ABSTRACT

GM2 gangliosidosis variant 0 (Sandhoff disease, SD; OMIM#268800) is a fatal, autosomal recessive, lysosomal storage disorder resulting from excessive accumulation of the GM2 ganglioside in the neurons. The disease is caused by deleterious mutations of the *HEXB* gene encoding the β-subunit of lysosomal β-*N*-acetylhexosaminidase (Hex, EC 3.2.1.52) and affected individuals die prematurely of brain damage through progressive neurological signs. The objectives of my thesis are clarification of molecular bases of the disease, development of new molecular diagnostic methods and finally an epidemiological survey in the respective populations of the disease aiming to prevent and/or control the disease in dogs and cats.

In order to clarify the molecular basis of the disease, I collected tissue or blood samples of Toy Poodles, a Japanese mixed-breed dog and Japanese domestic cats that had already been reported and suspected as SD affected. Genomic and complementary DNA sequences covering exonic regions of the canine and feline *HEXB* gene were analysed using DNA and RNA in the affected animals. In a feline case suspected with SD, biochemical and pathological investigations were also done to definitively diagnose SD in the cat. In addition, several genotyping methods for canine and feline SD were developed using theories of length polymorphism-based polymerase chain reaction (PCR) coupled with a microchip electrophoresis, PCR-primer introduced restriction analysis, mutagenically separated-PCR and real-time PCR with TaqMan minor groove binder probes. These genotyping assays were evaluated and used for pedigree analysis and/or epidemiological survey of a previously identified mutation (c.667C>T) in Japanese domestic cats with SD and 3 novel mutations in canine and feline SD identified in the present study.

A novel homozygous single base pair deletion of guanine (c.283delG) was identified in exon 3 in the canine *HEXB* gene in Toy Poodles with SD. This mutation has the potential to

cause a frameshift resulting in the alteration of valine to a stop codon (p.V95fsX). In a mixed-breed dog with SD, a novel 20-base pair deletion (c.791_810del20) was identified in exon 8 in the *HEXB* gene. This mutation also has the potential to cause a frameshift resulting in a premature termination with a stop codon (p.I264fsX12). The mRNA could not be detected by reverse transcription (RT)-PCR in the affected mixed breed dog. Therefore, the degradation of the mutant transcript is the most probable to occur through nonsense-mediated mRNA decay. Feline SD was newly diagnosed with clinical, biochemical and pathological features in a Japanese domestic cat. The feline *HEXB* gene analysis suggested a novel 4-base pair deletion (c.996-23T[7]) at the polypyrimidine tract in intron 9 may cause skipping of exon 10, which is a possible cause of a frameshift creating a premature stop codon (p.K332fsX29). The exon skipping was confirmed by RT-PCR in the cat with SD.

All assays developed for genotyping the 4 kinds of mutations were available to discriminate all 3 genotypes, i.e., wild-type, carrier and affected animals. Of all assays, real-time PCR methods were the most suitable for large-scale examination because of its high throughput. The genotyping survey among 497 Toy Poodles demonstrated that carrier frequency is 0.20% in Japan. This finding suggested that the mutant allele frequency of SD in Toy Poodles is not so high in Japan, but measures to prevent and control the disease would be warranted because Toy Poodles is one of the most popular breed in Japan and Western countries. The genotyping surveys for feline SD among approximately 1,800 mixed-breed cats detected 1 carrier of c.667C>T and no carrier of c.996-23T[7] suggesting that the allele frequencies are extremely low in southern Japan. Therefore, genotyping assays for feline SD can be used for only diagnosis and genotyping of affected cats and their blood-related cats, not for prevention of the disease.

In conclusion, the molecular pathogenesis and epidemiological information clarified in

the present study will contribute to prevent and/or control canine and feline SD. Furthermore, these molecularly-defined animals are the appropriate models of human SD to test therapeutic approaches to cure both human and animal patients in the near future.