別記様式第５号(第６条、第 12 条関係)

学 位 論 文 要 旨

氏名 Tofazzal Md Rakib（トファザル・モハメド・ラキブ）

題　　目： Study on the molecular bases of feline Pompe disease and Niemann-Pick disease type C
（猫のポンペ病およびニーマン・ピック病C型の分子基盤に関する研究）

論文要旨：

Lysosomal storage diseases refer to a collection of uncommon, inherited genetic disorders that affect cellular catabolism that usually arise from autosomal recessive inheritance and are caused by mutations or alterations in the cording sequence of acid hydrolases, or their activators located in the lysosome. These genetic defects lead to a decrease or complete loss of catalytic activity of the particular enzymatic reactions, causing an accumulation of the substrates of those reactions within the lysosome. Therefore, many lysosomal storage diseases present as progressively worsening, neurological conditions that can ultimately be fatal. Pompe disease (PD) and Niemann-Pick (NP) disease are rare genetic disorders found in cats that resemble their human counterparts. The elucidation of the genetic defects responsible for these diseases can aid in understanding their pathophysiology, development of new treatments and therapies, and potential benefits for human medicine.

Chapter 1: PD is an autosomal recessively inherited fatal genetic disorder that results from the deficiency of a glycogen hydrolyzing enzyme, acid α-glucosidase encoded by the *GAA* gene. Here, we describe the molecular basis of genetic defects in an 8-month-old domestic short-haired (DSH) cat with PD. The cat was previously diagnosed with PD based on the clinical and pathological findings of hypertrophic cardiomyopathy and excessive accumulation of glycogen in the cardiac muscles. Sanger sequencing was performed on 20 exons of the feline *GAA* gene using genomic DNA extracted from paraffin-embedded liver tissues. The affected cat was found to be homozygous for the *GAA*:c.1799G>A mutation resulting in an amino acid substitution (p.R600H) of acid α-glucosidase, a codon position of which is identical with three missense mutations (p.R600C, p.R600L, and p.R600H) causing human infantile-onset PD (IOPD). Several stability and pathogenicity predictors have also shown that the feline mutation is deleterious and severely decreases the stability of the GAA protein. The clinical, pathological, and molecular findings in the cat were similar to those of IOPD in humans. To our knowledge, this is the first report of a pathogenic mutation in a cat. Feline PD is an excellent

model for human PD, especially IOPD.

Chapter 2: NP disease type C (NPC) is an autosomal, recessive, and inherited neurovisceral genetic disorder characterized by the accumulation of unesterified cholesterol and glycolipids in cellular lysosomes and late endosomes, with a wide spectrum of clinical phenotypes. This study aimed to determine the molecular genetic alterations in two cases of felines with NP in Japan, a Siamese cat in 1989 and a Japanese domestic (JD) cat in 1998. Sanger sequencing was performed on 25 exons of the feline *NPC1* gene and 4 exons of the feline *NPC2* gene, using genomic DNA extracted from paraffin-embedded tissue specimens. The sequenced exons were compared with reference sequences retrieved from the GenBank database. The identified mutations and alterations were then analyzed using different prediction algorithms. No pathogenic mutations were found in feline *NPC1*; however, c.376G>A (p.V126M) was identified as a pathogenic mutation in the *NPC2* gene. The Siamese cat was found to be homozygous for this mutation. The JD cat was heterozygous for the same mutation, but no other exonic *NPC2* mutation was found. Furthermore, the JD cat had a homozygous splice variant (c.364-4C>T) in the *NPC2* gene, which is not known to be associated with this disease. The *NPC2*:c.376G>A (p.V126M) mutation is the second reported pathogenic mutation in the feline *NPC2* gene that may be present in the Japanese cat population.

In conclusion, the articles discussed the molecular basis of two different genetic disorders in cats. The first study that focused on PD identified a pathogenic mutation (*GAA*:c.1799G>A, p.R600H) in the feline *GAA* gene of a DSH cat with PD. This is the first report of a cat with PD carrying the same mutation as reported in a case of human classical IOPD. The clinical and histological findings in this cat with PD were similar to those in humans with IOPD. Therefore, this feline PD is an excellent model of human PD, especially IOPD. The second study that focused on NPC identified a pathogenic mutation (*NPC2*:c.376G>A (p.V126M)) in the feline *NPC2* gene of a Siamese cat and a JD cat with NP. The Siamese and JD cats were homozygous and heterozygous for this mutation, respectively. No other exonic *NPC2* mutation or deleterious splice variant was found in the JD cat, suggesting that this cat was a compound heterozygote of the identified *NPC2*:c.376G>A mutation and another pathogenic mutation that may be located in intronic regions. The c.376G>A (p.V126M) mutation is the second reported pathogenic mutation in the feline *NPC2* gene that causes NPC, and it may be present in the Japanese cat population. This feline models of PD and NPC may contribute to the development of new therapeutic strategies for treating those human diseases.

(和文2, 000字又は英文800語程度)