

Cat Health Network Feline SNP Chip Studies
Lay Language Progress Report Updates
March 29, 2012

D12FE-503, Gerhard Wess, University of Berne

“Genetic Analysis of Hypertrophic Cardiomyopathy (HCM) in Maine Coon Cats”

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

Hypothesis: HCM in Maine Coon cats is not or at least not only caused by the previously published mutations

Objectives:

- (1) Mapping of HCM mutation(s) by genome-wide association studies (GWAS)
- (2) Mutation analysis of positional candidate genes
- (3) Collection of further samples

We would like to find the causative mutation(s) for HCM in Maine Coon cats. Ultimately, this should lead to the development of one or more genetic tests that will allow the reduction of this disease by breeding measures. Until now, we have isolated DNA from 35 affected cats and 49 control cats and sent it for genotyping on the feline SNP chips to Neogen/GeneSeek. All samples were purebred Maine Coon cats of European origin. We received the data and performed a preliminary genome-wide association study (GWAS). Surprisingly, this analysis did not yet yield an unequivocal association signal. On the one hand, this is clearly disappointing to us and means that our research project is not as straightforward as we had hoped for. On the other hand, our results also clearly show that the published HCM variants in the MYBPC3 gene are not responsible for a large fraction of the HCM cases seen in European Maine Coon cats. Thus, there clearly is an urgent need for further research in Maine Coon HCM.

We will genotype 60 additional Maine Coon cats in the course of this project. The increased cohort size will improve the power of our GWAS. Thus, we will have better chances of success at the end of the planned project.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

Cat, Maine Coon

It is currently not known whether the specific form(s) of HCM, which we are investigating, exist in other breeds as well. If so, then any results could be applied to those breeds as well.

C. How will your project help owners/stakeholders?

If our project is successful, a genetic test can be developed, which will allow the reduction of this disease.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

If our project is successful, a genetic test can be developed, which will probably facilitate an earlier and more accurate diagnosis of HCM.

E. Note any problems you've encountered and solutions to those problems.

So far, we don't see a clear association signal. We have plans to increase our sample size, which should help to overcome this problem.

D12FE-504, Tosso Leeb, Be, University of Berne

“Genetic Analysis of Polycystic Kidney Disease (PKD) in Maine Coon Cats”

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

Hypothesis: PKD in Maine Coon cats is not caused by the known PKD1 mutation found in Persian cats and is inherited as a monogenic autosomal recessive trait.

Objectives:

- (1) Mapping of the mutation by GWAS and homozygosity mapping
- (2) Mutation analysis of positional candidate genes
- (3) Collection of further samples

We would like to find the causative mutation for an inherited form of polycystic kidney disease (PKD) in Maine Coon cats. This form of PKD is different from the well-known PKD of Persian cats. Ultimately, this should lead to the development of a genetic test that will allow the eradication of this disease.

Until now, we have isolated DNA from 6 affected cats and 6 control cats and sent it for genotyping on the feline SNP chips to Neogen/GeneSeek. We received the data and performed a preliminary genome-wide association study (GWAS) and a homozygosity analysis. Unfortunately, this analysis did not yet yield an unequivocal association signal. This is not all that surprising as we genotyped only a very small number of cats.

We will genotype additional Maine Coon cats in the course of our related project on HCM (D12FE-503). This will allow us to also include additional cats with PKD phenotypes. Thus, we will have much better chances of success at the end of the planned project.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

Cat, Maine Coon

It is currently not known whether the specific form of PKD, which we are investigating, exists in other breeds as well. If so, then any results could be applied to those breeds as well.

C. How will your project help owners/stakeholders?

If our project is successful, a genetic test can be developed, which will allow the eradication of this disease.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

If our project is successful, a genetic test can be developed, which will probably facilitate the accurate diagnosis of this specific form of PKD.

E. Note any problems you've encountered and solutions to those problems.

So far, we don't see a clear association signal. We have plans to increase our sample size, which should help to overcome this problem.

D12FE-505, Leslie A. Lyons, Ph.D., University of California/Davis

“Genetic Estimation of Introgression Between Domestic Cat and Wildcat Populations”

The progenitor of the domestic cat, the wildcat, lives in the same areas as the domestic cats throughout Europe, the Mediterranean, and North Africa. Several subspecies of wildcats occupy these areas, the African wildcat (*Felis silvestris libyca*) is the subspecies considered to be most influential in cat domestication. However, because of the extensive habitat overlaps, populations of European wildcat (*Felis silvestris silvestris*) are breeding with domestic cats, causing hybridization between the populations. Conservation programs throughout Europe are attempting to preserve true wildcat populations; however, identifying “pure” wildcat individuals can only be accomplished by genetics. Body size and coat colors have wide variation between the wildcats and domestic cats, thus, cannot always be used to delineate a particular individual of wildcat from domestic cat. Several studies have used different types of genetics markers to decipher the amount of breeding between the species. Conservation programs are attempting to find the most diagnostic markers that are within the funding available to help develop population management programs for the wildcats. The new Illumina Infinium Cat DNA array was designed with ~5,000 SNPs for the analysis of wildcat populations. Thus, data from the array could provide the most accurate measure for wildcat and domestic cat hybridization. The hypothesis of this study is that wildcat and domestic cat hybridization can be more accurately estimated by extensive DNA marker comparisons. The long-range goal of this study is to identify pure wildcat population for conservation within Europe. The rationale for the project is that wildcat populations will become less able to survive in the wild if domestic cat diseases, included inherited and acquired, are transmitted to wildcat populations, thus, the most vulnerable populations should be protected from further domestic cat mixing. A secondary goal of the research is to identify regions of the genome that may be involved with the domestication process of the cat. The project could identify genes that are contributing to boldness and tameness that are influencing cat domestication. Genetic tests could feasibly be developed that would help select the more tame and social cats that would be more suitable for adoption and nursing care interactions. The investigators already have the required samples available and have collaborated on similar studies in dogs. The data analysis would be feasible within the year. Comparisons to the previous DNA marker database will help determine the accuracy of each method for the detection of hybrids. The work can help identify the most threaten wildcat populations and support the development of the population management plans.

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

The hypothesis of this proposal is introgression and hybridization of wildcat populations with domestic cats can be more accurately estimated by genotyping the ~5,000 wildcat specific SNPs in the feral and wildcat populations. The objective of this study is to determine the most efficient markers for estimating introgression between the domestic and wildcat populations and to identify pure and hybridized individual wildcats and their populations throughout Europe. The long-range goal of this study is to assist the development of species survival and population management plans for the wildcats of Europe. Sample selection and preparations are underway. A secondary goal is to further refine the domestication events that have led to the diversified cat populations between Western Europe and Southern Asia. Additionally, haplotype analyses may indicate regions of selective sweeps between domestic and wildcats, indicating regions of the genome that may be important in cat domestication and taming. This project has not yet been initiated. The wildcat samples are in the laboratory and are being selected for analysis. An Italian student from the collaborators has been awarded a VISA and will be joining the Lyons laboratory in May, 2012 for the implementation of the project.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

Wildcats and domestic cats. The data will be specific to cats, but theories and approaches could be applied to other species.

C. How will your project help owners/stakeholders?

The goal will be to use the information to identify hybrid cats and develop conservation programs for the pure wildcats within specific regions of Europe.

D12FE-506, Leslie A. Lyons, Ph.D., University of California/Davis
“Genome-wide Association Studies of Brachycephaly in Domestic Cats”

Cat and dog breeding has become the focus of significant scrutiny with the realization that humans are purposely selecting and creating certain breeds and extremes of conformation that can have inherent health problems. In 2008, the BBC aired a documentary entitled “Pedigreed Dogs Exposed” that highlighted a variety of genetic problems in dog breeds that compromise an individual animal’s health. The British Bulldog has been bred to have such a deep chest that natural mating is physically impossible for the breed. Cat breeders also have received scrutiny as some cat breeds are associated with genetic mutations and heritable factors that also can compromise health. The tailless mutation of the Manx cat breed affects spine and tail development, the breed being purposely selected to have no tail. If the tail is too short, vertebrae of the lower back are missing, leading to poor development of nerves, thus cats can be lame and not be able to control their bladder or bowels. Kittens with two copies of the tailless mutation die in the womb. The Scottish Fold cat breed has an inherited mutation that causes the ears to fold, but also can cause a joint condition similar to osteoarthritis. Cats with two copies of the mutation can have very severe problems and be very painful with a crippling lameness. These two breeds are not amongst the most popular of cat breeds and some cat breed associations ban their registration. However, a very short face is common to many breeds, including one of the most popular breeds, the Persian. The short face is a highly desired trait as it makes the cats have big round faces with big eyes, like a baby (the Lorenzian theory of beauty). Short faces likely cause significantly more health problems than the ear fold and tailless mutations combined. The secondary health effects of the extreme shortening of the facial bones leads to constant tearing, nasal discharge, eye problems, chewing problems and eye issues. Sometimes cats have surgery to increase the size of the nostrils. The cats often require daily management. Some newly developing breeds use Persians as crosses to obtain the style. Breeders of even some historical breeds, such as the British Shorthair and American Shorthair, are also crossing with Persians. This proposal seeks to identify the major gene(s) controlling short faces in domestic cat breeds. Breeding studies have shown that the short face is likely a semi-dominant trait in cats. Cats having two copies of the gene have shorter faces than cats with one copy. The study will use short-faced cats and long faced cats from different breeds, similar to a successful study in domestic dog breeds with short faces, such as Pugs. The goal of the project is to identify genes and mutations that affect facial structure of the cat, with the potential to develop genetic tests to monitor these mutations in the breed populations. The long-range goal is to decipher the genetic control of facial structure so that breeders may have more control over producing healthier cats. These same genes may be involved with facial development in humans. The GWA study should support a regional location of genes of interest within the year.

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

The hypothesis of this study is brachycephaly of the domestic cat is strongly influenced by a major gene. The goal of this proposal is to identify chromosomal regions for cat brachycephaly using genome-wide association (GWA) studies in domesticated cat breeds that display the extremes of facial types. The rationale for this project is brachycephaly and to a lesser extent, dolichocephaly, cause chronic health issues that are due to the extremes of the facial structures. Resources were requested to test 96 cats for this project; however, data from other studies would be combined for the analysis of brachycephaly in Western and Eastern cat breeds. Thirty-three (33) samples specific for this project have been submitted to Neogene, data has not yet been received. As data is accumulated from different projects, the remaining arrays will be tested with cats that may strengthen the preliminary results.

The long-range goal is the eventual identification of the genes that will predict the major control of the phenotypes. Further studies can then decipher the interplay of minor gene contributions to the minor variability in the facial morphologies. The long range goals will require additional funding sources.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

The study has focused on the domestic cat. Any other species, dog, human, horse, that has abnormalities with facial development, both desired and undesired, will benefit from the work.

C. How will your project help owners/stakeholders?

The overall hope of this project will be to use genetic tests that will help cat breeders select cats that are brachycephalic, but not to an extreme that causes other health issues for the individual animals. Similar health issues and abnormalities are seen in many breeds of other species that are brachycephalic.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

Much of the health issues with Persian cats are due to the extreme facial and head structures of brachycephaly. If veterinarians can help breeders realize that genetic tests are available, perhaps we can pressure breeders to not produce cats as extreme.

This will hopefully reduce health issues associated with the head type.

D12FE-507, Leslie A. Lyons, Ph.D., University of California/Davis

“Construction of a High-Resolution Map for Assisting Cat Genome Sequence Assembly”

Genetic maps determine the correct order of genes on a chromosome and the distance between those genes. The ultimate genetic map for a species is the complete DNA sequence from the beginning to the end of each chromosome. The cat has 18 pairs of chromosomes and the sex chromosomes, XX or XY. Thus a complete cat genome sequence would be twenty complete strings of DNA. However, even the most intense genome projects, such as the human genome project, fall short of this measure. The genome is sequenced in small fragments that then need to be re-assembled to reconstruct the sequence in the correct order. Like putting a puzzle together, once the sequences are generated, computer algorithms attempt to put the pieces of sequences back in the correct order. Historical sequencing methods have produced long fragments of sequence. The newer Next Generation technologies on the other hand, allow a higher volume of sequence for less cost, but the sequence fragments are shorter with an error rate higher than the old method. Thus, the same complex puzzle needs to be put back together with a 100 times more pieces that are all smaller. The sequence for the domestic cat has a base assembly using the old method, but most of the additional sequence is based on NextGen technologies. Assembly techniques take advantage of other genetic maps to facilitate the correct order and distance of the genes in the genome. Typically, genetic maps are made by typing markers in large families, and radiation hybrid panels, which use X-rays to fragment DNA. Family maps generally have a resolution to approximately 1 – 10 Mb, limited by the number of individuals in the pedigree and DNA variation of the families. The resolution of a radiation hybrid panel can be adjusted by the dosage of radiation and is not limited by DNA variation. These maps can then be used as a secondary validation for the genome assembly, as in general, the sequence assembly and the genetic maps should match. Thus, maps are like knowing which pieces of a puzzle are the ends and the corners or are specific colors that mark regions of the puzzle. The current maps and their resolution power provide minimal support to the genome assembly of the cat. A new high-resolution radiation hybrid panel has been constructed for the domestic cat with an average resolution power 20 times more powerful than the current panel. Approximately ~1,000 markers have been typed on the new panel, defining its resolution and the feasibility of using chip technologies. The focus of this proposal is to type the new RH panel with the cat arrays so that a high-density genetic map can be constructed. This independent mapping effort can then support the genome assembly of the cat sequencing, and likely supporting or correcting gene order and distances in the genome. This data will be of use to the entire community, all researchers will benefit from a more accurate genetic map of the cat. A more correct genome assembly will facilitate gene hunting and mutation screening efforts for any disease, regardless of breed or population. The production of the new map was funded by the NIH, thus no funding is requested for this project. The RH map construction should be feasible within one year once array data is completed. This data will be shared with the genome sequencing facilities of the cat to assist the next version of the cat assembly.

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

The hypothesis of this project is that the high-resolution cat radiation hybrid panel developed by UC Davis can be genotyped on the DNA array to construct a genetic map of the cat. The assay of the cat RH panel was supported by funding from the National Institutes of Health. The samples for the panel have been processed, submitted to Neogene for assay, and the data has been received. The objective and goal of the project has been successfully completed.

The long-range goal is to make a ~30,000-50,000 SNP map and then use this map to assist the future genome reassembly for the cat.

The data analysis for this project will be ongoing work for doctoral dissertation work.

D12FE-508, Leslie A. Lyons, Ph.D., University of California/Davis

“Genome-wide Association Study for Hypokalemic Polymyopathy in Burmese cats”

Potassium is an important mineral required for normal cell excitability. Generalized muscle weakness can occur in any species with low intake of dietary potassium or abnormal metabolism or elimination of potassium due to health problems. Low potassium (hypokalemia) can be a secondary condition to a more significant health concern, such as anorexia, diarrhea, vomiting, and kidney function abnormalities. However, low potassium levels can also be due to an inherited defect. Clinical signs are first evident between 2 to 6 months of age and may be induced by stress or exercise. During episodes, cats have a characteristic flexing of the head downwards following the pull of gravity. Cats can have a variable course of improvement, followed by relapse that may not occur for weeks to months. Two types of hypokalemia have been documented in humans, caused by different genes that affect the channels in the cell walls that allow potassium transfer for normal cell and muscle function. Hypokalemia has been documented in Burmese cats and is characterized by periodic muscle weakness associated with intermittent low potassium levels. The past and present breed prevalence and pedigree analysis has strongly suggested hypokalemia in the Burmese to be an autosomal recessive condition. Although the disease may only show on occasion, the condition is easily diagnosed and a cat would not advance to an older age with being diagnosed. Cases and controls can be readily determined and easily distinguished from secondary causes of hypokalemia. Previous studies of Burmese cats with low potassium levels have excluded the genes that cause some similar conditions in humans. The cats from the previous study are available for this project as well as previously collected cats from the England. Novel cases from within the past two years have also been collected from the England and Australia. The hypothesis of this proposal is the heritable form of hypokalemia in non-USA Burmese cats is controlled by a simple genetic mutation. The objective of this proposal is to identify the chromosomal region of the causative gene for the autosomal recessive Burmese hypokalemia via a case-control study that will evaluate ~64,000 DNA markers across the entire genome of the cat. The study will be performed in the same fashion as in horses and dogs; following the recommendation for the Europe Union dog consortium “LUPA” that suggests 30 cases and 30 controls for autosomal recessive traits. The long-range goal is to identify the gene and mutation for Burmese hypokalemia to develop a genetic test for the eradication of the disease in the Burmese breed and other populations of cats. The population of Burmese is non-USA cats. Burmese cats in the USA have an inherited craniofacial defect. Thus, Burmese cats are not shared between the USA and non-USA countries due to the fear of the heritable defects, resulting in increased health problems and loss of genetic variation in both populations. Overall, the identification of the Burmese hypokalemia mutation will allow the development of a genetic test. The genetic test could be used in random bred cats to help decipher a heritable, primary cause of hypokalemia and the test could be used to eradicate the disease in the Burmese breed. Once this disease is eradicated or can be monitored via genetic testing, more outcrossing could be performed to increase the genetic health of worldwide Burmese populations. Also, the test would prevent the spread of the disease to other breed populations that use Burmese, such as Bombay, Burmilla, Tonkinese, Singapura and Australian Mist. Finally, the identified gene could always be considered for other species that have potassium channel anomalies.

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

The hypothesis of this proposal is the heritable form of hypokalemic polymyopathy in non-USA Burmese cats is controlled by a simple genetic mutation. The objective of this proposal is to identify the chromosomal region of the causative gene for the autosomal recessive Burmese hypokalemia via a case-control SNP-based genome-wide association study. The original application requested funds to prepare DNA and process 60 DNA arrays, which was then extended to 72 arrays as more samples became available. Cat DNA samples were submitted to Neogene for processing and data was received. Data analysis implicated a chromosomal region on a specific cat chromosome for the cause of Burmese hypokalemia. The objective and goal of the project has been successfully completed.

The long-range goal is to identify the gene and mutation for Burmese hypokalemia to develop a genetic test for the eradication of the disease in the Burmese breed and other cat populations. With the assistance of additional funding sources, a candidate gene for Burmese hypokalemia was identified and a specific – causative mutation

has been identified. The broader and long-range objective and goal of the project has been successfully completed.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

The study has focused on the domestic cat. Any other species, dog, human, horse, that has abnormalities with potassium metabolism will benefit from the work.

C. How will your project help owners/stakeholders?

A genetic test will be developed and breeders can now prevent their cats from having the disease – which can lead to the eradication of the ailment in the cat population – the ultimate cure.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

A genetic test will be developed and veterinarians can use the test to distinguish between other causes of hypokalemia.

Current diagnosis will be improved as well as prognosis and treatment.

E. Note any problems you've encountered and solutions to those problems.

No problems – project was fully successful

D12FE-509, Leslie A. Lyons, Ph.D., University of California/Davis

“Genome-wide Association Studies for Progressive Retinal Atrophies in Cats”

Three types of inherited retinal degenerative diseases, collectively called progressive retinal atrophy, are well documented in domestic cats and ultimately cause blindness. Two forms are in Abyssinian cats and the causative mutations are identified in two different genes. The third form is in Persian cats, is autosomal recessive with early-onset and fast progression, culminating in blindness by 12 – 16 weeks of age. This disease is shown to be a degeneration of the photoreceptors and neither of the Abyssinian mutations account for the Persian disease. A fourth form of PRA in the Bengal cat breed has also been identified by our research team and has been characterized clinically and using electroretinograms (ERGs). Visual impairment begins at ~ 4 - 5 months of age and progresses slowly, with most cats having severe visual compromise by 1.5 – 2 years of age. Breeding studies have proven this disease to be autosomal recessive and the blindness is complemented by breedings to Persians, thus is not the result of a mutation at the same locus. Likewise, neither of the Abyssinian mutations account for the Bengal form of PRA. A research colony at UC Davis is established to examine the inheritance patterns of the Persian and Bengal PRA, clinically define the diseases, and to build pedigrees for linkage analyses to identify the causative genes. To date, a large pedigree of over 250 individuals has been developed for the Persian form of PRA. Three founders, Persian cats from different lines with PRA, were donated to the program. These cats have been outcrossed to cats of different breeds and then backcrossed to produce offspring that are either affected or obligate carriers. Within the last four years, three founder Bengal cats were added to the colony, following the same breeding scheme used for the Persian cats. Currently, the Persian and Bengal pedigrees have been interbred to produce an efficient colony of cats that carry genes for both forms of PRA. In addition, eleven additional cases of PRA in Bengal cats have been ascertained from the breeding community. The hypothesis of these studies is that the Persian and Bengal forms of PRA are caused by different single gene mutations. For this application, we propose to genetically map the two forms of PRA using the Illumina 62K Infinium cat arrays. Because the cats have been cross-bred, population stratification will exist. Both traits are autosomal recessive with full penetrance. The Bengal disease has more variable age of onset and rate of progression; however can be clearly defined clinically via fundic examination. Basic linkage analysis and homozygosity mapping will be used to localized the genes for the Bengal form of PRA, likely within one year. These are important traits to eliminate from the cat breeding populations since the Persian and Bengal breeds represent two of the most popular breeds worldwide. Persian cats are used to modify conformation of many other breeds, thus, the disease could easily spread to these breeds. The founders for the colony were found from unrelated lines from different parts of the USA, although a common founder could be identified. Because of the general behavior of Persian cats, many blind cats may go undiagnosed. The ascertained Bengal cats are from three different continents, thus, this disease is rapidly spreading in the Bengal breed. A development of a genetic test would be popular and useful for both breeds and if implemented quickly, could stop the widespread dissemination of PRA. The autosomal recessive form of blindness in Persian cats is an excellent model for potential gene therapy, thus disease could be corrected in affected cats once the gene and mutation are identified.

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

The hypothesis of this proposal is the heritable forms of progressive retinal atrophy (PRA) in Persian and Bengal cats are controlled by two different simple genetic mutations. The objective of this proposal is to identify the chromosomal region of the causative genes for the autosomal recessive Persian and the autosomal recessive Bengal forms of PRA via a SNP-based genome-wide association study using homozygosity and linkage mapping. A chromosomal location for the Bengal PRA has been determined.

The long-range goal is to identify the genes and mutations for these forms of PRA with the aim of developing genetic tests for the eradication of the disease in the breeds and other populations of related cats. The Bengal gene and mutation discovery has commenced, Persians are still being analyzed.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

Any species, dog, human, will benefit as this project will discover genes involved with retinal degeneration.

C. How will your project help owners/stakeholders?

A genetic test will be developed and breeders can now prevent their cats from having the disease – which can lead to the eradication of the ailment in the cat population – the ultimate cure.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

A genetic test will be developed and veterinarians can use the test to distinguish between other causes of blindness. Current diagnosis will be improved as well as prognosis and treatment.

D12FE-510, Robert A. Grahn, Ph.D., University of California/Davis

“Genome-wide Association Study for Congenital Muscular Dystrophy in Sphynx and Devon Rex Cats”

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

Two related breeds of cat, the Sphynx and Devon Rex, have an inherited recessive form of muscular dystrophy. Since Sphynx breeders use Devons for outcrossing, the disease is most likely caused by the same genetic mutation in both breeds. This form of dystrophy presents as a progressive muscular weakness first evident at about 3 months that continues to 9 months, at which time the rate of deterioration decreases. Cats with this form of dystrophy have difficulty supporting their heads, have protruding shoulder blades and commonly die from choking while eating. We have identified a single muscle protein, α -dystroglycan, which is present at drastically reduced levels in affected cats. However, no mutations were found in this gene or any of the genes that directly modify this protein. The hypothesis of this proposal is that the autosomal recessive congenital muscular dystrophy found in Sphynx and Devon Rex is caused by a single genetic mutation. The objective of this proposal is that a genome wide association case control study of cats with congenital muscular dystrophy will identify the genomic region associated with the disease. We propose to use affected cases and unaffected controls in a genome wide association study to identify the region of the genetic code where the mutation causing this disease is located. By narrowing the region, a candidate gene can be identified and sequenced to determine the causative mutation. Moreover, candidate genes interacting with the α -dystroglycan can be eliminated from consideration without the laborious task of direct sequencing. Finding this mutation will allow owners and breeders to test for carriers of Devon Rex and Sphynx muscular dystrophy and eliminate it from the population.

To date, 36 of 48 samples have been sent for genome wide association analysis. Many associated genomic regions have been identified but none stand out more than any other. However, one of the most strongly associated regions does have a plausible candidate. The remaining 12 samples submitted will be controls and will further eliminate background.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

The breeds specifically being analyzed are Devon Rex and Sphynx, but all species with muscles will benefit as this project will identify yet another gene involved with muscular dystrophy.

C. How will your project help owners/stakeholders?

CMD is an autosomal recessive disease. As such, carriers are not detected until breeding with other carriers produce affected kittens. This research will produce a genetic test allowing breeders to identify carriers and remove them from their breeding program. This will ultimately give owners the tools necessary to eliminate the disease from the population.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

Diagnosis and treatment will not change. This is an autosomal recessive disease, as such; carriers are not detected until breeding with other carriers produce affected kittens.

E. Note any problems you've encountered and solutions to those problems.

The background noise is higher than anticipated in the samples chosen. This is probably an artifact of the relatedness of the affected samples. Twelve chips were not used to determine in case more cases or controls were needed. Adding more controls will reduce the background. Additionally, the most closely related breeds based upon genetics (Birmans), have been added to help with the noise issue.

D12FE-511, Hannes T Lohi, PhD, University of Helsinki

“Identification of a Novel Gene for a Familial Reflux Nephropathy in Ragdoll Cats”

Background:

Reflux nephropathy is a disease associated with vesicoureteral reflux (VUR). It is caused by malfunction of the vesicoureteral junction resulting in retrograde flow of urine from the bladder into the kidneys. VUR has been reported in cats but presently there is no reliable method to routinely detect abnormal vesicoureteral reflux. Our group has been studying the clinicopathological features of this kidney disorder in altogether 261 ragdolls including 52 post mortem cases. Our data shows that typically both kidneys are affected with marked polar atrophy and nephritis. No cases of polycystic kidney disease have been found. The average age of euthanasia due to renal failure is 6 years. Our pedigree analyses indicate a possible autosomal dominant mode of inheritance of the disease.

Objective:

We were awarded a genotyping grant for 60 cats including 30 cases and 30 controls to perform a genome-wide association study (GWAS) using the new feline SNP arrays. GWAS should help us to map the disease locus. Completed experiments. Towards these aims we have been actively validating and expanding our ragdoll sample collection for genotyping. Overall, we have collected DNA samples now from 305 ragdolls in Finland and more than half of those cats have been sampled over the past 6 months. We have DNA samples from 67 confirmed reflux cats and 34 non-reflux controls. Due to the variable age of onset of the reflux in the breed, we required controls to be over 8 years old. Breed club has funded a separate ultrasounds study and we have received samples from 30 cats that can be utilized in this study over the past 6 months. We are delayed in submitting the samples for genotyping due to quality issues of some of our DNAs. However, we just completed our cohort and will submit our samples to GeneSeek by early next week (second week of March 2012).

Future plans and expected results:

We expect to get the genotyping data back from GeneSeek for statistical analyses in two months. We will then perform careful association and linkage-based analyses to uncover the potential disease locus in the feline genome. Identification of the disease locus would be a breakthrough for the project and would result in a deeper analysis of the target region to identify the causative variant.

Expected outcome:

The identification of the VUR gene would enable the development of a DNA test for breeding purposes to eradicate the disease from the breed and to test its possible presence in other breeds with renal failures. The early diagnosis of the disease by ultrasounds remains challenging and the development of a DNA marker would help in this regard. Identification of the underlying gene(s) would also facilitate the understanding of the disease mechanisms involved in the reflux and enable us to study the pathogenesis of VUR in a spontaneous animal model.

Time schedule:

We are delayed by 2 months from our original plan, and although it is still likely that we will complete the planned analyses in the intended 12-month timeframe, we have asked a 6 month non-cost extension for the project.

D12FE-513, Bianca Haase, Dr.med.vet, The University of Sydney

“Bodyweight: Investigation of Genetic Aspects in an Experimental Cat Population”

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

Obesity and obesity-associated diseases are growing problems for feline health and have become a serious veterinary problem. Multiple diseases, such as type II diabetes mellitus and dermatosis, are associated with excess bodyweight and may result in a lowered quality of life and potentially lead to an early death. A striking overweight phenotype could be observed in an experimental cat population hosted at the Institute of Animal Nutrition, Vetsuisse Faculty, Switzerland. As all cats share common housing facilities and fed similarly, environmental factors can most likely be excluded as the cause for this observation. Differences in feeding behavior due to the order of precedence could also be excluded as at least two feeding places were available in each group. Preliminary investigations on a subset of cats revealed significant differences between the two observed phenotype classes. As the two phenotypic groups were clearly distinguishable from each other, the result prompted us to hypothesize that a single major gene may be responsible for a large fraction of the observed variation in body composition. To test for the mode of inheritance all cats were classified into overweight and lean groups according to their lean body mass. A computer based complex analysis confirmed our hypothesis that a major genetic component is responsible for the majority of observed phenotypic difference and that the condition is heritable.

Objectives of the project:

Testing all individuals belonging to the experimental cat population on the new feline 60k genotyping array will allow testing of about 60.000 variable positions in a cat's genome and will give a high resolution genome map. A linkage study will identify the putative gene with large impact on body composition in cats. This comparison will highlight genomic regions that differ between the two groups. Subsequently, these regions can be further analyzed.

To date, all animals belonging to the experimental cat population were genotyped on the feline 60k genotyping array and a first analysis was performed.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

The studied experimental cat population is a mixed breed group. Identification of a causative mutation would allow genetic testing of cats from the general cat population, regardless breed.

C. How will your project help owners/stakeholders?

A better understanding of the control of body mass may highlight better management options, such as diet and will facilitate the breeding of healthier cats.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

A better understanding of the control of body mass may highlight better management options, such as diet. In addition, as multiple diseases, such as type II diabetes mellitus and dermatosis are associated with excess bodyweight, the outcome may provide better treatment options for already affected cats.

E. Note any problems you've encountered and solutions to those problems.

The feline genotyping array is a powerful tool to map inherited diseases. Biggest problem/challenge was and still is the low quality of the publicly available feline genome assembly. At the 5th international conference for “Advances in canine and feline genomics” in Baltimore it was announced that a 10x assembly of the feline genome would be available within the next months. Unfortunately, the assembly is not just yet available.

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

Two well-loved breeds of cats have folded ears. The Scottish fold has ears which fold forward, making their face look sweet and owl like. The American curl has ears which fold in the opposite direction, which makes them look peculiar and somewhat quaint. There is convincing clinical, radiological, histological and genetic evidence that Scottish fold cats have an underlying defect which affects the structure and function of cartilage, resulting in crippling lameness in homozygous individuals and early onset of degenerative joint disease in heterozygous individuals. Heterozygous individuals i.e. Scottish fold, have a variably severe arthritis which causes significant pain and discomfort as they age, causing osteoarthritis at a much accelerated rate compared to domestic cross bred cats. To convincingly demonstrate that there is a problem in cartilage development, and thereby provide authorities considering breeding schemes/regulations the information that they need to make informed policy. To do this we must identify the exact molecular defect that underlies the exhibited cartilage weakness.

To do this, we plan:

a) To use the newly available feline microarray chip that will permit us to scan the whole genetic code of affected cats and closely related but normal individuals.

This should permit us to rapidly identify key candidate genes which are responsible for the deficiency in cartilage function.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

The major focus is the identification of the underlying mutations causing folded ear phenotypes in Scottish fold and American Curls. In addition, the outcome of this study is of particular importance in understanding cartilage physiology. It might inform on the much broader problem of osteoarthritis in cats.

C. How will your project help owners/stakeholders?

Identification of the causative mutation for the folded ear phenotype will clarify whether the same mutation is responsible for osteochondrodysplasia, or whether the disease phenotype is caused by another mutation nearby. This will facilitate the breeding of healthier cats.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

The much broader goal is the understanding of osteoarthritis in cats. Insight into underlying mechanism might help to develop new treatments.

E. Note any problems you've encountered and solutions to those problems.

The feline genotyping array is a powerful tool to map inherited diseases. Biggest problem/challenge was and still is the low quality of the publicly available feline genome assembly. At the 5th International Conference for “Advances in Canine and Feline Genomics” in Baltimore it was announced that a 10x assembly of the feline genome would be available within the next months. Unfortunately, the assembly is not just yet available.

D12FE-515, Kathryn M. Meurs, DVM, PhD, North Carolina State University
“Genome Wide Association of Hypertrophic Cardiomyopathy in the Sphynx Cat”

A. Summarize the hypothesis and objectives of your project.

We hypothesize that we can use a genome association approach to identify a specific chromosomal region of interest associated with the development of familial feline hypertrophic cardiomyopathy. The objective of this study was to perform an association analysis using the new feline SNP array and previously collected DNA samples from Sphynx cats with and without familial hypertrophic cardiomyopathy collected within the United States and Europe.

Which objectives have you completed to date?

We have completed the objective above. We have collected all cats, performed the array with Geneseek (Neogen) and have completed data analysis with PLINK. Using this approach, we have identified 2 extremely statistically significant regions and 5 that are significant but less.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

Sphynx cat

C. How will your project help owners/stakeholders?

We hope that this work will ultimately help us identify a causative genetic mutation. If so that will allow the development of a genetic screening test for this disease

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

At this time, it does not change patient care. However, if we are able to identify a causative mutation for the disease in the future from this data, it will enable genetic testing.

E. Note any problems you've encountered and solutions to those problems.

Running the samples on the SNP array went very well. However, being able to move forward with the results is proving complicated. The stated location of the SNP is incorrect based on the current feline Map (the location of the SNP is about 30,000 base pairs from the location it is identified as holding, some SNPs do not align with actual areas of the feline chromosome (when I look in the area of its stated location, the feline genome only has NNNNNN listed). This has led to some (great concern) about the accuracy of the feline SNPS. I am making do but I have great concerns that whoever selected the feline SNPs for the array was a bit careless (in my opinion).

D12FE-516, Niels C. Pedersen, DVM, PhD, University of California/Davis

“Genetic susceptibility to Feline Infectious Peritonitis (FIP)”

A. Summarize the hypothesis and objectives of your project. Which objectives have you completed to date?

Hypothesis - Simple or complex (polygenic) genetic factors underlie a significant portion of the susceptibility to Feline Infectious Peritonitis in pedigreed cats such as the Birman.

Objectives –

1. To engage Birman breeders in Denmark to provide DNA samples and pedigrees from 500 cats that have FIP, that are healthy but closely related to cats that have died of FIP, and healthy cats free of FIP for at least three generations.
 - Birman cat breeders and owners from Denmark, and also some from Sweden and Norway, have responded beyond our most optimistic projections and have already contributed samples from over 400 cats in the three risk groups. Sufficient DNA has been already extracted from all of the submitted buccal swabs with a success rate over 90%. Birman breeders in Scandinavia, with the assistance of Dr. Anne Sorensen and Susanne Wehnert, continue to send new samples at the rate of around 10-25 per month.
2. To test DNA using Illumina high density SNP array chips to identify possible genetic associations with FIP susceptibility or resistance.
 - DNA from 192 cats has been submitted to GeneSeek (NeoGen) for testing on the cat SNP arrays and useable data obtained from over 98% of the submitted samples. We are now in the process of identifying potential genetic associations with FIP susceptibility and have identified 6 or more possible genes of interest in regions of the genome where strong associations were identified. Almost all of these genes are associated with various aspects of the innate and adaptive immune responses.
3. To apply any genetic associations gleaned from Birman cats to other breeds and to random bred cats
 - We have extracted DNA from over 200 cats of various breeds, as well as from random bred cats, for this phase of the study. We will develop in-house genetic tests that target potential resistance/susceptibility genes identified in Birman cats to see if any of these same genes are associated with FIP in non-Birman breeds and in random bred cats.

B. Note the species/breed that is being studied and discuss other species/breeds that this work will benefit, if applicable.

We believe that susceptibility to FIP is common among all cats and that only about 20% of the random bred population is genetically resistant to the FIP virus. The degree of resistance may vary from breed to breed, with some possibly being more susceptible or resistant than others, depending on how the various susceptibility/resistance genes have been parceled out during breed development. Although we are concentrating on the Birman breed for this study, we believe that susceptibility/resistance genes discovered in Birman cats will also be involved in FIP in other breeds and in random bred cats.

C. How will your project help owners/stakeholders?

Identifying genes that influence susceptibility or resistance to FIP will allow us to develop specific tests for these various genes. Such tests will allow pure breeders to determine the genetic status of their breed to FIP and to either pick matings that will minimize FIP risk or identify cats that will allow resistance to be reintroduced.

D. How might veterinary professionals use information gained from this study to improve patient care? Will it change current methods for either the diagnosis or treatment of disease? If so, how?

Genetic testing of pedigreed cats will not improve the care of cats with FIP, or change current diagnostic or therapeutic approaches. However, if pure breeders can significantly reduce FIP incidence, it will have a direct impact on the numbers of FIP cases that are seen by practitioners.

E. Note any problems you've encountered and solutions to those problems.

The project has gone amazingly well and Scandinavian breeders have been extremely cooperative. This is due to large part to the willingness of Birman breeders in this region to end what they perceive as a significant health problem in their breed. In addition, the success of the project up to this point is a tribute to the participation of a leading Birman veterinarian in Denmark (Dr. Anne Sorensen) and a leading Birman breeder (Susanne Wehnert).