Molecular Genetic Studies of Inherited Cataracts in the American Cocker Spaniel - progress report

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Objectives

The principal objective is the identification of the genes and genetic variants responsible for inherited cataract in the American Cocker Spaniel(ACS) dog breed. Our group was granted additional funding and support by the ACSF to map and characterize the genetic origin of the cataract in the ACS. The ultimate goal of our project is to detect the gene and mutation, i.e. the genetic marker, to be used for a DNA-based test that would prevent the production of ACS puppies with this condition. In the initial stages of our investigation, we were assuming a simple disease scenario for the cataract in ACS, with an easily identifiable inheritance mechanism and markers. However, further analysis has revealed this assumption is incorrect, which led to a prolongation of our effort and intensification of sample gathering. In this report we describe our findings, the progress achieved to date, and the last challenges faced, as well as explain our recent proposal and future goals.

Background

Cataracts are the most common cause of vison impairment in humans and other mammals, as well as frequently occur in dogs. Several dog breeds are affected by this condition, including ACS, with an estimated prevalence of 8-11%. This percentage range describes both acquired and inherited cataracts. However, the latter category contains cataract phenotypes that are clinically similar but may have a different genetic etiology and only a superficial clinical similarity. ACS 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.

The mechanism of inheritance in ACS has been previously proposed as being

autosomal recessive, but our ongoing studies indicates a situation more complex than the one predicted in the preliminary phase of the project. We have observed that the most likely mechanism involves the presence of potential risk factors based on the sub-population observed. A significant element in the progress of our project is the thorough classification of suitable and verified samples in the ACS population. Our constant re-analysis and update of the cases and controls present in our database would not be possible if not for the outstanding cooperation of the ACS owners and breeders. This allowed us to pinpoint specific areas of the genome associated in varying degrees with the condition, and to confirm or remove such association with each iteration of the analysis.

Our final aim remains the identification of gene(s) and vulnerability loci associated with the most common form of cataract in ACS and on validating its inheritance mechanism. We achieved such analysis of the database through tight communications with the owners and the breed association. After reaching a sufficient number of samples, we planned and executed the use additional resources and techniques in order to move the project forward. Nonetheless, we concluded that the complexity of the issue requires additional data in order to pinpoint the exact genetic cause of the condition.

Cataract in ACS – nature of the samples

Cataracts are often inherited conditions. They are characterized by opacity/cloudiness of the lens, arising due to lens protein misfolding, solubility changes and aggregation leading to vision impairment of progressive severity, occasionally demanding surgical intervention. American Cocker Spaniels are among the most commonly cataract-affected dog breeds.

As previously reported, we acknowledged a spectrum of cataract phenotypes differing in location, progression rate, whether they are unilateral or bilateral, genetic background and age of onset. We considered the latter parameter, above the rest, as a most crucial factor for the classification and grouping of our samples. Specifically, inherited cataracts in ACS are thought to appear sometime around 2-5 years of age and progress. Nonetheless, we have found a subset of cases where cataracts, presumably inherited, begin between 5-8 years of age.

We focused on very detailed collection of information about the affected and unaffected dogs, and for a precise assessment of the phenotype and the selection of a

good control sample group, essential in order to select candidate cases for cataracts predictable as having a genetic etiology. We also eagerly accepted input and suggestions from breeders in order to explore additional options and compare their observation with our statistical data (see below).

Cataracts can be caused by environmental effects such as UV light exposure, mechanical trauma, poor nutrition, or exposure to toxic substances. They can also occur as secondary effects of other ophthalmic diseases, such as uveitis or glaucoma. We used the maximum care in excluding any possible secondary cataract phenotype with a high likelihood of not having a genetic etiology, and thus lowering the quality of the dataset.

Research on genetic diseases in companion animals

Current research in genetic diseases in domestic animals is based on three main principles: (I) Construction of a suitable dataset, obtained through the identification of cases and valid controls, (II) Mapping of the variants associated with the condition studied, (III) Validation through sequencing.

The importance of (I) is described and explained in the above paragraph, and we previously described the steps that have been made thanks to this approach including the addition of new cases.

(II) is generally achieved using SNP genotyping. The method uses purified DNA, preferably obtained from blood samples of cases and controls, that is placed on 'chips'-specific platforms scanned for strategically selected genetic variation markers, called single nucleotide polymorphisms (SNPs). Using the information obtained by this experiment, we can explore the presence of common (and ideally, exclusive) shared regions among the cases. Such region could be, for example, a common homozygous interval (as it happens in fully penetrant recessive diseases). Analysis of markers inherited from parents and identical by descent can even pinpoint shared linked interval in heterozygous regions of the chromosome (as in dominant diseases). Research is constantly trying to improve such technologies with denser chips, that increases the amount of information contained.

Another common type of analysis is the Genome Wide Association Study (GWAS) that uses the SNP chip platform. Such study pinpoints higher frequency of certain SNPs in cases vs. controls, associating these variations with the disease. GWAS

can be implemented on a wide population of dogs with reasonable computation time, and regardless of the family information about the samples. Moreover, GWAS can better predict variable degrees of association of a locus with the condition, giving away vital information in the investigation of a more complex inheritance mechanism. In fact, GWAS has been a vital part of our approach, since there is no perfect segregation of the markers between cases and controls. Often, the dataset generated for GWAS analysis is also used to search shared homozygous regions among the cases, which we routinely do in our analysis. However, so far, we did not notice any detectable trend between cases and controls.

Sequencing (III) determines the exact DNA sequence of a given genomic region (of variable size, included a genome in its entirety). A common and fast sequencing method is the Sanger sequencing used for comparison of candidate mutations in cases and controls (that is, to validate whether a given mutation is associated with the condition, thus possibly being the causative one). Sanger is often used even for the development and execution of a genetic test for a disease. We are working on its complete implementation on specific candidate markers in order to assess their frequency and segregation (between cases and controls) of several candidate variants in our population.

A limited and targeted use of Sanger sequencing is relatively cheap, but the exploration of a whole genome sequence would make it impractical and too expensive. On the other hand, Whole Genome Sequencing (WGS) methods have brought a whole new level in the exploration of genetic defects, because they allow us to obtain detailed information about the genome of a sequenced animal. WGS is particularly useful when the sequencing of a high amount of candidate variants in one or more cases would be time and cost prohibitive if done using more conventional approaches. Additionally, the cost of WGS is decreasing over time – the analysis of a single genome dropped by $1/5^{th}$ or even $1/10^{th}$ (depending on the specific type used) in the last 10 years. This is especially crucial when a lot of data is required due to the complexity of the inheritance.

An ideal scenario in the study of a genetic defect involves the use of SNP chip for the mapping the disease to a specific chromosomal region, and sequencing a putative candidate gene(s) for the validation of the data once the genomic region is identified. Even in case of more than one associated/implicated region, a careful evaluation of the samples selected for WGS, a consistent dataset and a high number of controls can finally unveil the genetic etiology of the disease. Our approach is flexible and related mostly to the GWAS results – candidate haplotypes detected will dictate the number

and specific dogs sequenced. As will be seen below, this approach has worked. GWAS and haplotyping has identified two chromosomal regions of interest, and WGS is progressing to examine potential candidate genes and variants in these regions.

Summary of the previous work (and progress to date):

COVID-19 and ACS cataract research:

During the peak of the COVID-19 pandemic we implemented several strategies in order to face the limitations brought upon us by the lockdown and university closure, and implemented a 'workaround' plan so that the research progress would not be impacted adversely. Video conferencing like “Zoom” or “Skype”, as well as shortages and backorders in PPE and lab consumables, proved to be sometimes challenging. However, we maintained our determination to push the project forward. Even during the initial lockdown phase where most research activity diminished, we were able to send samples to be run in the high-density SNP chip and continued some of the WGS analysis on samples already collected and run. Now that many restrictions are lifted, new clinics could be planned and an exam/sample collecting pipeline closer to the pre-pandemic situation can be implemented.

Candidate genes and Pedigree analysis

A deep analysis of the data suggested that a common, shared genetic variants causing *all* the genetic cataracts in the ACS population is unlikely. While in the ongoing process of collecting sufficient samples needed for detailed genomic studies, we carried out a preliminary candidate gene analysis in order to exclude more obvious genes with negative results. Nonetheless, functional role of genes showing to be mutated in WGS data is indeed taken into account when the variant has to be considered as a candidate for testing. Our current approach is population-based but as shown below, we are eager to listen to observations from breeders and Club.

Samples received

Compared to the previous report, the number of dogs participating the study increased to 943 from the 869 reported last time. This is a significant increase and many of the new samples are from the older population (>8-9 yrs of non-affected dogs). Figure 1 lists the states from where the samples have been obtained. A short breakdown of the samples follows:

Total dogs	**943**
Total of Informative dogs	**580**
Potential cases	***116***
Bilateral	80
Unilateral or very Asymmetric	36
Controls	***463***
Too young to be properly assessed for study inclusion at this time	281
Total of Excluded dogs	**363**

Table 1 –Total of dogs included in the dataset. Count of dogs that are sufficiently informative, type of cases, potential controls and dogs not suitable for the study. Causes for exclusion: co-morbidity with another eye condition, doubts about diet, the dog prematurely deceased (especially if DNA/blood is missing), lack of feedback on updates (fortunately, this now is a very rare occurrence), lack of an official diagnosis by a certified veterinary ophthalmologist (or of monitoring post diagnosis), inconsistent records (very rare occurrence). Of the dogs shown above, only the ones with consistent records over time can be genotyped. It must be noted, the number of dogs genotyped is not only linked to new potential cases and controls, but also to updates and new samples for dogs already in the study!

Currently, all ACS DNA used in our study is isolated from blood samples received by our group from breeders. All of the blood samples have been sent to us in EDTA lined tubes, to prevent clotting. We extracted the DNA from the blood samples of cases and controls considered suitable for the study. The overwhelming majority of samples were of freshly drawn blood samples, which are easier to work with and generally give better DNA yield. This is greatly appreciated since the DNA yield and quality from blood extraction is drastically superior, and is useful if multiple genetic tests have to be carried out.

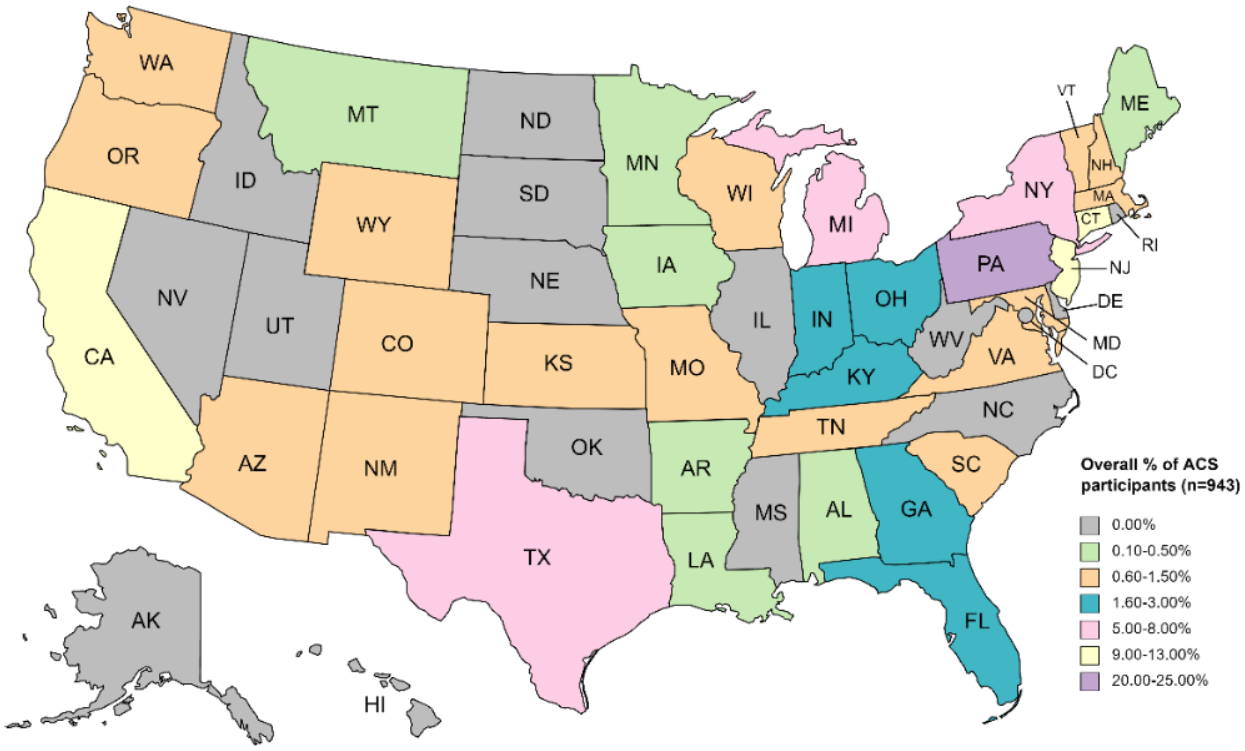

Figure 1 – Breakdown of the study samples received by state. >99% of the samples are from the US, and all of them are from North America.

Phenotype reassessment

We previously reported the development and use of a standardized eye exam research form. We wish to stress again that the forms are extremely useful and important to the study. It has been noticed that still not every veterinary ophthalmologist uses them. This has been a problem as the forms used-OFA/CAER-are inadequate for consistent diagnosis. A proper form can be downloaded through the following link:

https://drive.google.com/open?id=1c-hbLI2sdgMyVtb1jz7gSkO9v8AAme5V
Clicking on this link will direct to a page with the document. It can be downloaded (top right) and/or printed. Please note this is an updated version of the link and the form (Jan 2021)

Each time new samples are added, and a sufficient number of updates is gathered, we analyze the new information and re-classify the dogs. We make use of our carefully organized archive and classify the samples as Cases, Controls, and Excluded (due to the phenotype being probably explained by a non-genetic etiology), as well as

samples from dogs simply too young to be evaluated with certainty (therefore the assignment is to temporarily not use the samples awaiting future clinical updates).

In this recent iteration, an improvement concerning the sample gathering has been implemented thanks to the collaboration of the Club, with incentives and refunds for breeders that wish to submit samples to our lab. We are especially grateful for such responsiveness to our plea for a high number of good quality cases and controls. We are confident such measure increased the total sample number. Please note: we still reserve the right to exclude any submitted reports/samples from the study if the specific participant sample cannot contribute to our research (not enough information was provided, sample was sent to us in an inappropriate way causing it to degrade, paperwork/sample not submitted, dog is not a good candidate for the study, etc.).

ACS dogs seem to exhibit distinct sub-types of phenotypes of inherited cataract. Primarily, we registered (I) a possible stratification of the phenotypes in regard of the age of onset. We also (II) noted that there seems to be a second type of classification of the cataract phenotype, where one eye develops a cataract at an early age and several years later a second cataract appears in the fellow eye. We also (III) considered the anteroposterior position of the cataract onset for the classification of the phenotype.

Our principal means of classification of the phenotypes is still based on age (I). In fact, since we started to carefully re-assess the phenotypes of the dogs, this element was our primary concern in order to include a sample in the “Cases” or “Controls” cohorts, and more importantly, asses the quality of the “Case” with a relevant score. Such subdivision is distinct and both groups consist in a high number of samples. In case of (II) and (III), we considered the conditions separately (sub-phenotypes) in the initial iterations of the analysis, but we were unsure about our preliminary results because of the lower amount of samples for a given subset (e.g. “anterior unilateral cataract samples”).

Thus, adding new and reassessed samples, we compared multiple sub-groups within themselves, as we have done for the previous report (Fig 1). The new samples added power to our analysis, and just like last time our investigation has shown that inherited cataracts within the 5-8 year-old population are more likely to be bilateral (>75%) than asymmetrical (<25%) in appearance. However, thanks to the added power, a statistically significant difference between the two phenotypes in this sub-group has been realized, which acts as a validation to the above stated finding.

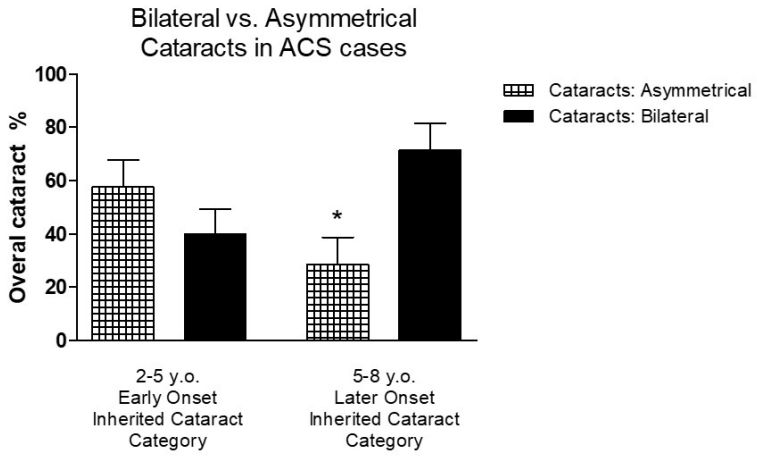

Figure 2 – difference of prevalence of asymmetrical and bilateral cataract in early and late onset case sub-populations.

Importantly, we did not ignore the possibility of taking in account the phenotype sub classes (I-II-III) in light of the population structure of the dataset after our PCA analysis (see previous report). We maintain that additional cases should be gathered to confirm any trend detected so far.

Cataracts and coat color

Recently, we have received a question from the Foundation on whether any association between inherited cataracts in ACS and their coat color was spotted and tested. We have found the idea of interest, thus segregated dogs in each cataract case sub-group, as well as in the controls, by coat color. A statistical analysis between each cohort and the coat color was then performed (Fig. 3).

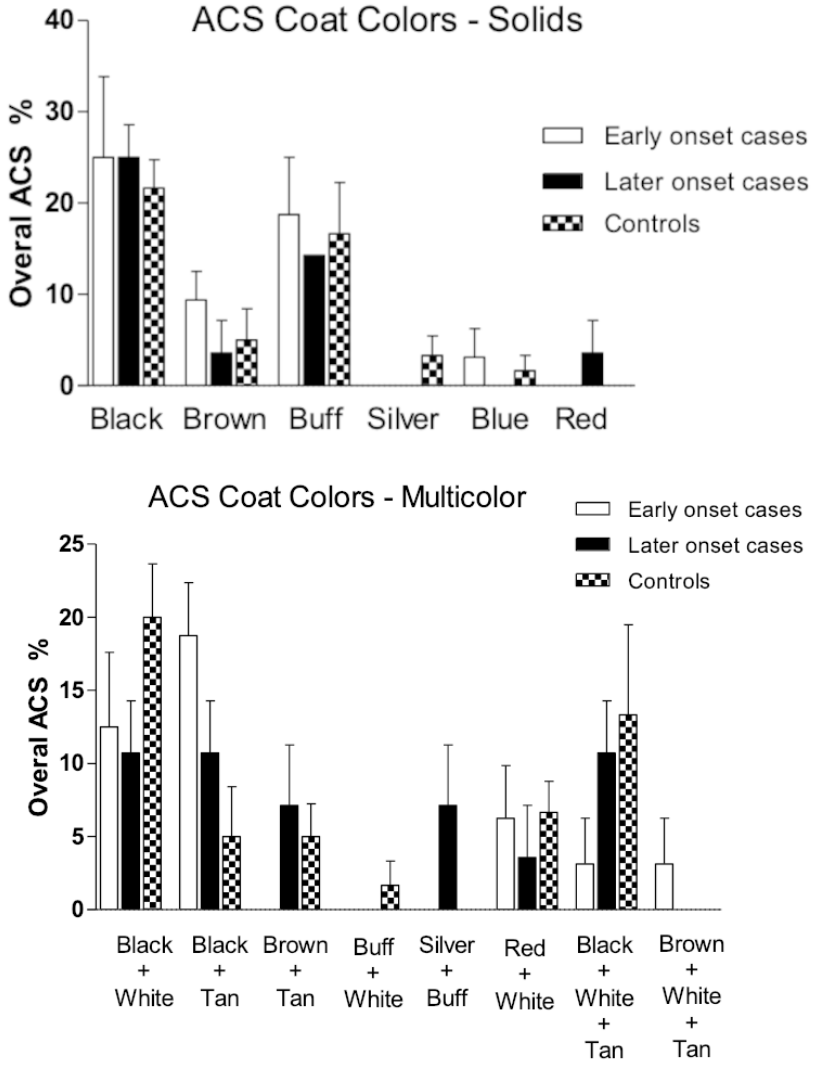

Figure 3 – Prevalence of cases (early and late onset) and controls among the different coat color. Top: counts done for solid-colored dogs. Bottom: calculations done for dogs with a coat consisting in multiple color combinations.

Overall, we found no statistical significance and no direct association between cataract occurrence and the coat color. We found interesting details concerning the sub-population structure, however even comparing the two case sub-groups to each other no tie in of the dog's coat coloration and the pathological condition was found. The prevalence of cases or controls had no trend within specific colors or color combination. Important to note that while some coat colors cluster in one of the populations, e.g. Buff in the left population (Fig. 4, top image) a look at the data in Figure 3 shows that for this coat color there is no difference in disease incidence between cases-early onset and late onset- and controls.

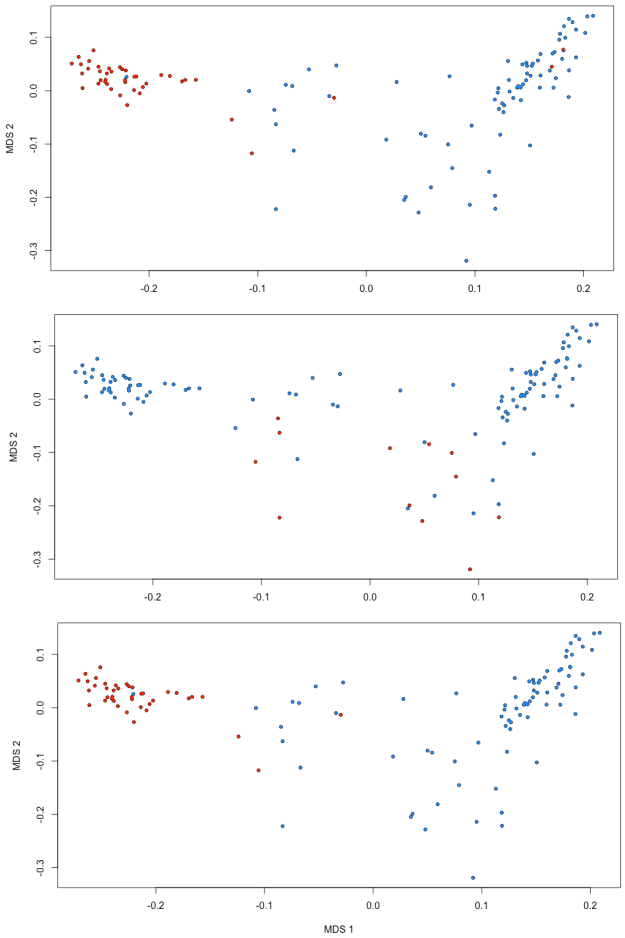

Figure 4 – Principal Component Analysis (PCA) of the ACS cataract population as introduced in previous reports. We can observe the two sub-populations clustering on the right (population A) and left (population B) zones of the plot. In red, we can see marked dogs with a specific coat color (whole coat or as a component). Interestingly, Buff (top) and white with other colors (bottom) tend to cluster in population B and brown dogs (plot in the middle) cluster to the central "outliers" population.

Nonetheless, this analysis did give us ideas regarding the sub-group structure and did cast some light on the 2 subpopulation components and the possible reasons behind their stratification. Albeit this is unlikely to be directly associated with any pathological condition, in the future we will take this information into account to avoid any possible false positives due to segregation in coat color. Note that, as reported previously, roughly 80% of the total individuals would fall within one of the two sub-populations of uneven size.

SNP genotyping and data analysis

Since our last report, we outlined a considerable improvement in our SNP chip dataset. We report a similar improvement in this subsequent iteration. In fact, several dogs received updates, and new batches of dogs to be genotyped have been added. In specific, we were able to review our records and improve the total amount of cases to 66 and of controls up to 60. Few updates led us to set to 60 the number of excluded. Once again, the total of high-quality cases increased. Because of the new surge of samples and updates, we are going through phenotype assessment and exam record analysis of dogs previously not included in the study. As we write this report, the data from the last SNP set group has been processed, and we also are reviewing samples

for a new SNP batch (8-16 dogs)-mostly high quality controls (which is at the moment, desired).

As noted previously, we took advantage of the new, higher density (220k vs 170k) canine SNP chips. The new chip is ~30% more informative, with no information loss compared to the older one (that is, more SNPs were added to the new version but with full compatibility with the older one). Specific computational techniques were used to raise the information density of the old dataset at the level of the new one ("imputation", through the popular software Beagle, extensively used by our group in other projects).

As well, before the lockdown period we managed to select dogs from our best cases and controls and send them to be processed for a third type of SNP chip using a new technology. Such technology allows the genotyping of the selected samples for 712k SNPs, more than three times the original information. As for the previous batches, the new set of genotyped dogs (220k, see below) would be added to this dataset and imputed as well. Any tentative haplotype obtained would be noted and used for whole genome sequencing sample selection.

Each case and control subset was classified on the basis of the age of onset, laterality, anterior-posterior side of development of the cataract, and reliability of the sample (generally age-related). We also once again checked for any sex or age bias in the ratio of bilateral and unilateral cataract.

GWAS: We carried out a whole new series of Genome Wide Association Studies (GWAS), with another series of analyses using all the cases (60) and controls (66) within the whole population. As done previously, we used the excellent R package GenABEL (used in numerous animal genetics studies). The aim of such studies is to associate a specific genomic region and its markers to a cohort of study cases. In addition, we used association analysis packages from plink 2.0 in order to validate the findings and check whether the association found is consistent with one carried out with a different program. The two sub-populations were used in both separate analyses and grouped together. In the case of the larger sub-population ("population A"-see Fig. 4, larger population on the right), the peaks obtained, and the analysis of the quantiles once again confirmed the clear improvement registered in the last report (Figure 5). Additionally, we tentatively observed association with specific sub-phenotypes. We are still working on these trends, which MUST be confirmed with additional genotyping in the

immediate future. In this regard, we state that the quickest outcome could be obtained with an high number of good quality controls at this stage of the study.

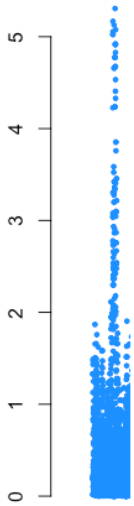

Figure 5 – Partial Manhattan plot of the case/control association for a specific sub population (based on symmetry and PCA results). It is possible to observe a suggestive peak. Only the chromosome of interest, plus two other flanking it, are shown as a reference. Chromosomal number is not shown. The $-\log_{10}$(P-value) shown on the Y-axis is a function of the association – the higher, the better the association of a given genomic region with the cataract phenotype.

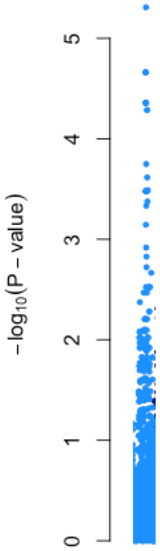

Figure 6 – Partial Manhattan plot of the case/control association for a specific large sub-population (based on symmetry). It's possible to observe a novel suggestive peak. Only the chromosome of interest is shown as a reference. Chromosomal number is not shown. The $-\log_{10}$(P-value) is a function of the association – the higher, the better the association of a given genomic region with the phenotype is.

Homozygosity mapping: It is possible though extremely unlikely that two regions apparently identical between cases and controls are in fact distinct at the fine molecular level. We count on whole genome sequencing data also to elucidate this possibility. For this reason, we are still considering the possibility that regions in which most dogs (cases and controls) are homozygous searching for exclusive markers to add to the pools of the one to be tested. This is in addition to the results obtained from GWAS. A first in-depth attempt in this regard will be tried after the current cycle of WGS data analysis.

Whole genome sequencing: We decided to send samples for WGS. Four cataract cases of the best quality were selected, taking advantage of convenient and timely offers existing in the platform used. These dogs are additional to the eight already

sequenced by WGS. Data will be available to us within the next 3-4 weeks.

Future prospects and plans

In the previous iteration, we focused on the following aspects of the cataract in ACS research; we re-analyzed and confirmed our case-control GWAS and genetic mapping. The first was updated and realigned. Additionally, the data was mapped to an updated canine genome reference. This is because more publicly available canine genetic data has been made accessible. Such data is going to be compared with the new samples whose sequencing is being finalized in the next few weeks, and the analysis of the sequence data will go through the same pipeline.

A complex disease: We hypothesized in the previous report that the occurrence of cataracts in American Cocker Spaniel is likely a complex of 2 or more diseases. As previously shown, a greater number of cases and controls leads to better and more encouraging results. The selection of the appropriate sub-populations of cases and controls moved forward the analyses and the project. We are now working on whole genome sequencing (WGS) on samples taking into account every candidate haplotype.

Tackling the complexity: In this report, we confirm that we have found that we can trace and identify trends and associations both under the assumption of a recessive disease, and under the assumption of a disease associated with loci of vulnerability not necessarily inherited in a recessive manner (we cannot, at this point, suggest a dominant inheritance – if such, the penetrance would be fairly low or dependent upon the co-existence of multiple factors, not necessarily all of them genetic).

We update our immediate and future objectives as listed below and compare them with what was stated in the last report.

. **A)** As always, we renew our stated intention to increase the sample number in the database: a greater number of cases means we will be able to enrich the specific sub-populations, and a greater number of controls allowing us to avoid false positives. The Research Scientist dedicated to the project spends a significant amount of time in the management of the database and in the interaction with the breeders and owners to obtain samples and updates, thus our database improved in numbers and diversity. Additionally, in order to carry out the updates of the status of dogs already included in the study and genotyped, the Research Scientist will contact owner of dogs of known status that need an update.

. We still welcome new participants to sign up to our study, and have expanded our sample intake to include frozen semen samples of deceased ACS that were known to have cataracts or produced/were related to cataract affected dogs (please contact the Research Scientist if planning to submit a frozen semen sample). We acknowledge that frozen semen of deceased sire dogs is very precious to breeders; however with well preserved and good quality sperm cells, a small amount can help. Blood samples are still very much welcomed and appreciated, and we wish to thank again the breeders and owners for their continuous contribution and support.

. The recent decision by the Foundation to provide 'reimbursement grants' to owner who bring additional dogs specifically needed for the study (>9 yr old control; early and late onset cataracts) has generated great interest and samples. These grants reimburse expenses in having dogs re-examined, and have blood samples collected and shipped, and applies only to the high quality cases needed for the study. This has increased the sample numbers for the study.

. The Foundation has suggested that veterinary ophthalmologists be contacted and asked to participate in the study by submitting samples and examination forms. Unfortunately, this has been tried before and was totally unsuccessful. As an alternative, Dr. Aguirre has been in contact with OFA/CAER and asked if an email with the letter attached could be sent to owners of ACS whose eye examination forms are submitted by either the owner or the veterinary ophthalmologist. OFA has been VERY supportive of this effort. A **draft** letter (included at end of Progress Report) has been sent to OFA for consideration. It will likely be modified, but the principles and goals of the letter should be achievable because it puts the responsibility on the owner and not the ophthalmologist to provide the samples.

. **B)** We previously stated that we would go through an in-depth analysis of the data output, never ignoring the slightest suggestive peak. Thanks to the contribution of the breeders we were able to add additional dogs to the analysis. We do think that now, due to the current state of the data, as well as comparing our results with other projects, this should be given the greatest priority. It cannot be denied that this new surge of samples has increased the power of our experiments and analysis. Thus, we ask for help in reaching an even greater number of cases and especially controls. Based on our experience in similar projects, we found that good quality control samples prove themselves more crucial at this stage of research. Hence, would like to encourage breeders and owners of ACS dogs considered to be a high-quality study controls to

enroll them in our study.

. **C)** We previously stated how our preliminary cross-reference of the data did not point out any specific correlation between laterality, i.e. unilateral or bilateral, and age of cataract onset. We repeated such analysis and also added data concerning the coat color. We found interesting details regarding the sub-population structure, but no association with the cataract. Moreover, with the help of additional samples and analysis power, a statistical significance was realized in the 5-8-year-old late onset cataract population and bilateral cataract phenotype. This acted as a validation that indeed within this sub-group of cases bilateral inherited cataracts are more prevalent.

. **D**) As previously reported, the whole genome sequencing data analysis will be the focus of our next steps. Indeed, we have added four high quality cases to our WGS pool (to the previous , thus validation will happen in two steps – through further sequencing, investigating the segregation of a candidate variant within the population, and/or with further 'wet' laboratory studies to confirm a supposed effect of the variant on gene expression, translation, splicing. We maintain our initial goal of a total of 12 new dogs sequenced, and to have half of these (6) as cases and the rest (6) as controls. Nonetheless, we think that now a greater priority should be given to mapping. For this reason, we reserved a second batch of WGS analysis after the results of the next GWAS (that is, after the current planned genotyping cycle).

E) As previously stated, the final strategy is to start with a large number of candidate markers, that would be then tested (Sanger sequencing) in small but significant groups of dogs belonging to the study. Once the marker “passes” this first selection, we plan a scenario in which fewer and fewer markers would be genotyped in a progressively greater number of dogs. The change is from most markers in few dogs, to few markers in most dogs. Based on the data currently available (see our last report), we gather that a given marker will be predictive of a cataract risk factor in dogs belonging to a given ACS sub-population. We also count on the fact that new GWAS and WGS data will help minimize the number of markers to be explored first, and speed up the project.

As in the last report, we share our excitement for the prospect of WGS of the ACS dog samples. We think that the current contribution of the breeders and owners is helping our plan to acquire a sufficiently informative dataset and will allow us to move the project forward and develop a DNA-based test.

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DRAFT

Date

Dear Cocker Spaniel owner/breeder,

The American Spaniel Club Foundation is funding a grant at the University of Pennsylvania for studies of inherited cataracts in the American cocker spaniel. The aim of the study is to identify the gene(s) and mutation(s) responsible for inherited cataracts, and develop DNA tests that identify affected, carrier and genetically normal dogs. With the support of the Foundation, we have made significant progress towards our goal. Now we are asking for your help to finalize the study and develop a DNA test that can be used to assist breeders in selection of dogs used for breeding. Our goal is to increase the number of samples from well characterized dogs in order to pinpoint with absolute certainty the genomic region of interest, and then focus on the genes located in the region to examine their association with the disease. To help with this effort, the Orthopedic Foundation for Animals/Companion Animal Eye Registry (OFA/CAER) has agreed to disseminate to the fancy information about the study and request your help.

OFA/CAER records indicate that your American cocker spaniel was examined by an ACVO diplomate, and the results of the examination submitted. The research investigators are asking you to collaborate in this study and provide a small sample if your dog fits in one of three criteria:

• dogs that are greater than 8-9 years of age and found to be normal in the most recent eye examination by an ACVO diplomate.

• dogs that are diagnosed with cataracts considered by the examiner to be inherited, and that fit in these two categories:

-Between 2-5 years of age.
Between 5-8 years of age.

For each sample, we will need the following materials:

• 2-3 ml blood sample in EDTA tube.

• copies of the OFA/CAER eye examination forms. For dogs diagnosed as having inherited cataracts, we would appreciate having copies of the previous eye examination results as well.

• pedigree.

NOTE: all the information received will be held in the strictest confidence and will only be available to the research investigators.

If you have any questions about the study or would like more information on shipping samples, please contact:
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Thanks so much for your help in getting this project across the finish line.

Gustavo Aguirre, VMD, PhD Principal Investigator	Leonardo Murgiano, PhD co-Principal Investigator	Katie Diehl, DVM, DACVO ACVO-OFA/CAER liason