# GRANT PROGRESS REPORT SUMMARY

**Grant:** 01425: *Identification of Epilepsy-Causing Mutations from the Associated Loci by Next-Generation Resequencing*

**Principal Investigator:** Dr. Hannes T Lohi, PhD

**Research Institution:** University of Helsinki and the Folkhälsan Institute of Genetics

**Grant Amount:** $86,400.00

**Start Date:** 1/1/2011 **End Date:** 6/30/2012

**Progress Report:** Mid-year 2

**Report Due:** 6/30/2012 **Report Received:** 10/10/2012

## Recommended for Approval:

*(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)*

## Original Project Description:

Epilepsy is the most common neurological disease in dogs and affects almost all breeds. Epilepsy runs often in families suggesting that disease risk is conferred by genes. To date, only a few recessive genes have been found in dogs in association with progressive myoclonus epilepsies but the genetic background of common idiopathic epilepsy (IE) remains unknown. We have embarked a large genetic study of epilepsy and have identified several new disease loci in many breeds including Belgian Shepherds, Kromfohrländers and Border Terriers. The aim of this project is to "zoom in" to the recently associated regions in these three breeds to identify the epilepsy causing mutations. We aim to use the latest deep sequencing technologies to scan the candidate genes and the entire regions for causative variants. Identification of the mutations will enable us to develop genetic tests for breeds and it will improve the understanding of the pathogenesis of the disease for better diagnosis and treatments. The associated regions in the three breeds under study are new IE loci and can be tested in other breeds and human epilepsy cohorts.

## Grant Objectives:

Objective 1: Identification of the Idiopathic Epilepsy mutation in Belgian Shepherds.

Objective 2: Fine-mapping and sequencing the Idiopathic Epilepsy locus in Kromfohrländers.

Objective 3: Fine-mapping and sequencing the Idiopathic Epilepsy locus in Border Terriers.

Objective 4: Testing the identified variants in other breeds with Idiopathic Epilepsy.

**Publications:**

- Lequarré AS, Andersson L, André C, Fredholm M, Hitte C, Leeb T, Lohi H, Lindblad-Toh K, Georges M. LUPA: A European initiative taking advantage of the canine genome architecture for unravelling complex disorders in both human and dogs. Vet J. 189(2):155-9, 2011.

- E. H. Seppälä, L. Koskinen, C. H. Gulløv, P. Jokinen, P. Karlskov-Mortensen, L. Bergamasco, I. Baranowska, S. Cizinauskas, A. Oberbauer, M. Berendt, M. Fredholm and H. Lohi. Identification of an idiopathic epilepsy locus in dogs.

**Report to Grant Sponsor from Investigator:**

The aim of this study was to use the latest sequencing technologies to capture the mutations in previously identified epilepsy loci in three different breeds, Belgian Shepherds, Kromfohrländers and Border Terriers. We have performed several experiments and resequenced the identified regions in Belgian Shepherd and Border Terrier breeds. We have found thousands of variants in each sample across the regions and are in a process of organizing them according to their types and functionality, e.g. coding variants, SNPs, indels, location at regulatory elements. This analysis is followed by comparison between cases and controls to identify those variants that appear only in the epileptic dogs. The most promising variants are validated in our larger cohorts to confirm their causality.

In our preliminary analysis, we found nine coding variants in epileptic Belgian Shepherds but none of them has validated as causative so far. Due to technical issues in the array-based capture experiment, the data quality was lower than expected, and we are performing a new capture and
resequencing experiment with more samples and improved technology to make sure we have the best possible data in the analysis.

The quality of our Border Terrier sequencing data of eight dogs is high and involves two chromosomal regions. We have found thousands of variants in each sample are in a process of validating the most promising candidate mutations in a larger sample cohort. Meanwhile, we have also genotyped a new cohort of epileptic and control dogs from UK and identified a third candidate locus at CFA24. This locus will be confirmed and further investigated in additional samples.

Our plan with Kromfohrländers included replication of the locus at CFA28 before its sequencing for mutation. The breed is small and sample recruiting has been slower and

prevented us from the replication experiment with a case-control approach. However, we have samples from dogs closely related with each other, and we performed the first replication attempt by using relatives to the dogs that were included in our initial genome scan, but this approach did not confirm our findings as the association remained modest. We also sequenced a candidate gene previously found in human in the region from affected dogs, but did not identify causative variants. Meanwhile we try to encourage the owners and breeders to participate actively to increase the size of our study cohort.

Besides the breeds included in our original application, we have made progress also in two other breeds with idiopathic epilepsy including Schipperke and Norwich Terrier. We have mapped putative loci also in these breeds and have performed similar capture sequencing experiments to identify the mutations. Finally, we have been able to collect hundreds of new cases in many breeds and if any mutations are found all breeds can be screened for them. Overall, we have progressed mostly as expected but need still experiments to identify the actual causative variants in the target breeds.