., Grall A., Hédan B., Dréano S., Seguin S.J., Delattre D., Thomas A., Galibert F., Paulin R., Lohi H., Sainio K., Andre C. 2009. Ancestral T-box mutation is present in many, but not all, short-tailed dog breeds. J. Hered. 100(2), 236–240. DOI: 10.1093/jhered/esn085.

Indrebø A., Langeland M., Juul H.M., Skogmo H.K., Rengmark A.H., Lingaas F. 2008. A study of inherited short tail and taillessness in Pembroke Welsh corgi. J. Small Anim. Pract. 49(5), 220–224. DOI: 10.1111/j.1748-5827.2007.00435.x.

Jesariew M., Linde C., Piasecki T. 2016. The issue of kinked tail in Syrian Hamster, https://podrikhamstery.weebly.com/the-issue-of-kinked-tail-in-syrian-hamster.html, access: 8.10.2024.

Kispert A., Herrmann B.G. 1993. The *Brachyury* gene encodes a novel DNA binding protein. EMBO J. 12(8), 3211–3220. DOI: 10.1002/j.1460-2075.1993.tb05990.x.

Martinelli C., Spring J. 2005. T-box and homeobox genes from the ctenophore *Pleurobrachia pileus*: Comparison of *Brachyury, Tbx2/3* and *Tlx* in basal metazoans and bilaterians. FEBS Lett. 579(22), 5024–5028. DOI: 10.1016/j.febslet.2005.08.008.

Packham E.A., Brook D.J. 2003. *T-box* genes in human disorders. Hum. Mol. Genet. 12(1), R37–R44. DOI: 10.1093/hmg/ddg077.

Papaioannou V.E., Silver L.M. 1998. The *T-box* gene family. BioEssays 20(1), 9–19. DOI: 10.1002/(SICI)1521-1878(199801)20:1%3C9::AID-BIES4%3E3.0.CO;2-Q.

Pflugfelder G.O., Roth H., Poeck B., Kerscher S., Schwarz H., Jonschker B., Heinserberg M. 1992. The lethal *optomotor-blind* gene of drosophila melanogaster is a major organizer of optic lobe development: isolation and characterization of the gene. Proc. Natl. Acad. Sci. U S A. 89(4), 1199–1203. DOI: 10.1073/pnas.89.4.1199.

Räber H. 2001. Encyklopedia psów rasowych. T. 1. Warszawa: Multico [in Polish].

Ruvinsky I., Silver L.M., Ho R.K. 1998. Characterization of the zebrafish *tbx16* gene and evolution of the vertebrate *T-box* family. Dev. Genes Evol. 208, 94–99. DOI: 10.1007/s004270050158.

Scholz C.B., Technau U. 2003. The ancestral role of *brachyury*: Expression of *NemBra1* in the basal cnidarian *Nematostella vectensis* (Anthozoa). Dev. Genes Evol. 212(12), 563–570. DOI: 10.1007/s00427-002-0272-x.

Schulte-Merker S., Ho R.K., Herrmann B.G., Nüsslein-Volhard C. 1992. The protein product of the zebrafish homologue of the mouse *T* gene is expressed in nuclei of the germ ring and the notochord of the early embryo. Development 116(4), 1021–1032. DOI: 10.1242/dev.116.4.1021.

Sebé-Pedrós A., Ariza-Cosano A., Weirauch M.T., Leininger S., Yang A., Torruella G., Adamski M., Adamska M., Hughes T.R., Gómez-Skarmeta J.L., Ruiz-Trillo I. 2013. Early evolution of the *T-box* transcription factor family. Proc. Natl. Acad. Sci. U S A. 110(40), 16050–16055. DOI: 10.1073/pnas.1309748110.

Sebé-Pedrós A., de Mendoza A., Lang B.F., Degnan B.M., Ruiz-Trillo I. 2011. Unexpected repertoire of metazoan transcription factors in the unicellular holozoan *Capsaspora owczarzaki*. Mol. Biol. Evol. 2(3), 1241–1254. DOI: 10.1093/molbev/msq309.

Sebé-Pedrós A., Ruiz-Trillo I. 2017. Evolution and classification of the *T-box* transcription factor family. Curr. Top. Dev. Biol. 122, 1–26. DOI: 10.1016/bs.ctdb.2016.06.004.

Showell C., Binder O., Conlon F.L. 2004.*T-box* genes in early embryogenesis. Dev. Dyn. 229(1), 201–218. DOI: 10.1002/dvdy.10480.

Silva D.M., Miguel G.G., Souza M.L., Cleveland H.P., Ramos C.A. 2018. Malformation of the tail in Labrador Retriever dogs caused by mutation C189G in the *T* gene. Pesq. Vet. Bras. 38(12), 2237–2240. DOI: 10.1590/1678-5150-PVB-5721.

Smith J. 1999. *T-box* genes what they do and how they do it. Trends Genet. 15(4), 154–158. DOI: 10.1016/s0168-9525(99)01693-5.

Smith J.C., Price B.M., Green J.B., Weigel D., Herrmann B.G. 1991. Expression of a xenopus homolog of *Brachyury* (T) is an immediate-early response to mesoderm induction. Cell 67(1), 79–87. DOI: 10.1016/0092-8674(91)90573-h.

Wilm B., Dahl E., Peters H., Balling R., Ima K. 1998. Targeted disruption of *Pax1* defines its null phenotype and proves haploinsufficience. Proc. Natl. Acad. Sci. U S A. 95(15), 8692–8697. DOI: 10.1073/pnas.95.15.8692.

Wilson V., Rashbass P., Beddington R.S. 1993. Chimeric analysis of *T* (*brachyury*) gene function. Development 117(4), 1321–1331. DOI: 10.1242/dev.117.4.1321.

Xu X., Sun X., Hu X.S., Zhuang Y., Liu Y.C., Meng H., Miao L., Yu H., Luo S.J. 2016. Whole genome sequencing identifies a missence mutation in *HES7* associated with short tails Asian domestic cats. Sci. Rep. 6(1), 31583. DOI: 10.1038/srep31583.

FUZJA I DYSMORFOLOGIA KRĘGÓW OGONOWYCH U BIAŁYCH OWCZARKÓW SZWAJCARSKICH – STUDIUM PRZYPADKU

Streszczenie. *T-box* należy do bardzo dużej rodziny genów z motywem T. Koduje czynniki transkrypcyjne kontrolujące przebieg procesu embriogenezy. Pełni główną rolę w tworzeniu się mezodermy – warstwy, z której rozwijają się struktury osiowe organizmu. U psów mutacja tego genu powoduje wystąpienie krótkiego ogona u heterozygot, często z występującym załomkiem, natomiast w układzie homozygotycznym recesywnym jest letalny. Homozygoty dominujące mają normalny fenotypowo długi ogon. Celem badań była identyfikacja nosicieli zmutowanego allelu genu *T-box* i sprawdzenie, czy u białych owczarków szwajcarskich warunkuje on wystąpienie braku ogona i załomka ogona. Pobrano próbki włosów, krwi, tkanki miękkiej oraz nabłonka z wewnętrznej strony policzka od 23 osobników rasy biały owczarek szwajcarski. Dwadzieścia dwa osobniki fenotypowo miały długi ogon, jeden był bez ogona. Wyizolowano DNA, przeprowadzono amplifikację za pomocą łańcuchowej reakcji polimerazy (PCR), poddano rozdziałowi elektroforetycznemu w żelu agarozowym, a następnie pocięto otrzymany produkt enzymem restrykcyjnym *Eco91*I (*BstE*II), by móc zwizualizować próby w żelu poliakryloamidowym. Wszystkie objęte badaniami psy rasy biały owczarek szwajcarski, których DNA przeanalizowano pod kątem nosicielstwa mutacji 259C>G genu *T-box*, okazały się homozygotami recesywnymi o długim ogonie, co jest zgodne ze standardem tej rasy. Przyczyną braku ogona u jednego z badanych osobników musiała być inna mutacja genu *T-box* lub mutacja w innym genie.

Słowa kluczowe: gen *T-box*, załomek ogona, biały owczarek szwajcarski.