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MOLECULAR DIAGNOSIS OF C.619C>T MUTATION IN THE CLN5 GENE IN *CANIS LUPUS FAMILIARIS*

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Abstract: Neuronal ceroid lipofuscinoses (NCLs) are a group of rare genetic disorders that affect the nervous system, and molecular research aims to distinguish them from other diseases such as transmissible lysosomal storage diseases, mucopolysaccharidosis and gangliosidosis. *Canis lupus familiaris* is a suitable candidate model for researching NCL conditions owing to the strong similarity of its' genome to the human counterpart. The target of the study is to identify the c.619C>T mutation in the CLN5 gene among Amstaff, Husky, Romanian Shepherd, French Bulldog, and Terrier breeds.

• Introduction

Neuronal ceroid lipofuscinosis (NCL) refers to a group of conditions affecting the nervous system, both in humans and animals. Signs and symptoms vary greatly between forms, but generally include a combination of dementia, vision loss and epilepsy. The present study focused on the identification of the c.619C>T mutation in the CLN5 (intracellular trafficking protein) gene, in several canine breeds with various neurological signs.

• Material and method

The PCR-RFLP procedure was used to detect the mutation, while the restriction products were separated through agarose gel electrophoresis to determine the genotypes. A fragment of 322 bp from the CLN5 gene was amplified with specific primers (g. 30,435,673-g.30,435,994, [GCF_000002285.5](https://www.ncbi.nlm.nih.gov/nuccore/GCF_000002285.5)/NCBI) and the amplimers were digested with the *MseI* enzyme, whose restriction site includes the c.619C>T polymorphism.

• Results and discussions

The potential mutation in the CLN5 fragment is located 63 bp downstream of the 5' end (>NM_001011556), resulting in different fragments for individuals affected/unaffected by the mutation. The affected homozygous individuals/heterozygous carriers of the mutation have an electrophoretic profile with fragments of 196 bp and of 63/63 bp, while wild-type individuals have the restriction fragments of 196 bp and 126 bp. Although the analysed patients did not exhibit the mutation in the CLN5 gene, other mutations within the CLN genes family could not be excluded (Fig.1).

• Conclusions

This study tested for the presence of a specific mutation in the CLN5 gene, in patients of various canine breeds, some of which exhibited neurodegenerative clinical signs (Husky and French Bulldog). The study confirmed the functionality of the differential molecular diagnosis protocol, useful for the early detection of the CLN5 mutation, prior to the appearance of the first clinical signs of the disease.

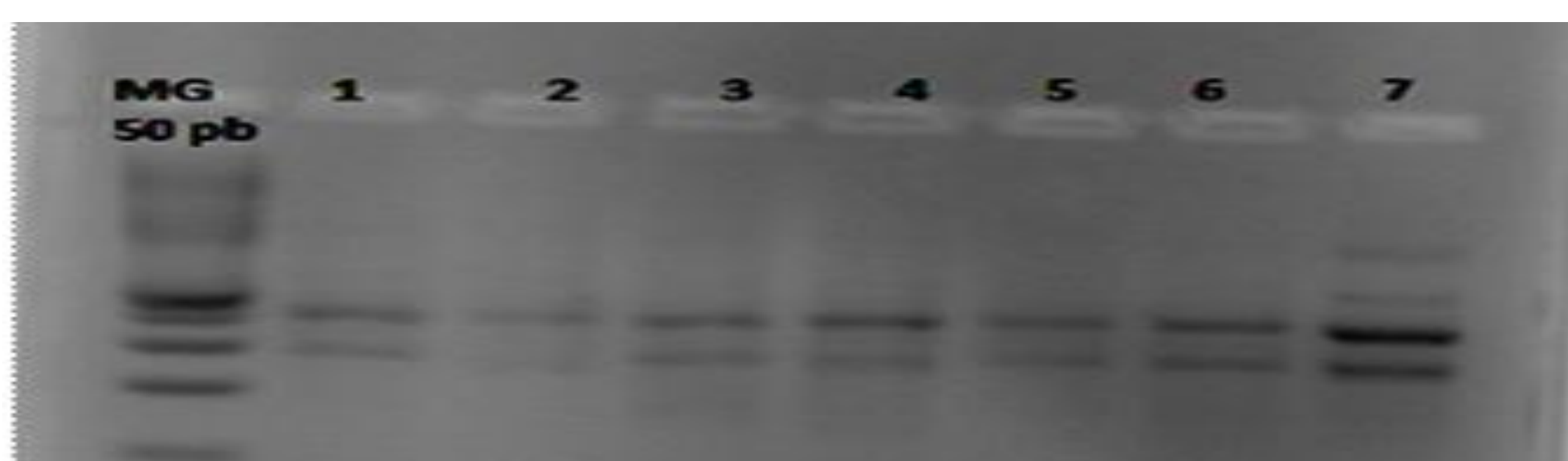


Fig. 1 The CLN5 electrophoretic profiles of canine patients. Line MM: 50 bp DNA step ladder (Promega, USA); **Lines 1-7:** profiles of 196 and 126 bp of wild-type individuals.