Molecular Pathological Evaluation of Familial Spontaneous Epileptic Cats

Summary of Doctoral Thesis

Yoshihiko Yu

Graduate School of Veterinary Medicine and Life Science

Nippon Veterinary and Life Science University

Epilepsy is one of the common neurological disorders characterized by recurrent epileptic seizures in dogs, cats and human, and its cause of disease occurrence involves genetic factor. Temporal lobe epilepsy (TLE) is the common form of epilepsy in human, and it includes lateral temporal lobe epilepsy (LTE) and mesial temporal lobe epilepsy (MTLE). It is known that both of them exist as familial forms. The causative variants of familial LTE (FLTE) are identified. While several loci are suggested in familial MTLE (FMTLE), however, none of the causative variants of FMTLE has identified.

Familial spontaneous epileptic cats (FSEC) is the unique feline strain with the familial accumulation of epilepsy and established as a colony by breeding epileptic cats selectively. FSEC included cats with spontaneous focal limbic +/- generalized seizures, cats with vestibular stimulation-induced generalized seizures, cats with both types of seizures, cats without seizures but with abnormal electroencephalography finding. Spontaneous seizure seen in FSEC does not only represent common seizure form in cats but also is similar to seizure form seen in human MTLE and feline limbic kindling/kainic acid model. Vestibular stimulation-induced seizure seen in FSEC resembles seizure form of EL mouse which is a mouse model of genetic epilepsy. Previous studies using electrpencephalography and magnetic resonance imaging suggested that FSEC is an animal model of FMTLE.

In this study, the purpose is to elucidate the genetic architecture in FSEC, and several studies including neuropathological evaluation in the hippocampus and the amygdala (Chapter 2), molecular cloning and mutational analysis in suggested candidate genes in human and animal model literatures (Chapter 3), detection of epilepsy associated loci using genome-wide association study (GWAS) (Chapter 4),

detection of loci using genome-wide linkage analysis (Chapter 5), and detection of unique variants to FSEC using whole genome sequencing (WGS) (Chapter 6).

Chapter 2: Neuropathological evaluation in the hippocampus and the amygdala in familial spontaneous epileptic cats

In this chapter, to characterize the pathological feature in FSEC, pathological evaluation was conducted in the hippocampus and the amygdala which were considered as the seizure onset zone of FSEC. FSECs showed neuronal decrease without gliosis in the hippocampal CA3 region and the amygdaloid central nuclei when compared with controls. FSECs with seizure history also showed gliosis without neuronal loss in the hippocampal CA4. Hippocampal sclerosis (HS) that is defined by neuronal loss and concurrent gliosis is well-known in human MTLE patients. In FSEC, the similar finding to the typical HS in human was not found. However, the unique findings such as the neuronal decrease in the hippocampus and the amygdala were seen in FSEC, and the genetic background may be involved in these findings somehow. In addition, gliosis without neuronal loss in the hippocampal CA4 in FSEC is also seen in human MTLE, which suggested the partial similarity between human MTLE and FSEC.

Chapter 3: Molecular cloning and mutational analysis of leucine-rich glioma-inactivated (LGI) gene family in familial spontaneous epileptic cats

In this chapter, candidate gene analysis was performed to evaluate whether candidate genes suggested by human and animal model literatures are associated with epilepsy occurrence in FSEC. Leucine-rich glioma-inactivated (LGI) protein family (LGI1–4) play role in development and function of the nervous system in the vertebrate such as synaptic transmission and myelination. Especially, *LGI1* is reported as the

causal gene of FLTE in human. Similarly, other LGI family genes and proteins are reported to be associated with epilepsy occurrence in human, dogs, and cats. Therefore, molecular cloning and mutational analysis in the LGI gene family in this chapter. Several nonsynonymous variants were found but there was discordance between genotype and phenotype. In this analysis, it was suggested that epilepsy in FSEC is not associated with a single mutation in the coding region of LGI gene family.

Chapter 4: Genome-wide association study in familial spontaneous epileptic cats

In this chapter, feline single nucleotide polymorphism (SNP) array was used to obtain SNP genotype data, and then GWAS was performed. Transmission disequilibrium test (TDT) and case control analysis was conducted in GWAS. Assuming that all the phenotypes were caused by the common variant(s), or each type of seizures (phenotype) was caused by (a) different variant(s), analyses included three patterns in total. TDT showed the highest association on chromosome B3 (136.1 Mb) when all the phenotypes are assumed to be caused by the same variant(s), on chromosome D4 (42.4 Mb) when only spontaneous seizure trait was analyzed, and on chromosome B3 (35.6 Mb) when only vestibular stimulation-induced seizure trait was analyzed. However, none of them obtained genome-wide significance. Case control analysis showed the highest association on chromosome C1 (48.3 Mb), chromosome A2 (133.5 Mb), and chromosome A2 (122.6 Mb), respectively.

Chapter 5: Genome-wide linkage analysis in familial spontaneous epileptic cats

In this chapter, genome-wide linkage analysis was performed using genotype data obtained in Chapter 4. Assuming that all the phenotypes were caused by the common variant(s), or each type of seizures (phenotype) was caused by (a) different variant(s),

analyses were conducted in different patterns in the same way described in Chapter 4. No genome-wide significant linkage was obtained. However, suggestive linkage was detected on chromosome B4 (79.6 Mb), C1 (78.1 Mb), C2 (9.1 Mb), and D2 (7.2 Mb) when analyzed in all trait. When analyzed in the spontaneous seizure trait, suggestive linkage was obtained on chromosome B1 (205.1-2 Mb), B2 (36.0, 108.4, 118.2-3 Mb), B3 (143.5-8 Mb, 146.0-148.3 Mb), D1 (5.1 Mb), D2 (5.6 Mb), D4 (23.8, 38.7, 39.0, 42.3 Mb), E2 (21.0 Mb), F2 (73.5-73.7 Mb), X (3.5-6 Mb, 3.9 Mb). When analyzed in the vestibular stimulation-induced seizure trait, suggestive linkage was detected on chromosome B1 (203.8 Mb), B3 (35.6, 65.4 Mb), B4 (74.3 Mb), C2 (9.5 Mb), D1 (109.3 Mb).

Chapter 6: Whole genome sequencing in familial spontaneous epileptic cats

In this chapter, total four FSECs; one cat with both seizure types, one cat with spontaneous seizures, and two cats with vestibular stimulation-induced seizures; were selected and WGS was performed on these four FSECs. WGS analysis was performed as a part of 99 Lives Cat Genome Sequencing Initiative that is a consortium of feline WGS projects. WGS data from 191 cats were compared with four FSECs' WGS data. Unique variants were detected from WGS data in the suggested region in Chapter 4 and 5. In this chapter, WGS data was generated from four FSECs, and no nonsynonymous variants that were common in the four FSECs and located within the suggested region by GWAS or linkage analysis were found, and this suggests the complexity of genetic architecture in FSEC. However, when analyzed in each trait, in the suggested regions by GWAS or linkage analysis, variants that showed concordance between phenotype and genotype were found, and there are genes that are considered to be associated with epilepsy. Most of those variants were intronic variants. It was considered that many

variants with small effect, rather than the single variant with large effect, may be contributed to epileptogenesis in FSEC.

In this study, the comprehensive genetic analysis was performed to elucidate the genetic architecture of FSEC that is characterized by spontaneous epilepsy with familial accumulation. Multiple loci detected by GWAS and linkage analysis in this study suggest that the genetic cause of epileptogenesis in FSEC is not simple or single but multiple. In addition, feline SNP array, that was used in this study, is low-density SNP array. It will be possible to perform GWAS and linkage analysis more precisely when a feline high-density SNP array will be available in the future. Identification of causal or susceptibility gene(s) of epilepsy would not only bring new insights about the biological pathway of epileptogenesis but also suggest the new treatment strategies including appropriate choice of drugs and drug repositioning. The comprehensive genetic analysis of FSEC may contribute to the understanding of genetic architecture in feline epilepsy and eventually in human epilepsy including FMTLE. Furthermore, it would be expected to elucidate the genetic architecture of FSEC more precisely along with the progress of genomic analyses in cats.