# Study of DNA Polymorphisms of the *CMAH* gene in Dogs and Cats

Abstract of Doctoral Thesis

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In the feline blood group system, N-glycolylneuraminic acid (Neu5Gc) and N-acetylneuraminic acid (Neu5Ac) are the type A and type B antigens, respectively, and the cytidine monophospho-N-acetylneuraminic acid hydroxylase (Cmah) enzyme is involved in the synthesis of Neu5Gc from Neu5Ac. Each type of antigen in this blood group system results from mutations in the *CMAH* gene that affect the production of Neu5Gc and Neu5Ac. A DNA screening scheme involving *CMAH* variants that can accurately determine blood types within the feline AB blood group system would be of great use and is highly desirable to complement phenotypic tests. Even though the *CMAH* gene has been well characterized in cats, not much is known about it in dogs. Furthermore, it was recently reported that canine and feline parvoviruses preferentially recognize Neu5Gc. Therefore, we characterized the dog *CMAH* gene for the first time. Additionally, we further studied the association between mutations and diplotypes of the cat *CMAH* gene and compared the results to those of the dog *CMAH* gene.

cDNA cloning showed that the dog *CMAH* gene contained a 1737-bp open reading frame that encodes a polypeptide of 578 amino acids; it was predicted to have high similarity to the *CMAH* gene of other mammals, including cats. The dog *CMAH* gene was expressed in many tissues, but not all tissues (n = 28); the cat *CMAH* gene has been reported to be expressed in almost all tissues. We also identified 15 single-nucleotide polymorphisms and an indel in *CMAH*. Focusing on c.554A>G (p.Lys185Arg), the G allele was widely distributed in western breeds (n = 229), although the Shiba dog was identified to be the most polymorphic at this locus among the breeds used in the study. To investigate the c.554A>G in dogs with the presence or absence of Neu5Ac expression, the phenotype of the binding of lectin to Neu5Ac (positive or negative) was determined in Shiba dogs and Labrador Retrievers. Thereafter, we genotyped the dogs at c.554A>G. However, the results did not clarify whether this mutation influences the expression of Neu5Ac.

We also investigated mutations and diplotypes of the cat *CMAH* gene in type B (n = 21) and AB cats (n = 6), and compared the results to those of a previous study (Omi et al., 2016). Results showed that the diplotype distribution in type B cats was not so discordant to that of the previous report, although some novel diplotypes were found. However, in type AB cats, some of the genotypes were discordant to those of the previous report as some showed diplotypes that were reported to have at least one intact allele (A type). Therefore, we need to analyze other exons in which other variants might be discovered.

Compared to the cat *CMAH* gene, the results on the dog *CMAH* gene suggest that variants with an amino acid substitution at the *CMAH* locus are not associated with the expression of Neu5Ac or Neu5Gc. Analysis of the regulation mechanism of mRNA expression may enable the cause of this difference to be identified.