別記様式第５号(第６条、第 12 条関係)

学 位 論 文 要 旨

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題　　目： Molecular epidemiologic study on canine neuronal ceroid lipofuscinosis and GM1 gangliosidosis
（犬の神経セロイドリポフスチン症およびGM1ガングリオシドーシスの分子疫学研究）

論文要旨：

Lysosomal storage diseases are a group of rare, genetic disorders of cellular catabolism. Most of them are inherited as autosomal recessive traits and result from mutations in the coding sequence of one of the acid hydrolases or their activators located in the lysosome. The genetic abnormality results in the reduction or elimination of the catalytic activity of the particular enzymatic reaction, and the reduction in catabolism results in the accumulation of the substrate of that enzymatic reaction within the lysosome. Neurons are affected in most of these diseases because they are postmitotic, permanent cell populations. Thus, many lysosomal storage diseases are manifested as progressive, neurological and eventually lethal disorders. Neuronal ceroid lipofuscinosis (NCL) and GM1 gangliosidosis are also lysosomal storage diseases that occur in several purebred dogs and are problems in producing healthy dogs. In this study, NCL in Chihuahua dogs (Chapter 1) and GM1 gangliosidosis in miniature type of Shiba Inus called Mame Shibas (Chapter 2) were investigated in order to analyze carrier rates and mutant allele frequencies, and to evaluate the necessity of control and prevention.

Chapter 1: NCL is a group of rare lethal neurodegenerative lysosomal storage diseases that occur in a range of dog breeds, including Chihuahuas. Recently, a homozygous single base-pair deletion (c.846delT), which causes a frame shift generating a premature stop codon (p.F282Lfs*13) in the canine *CLN7/MFSD8* gene, has been identified as a causative mutation for NCL in Chihuahuas. The objective of this study was to determine the frequency of the mutant allele and/or carrier rate of NCL in Chihuahuas in Japan using a newly designed real-time PCR assay. Samples of saliva were randomly collected from 1007 Chihuahua puppies during physical examinations prior to the transportation to pet shops. Screening results revealed a carrier rate of 1.29%, indicating a mutant allele frequency (0.00645) that is considered sufficiently high to warrant measures for the control and

prevention of this lethal disease. The genotyping assay designed in this study could make a valuable contribution to the control and prevention of NCL.

Chapter 2: GM1 gangliosidosis is a progressive, recessive, autosomal, neurodegenerative, lysosomal storage disorder that affects the brain and multiple systemic organs due to an acid galactosidase deficiency encoded by the *GLB1* gene. This disease occurs in the Shiba Inu breed, which is one of the most popular traditional breeds in Japan, due to the *GLB1*:c.1649delC (p.P550Rfs*50) mutation. Previous surveys performed of the Shiba Inu population in Japan found a carrier rate of 1.02–2.94%. Currently, a miniature type of the Shiba Inu called “Mame Shiba”, bred via artificial selection to yield smaller individuals, is becoming more popular than the standard Shiba Inu and it is now one of the most popular breeds in Japan and China. The GM1 gangliosidosis mutation has yet to be surveyed in the Mame Shiba population. This study aimed to determine the frequency of the mutant allele and carrier rate of GM1 gangliosidosis in the Mame Shiba breed. Blood samples were collected from 1832 clinically healthy adult Mame Shiba Inus used for breeding across 143 Japanese kennels. The genotyping was performed using a real-time PCR assay. The survey found nine carriers among the Mame Shibas, indicating that the carrier rate and mutant allele frequency were 0.49% and 0.00246, respectively. This study demonstrated that the mutant allele has already been inherited by the Mame Shiba population. There is a risk of GM1 gangliosidosis occurrence in the Mame Shiba breed if breeders use carriers for mating. Further genotyping surveys are necessary for breeding Mame Shibas to prevent the inheritance of this disease.

In conclusion, on the basis of the results obtained in this study (Chapter 1), it is established that in Japan, the carrier rate of NCL in Chihuahuas is currently 1.29%, and given the lethality of this disease, the corresponding mutant allele frequency (0.00645) is deemed sufficiently high to warrant measures for disease control and prevention. Ideally, in this regard, it is considered important to conduct extensive molecular screening of breeding Chihuahuas in related kennels throughout Japan. To this end, it is believed that the genotyping assay designed in this study will make a potentially valuable contribution to the control and prevention of NCL in Chihuahuas. Furthermore, the results of this study (Chapter 2) show that the carrier rate of GM1 gangliosidosis in the Mame Shiba in Japan is currently 0.49%, and, given the lethality of this disease and popularity of this breed, the corresponding mutant allele frequency (0.00246) is deemed sufficiently high to warrant measures for disease control and prevention. Ideally, continued genotype surveying should be performed on breeding Mame Shibas reared by breeders who undertake appropriate mating management.

（和文2, 000字又は英文800語程度）