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Molecular Genetic Studies of Inherited Cataracts in the American Cocker Spaniel

Personnel:

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Objective:

The project focuses on identifying the gene and mutation responsible for inherited cataract in American Cocker Spaniels (ACS), and subsequently developing genetic tests that can identify genetically normal, affected and carrier dogs.

Cataracts are an inherited condition characterized by loss of transparency in the lens. ACS have a spectrum of cataract phenotypes differing in location, progression rate, laterality, genetic background and age of onset. Dogs with inherited cataracts are born with normal lenses, which then proceed to opacify over time, leading to blindness by 2-10 years of age.

According to the ACVO “Blue Book”, 2014 edition, about 11% of ACS are diagnosed with cataracts. However, this number is not specific as it includes acquired and inherited cataracts; the latter category including cataract phenotypes that are clinically similar, but not identical and therefore may be due to different genetic causes. This project is focusing on the identification of gene(s) causing the most common form of cataract in the ACS. Once the gene and mutation are identified, ‘atypical cases’ will be included in further analyses. The mode of inheritance in ACS has been proposed to be autosomal recessive and there are no gene-based tests available.

Methods:

Research study forms

Even though every effort has been made to have Dr. Aguirre examine all the study dogs, we realized early on that this would be impractical, and that dogs examined by other ACVO diplomates will be included in the study. In order to obtain samples that are clinically characterized in a consistent manner, we developed separate clinical research forms for the cataract project. Such consistency in clinical ascertainment will be essential to properly develop the population for the research studies. The forms are attached with this Progress Report.

Sample collection and pedigree analysis

Samples from ACS diagnosed with cataracts and samples of their non-affected relatives together with their eye exam and pedigree information were retrieved from OptiGen archival DNA resource and collected at eye clinics organized by different dog clubs as well as the American Spaniel Club. We have also received samples (via OptiGen) from veterinary ophthalmologists both in US and Europe. DNA was extracted and used for PCR amplification. Different software was used for pedigree display and analysis, i.e. Cyrillic 2.1 and GenoPro.

Candidate gene analysis

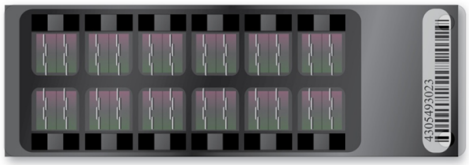
While in the process of collecting sufficient samples to carry out more detailed genomic studies, we first undertook a candidate gene analysis. Candidate genes were identified based on the reported findings about the association with the same or similar disease in the dog and other species as well as the knowledge about the gene's biological function. The exons of these genes were sequenced in cases and controls and were screened for associated variants to the disease. Candidate genes for cataracts include crystallins, gap junction proteins and transcription factors that are expressed in the lens.

Illumina CanineHD BeadChip

Featuring highly polymorphic SNP content and providing uniform genomic coverage, the CanineHD BeadChip array (Figure1) enables the interrogation

of genetic variation in any domestic dog breed. Importantly, this BeadChip presents an average of greater than 70 markers per megabase (Mb), providing ample SNP density for robust within-breed association. The BeadChip contains more than 170,000 markers placed on the CanFam3.1 reference sequence. Illumina developed the BeadChip in collaboration with the LUPA Consortium, which includes 22 European universities and other partners such as the Broad Institute.

Figure1. *Illumna CanineHD BeadChip*



Genotypic information will be used for a genome-wide association study (GWAS). The main advantage of GWAS approach is that it does not require any hypothesis regarding the location of the mutation or exact information on the genetics. Therefore, GWAS allows us to conduct an unbiased search for the areas of highest association throughout the whole genome.

To have a homogenous population of affected dogs we specifically chose samples from ACS diagnosed with bilateral inherited anterior and/or posterior cortical cataracts at the age of 2 to 5 years, and which were accompanied with extensive and reliable eye exams. Dogs that are at least 8 years of age and were free of cataracts were selected as controls. In total, 24 cases and 24 controls were sent to the Cornell University core genotyping facility, which provides high quality results at the most competitive price. In order to identify 24 cases and 24 controls, we screened the records of 96 cataract-affected cases and 186 normal controls for this initial analysis. Dogs not used in this initial analysis will be used in subsequent studies to validate the GWAS results.

Results:

Pedigree analysis

So far, pedigree analysis did not reveal a clear mode of inheritance if we include all dogs diagnosed with cataracts. However, pedigree analysis of dogs diagnosed with bilateral anterior and/or posterior cortical cataracts suggests a recessive mode of inheritance.

Candidate gene analysis

So far, we screened 23 cataract candidate genes (Table1). No association could be established between screened genes and the disease. However, we only screened the coding exons and the coding exon-intron boundaries of the candidate genes. Therefore, only exonic variants and splice site mutations can be excluded as cause for the disease at this time.

Table1. *Screened candidate genes for inherited cataracts in the ACS.*

Candidate genes	*HSF4*	*CRYAB*	*CRYAA*	*TMEM114*
	LIM2	*CRYBA1*	*CRYGS*	*SIX1*
	GJA3	*CRYBA4*	*CRYGD*	*SIX3*
	GJA8	*CRYBB1*	*CRYGC*	*SIX4*
	MIP	*CRYBB2*	*CRYGA*	*BFSP4*
	MAF	*CRYBB3*	*PITX3*	

Illumina CanineHD BeadChip

A sufficient number of phenotypically characterized normal and affected dogs were collected and sent for genotyping. Once the data is ready a GWAS/Linkage analysis will be performed. The results of the chip analysis will be available in early 2016. The data analysis and validation of results should take 8-12 months.

Perspectives:

Sample collection is ongoing. We have obtained great help from selected breeders, dog owners and the American Spaniel Club. It would be very helpful

if the American Spaniel Club continues to publicize the research effort, and encourages members to participate and assist in the research project. The project would also benefit from breeders and dog owners sending us the most recent eye exam and informing us of any change of their dog's phenotype. It is important to emphasize to all individuals that all information provided for this project will be kept in the strictest confidence, and no information will be released outside the immediate research group.

We will focus our pedigree analysis on dogs diagnosed with the same type of cataract in a consistently clinical manner.

Candidate gene sequencing will continue. The genome-wide association/linkage data will help to identify genomic regions of interest that will be screened for positional and functional candidate genes.

We have made excellent progress during the first year of work, and are grateful for all the help, interest and support of owners, breeders and the American Spaniel Club.

Expenditures:

Up to date $45,015.01 from $46,900 of the project budget was spent on Salaries, Equipment and Supplies. If the balance of $1,884.99 is not spent by the end of December, we would like to carry it over into the next year, as we expect to incur costs for sequencing and genotyping.