American Cocker Spaniel Cataract – brief progress report

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In this update of the American Cocker Spaniel (ACS) Inherited Cataract study report, we would like to again thank all the ACS breeders and owners for their continued support of our study. With their help by sending us samples and updates about dogs currently in the study, our dataset has improved and expanded. Compared to the report of July, 2021, the number of dogs participating in the study increased from 943 to 995.

Table 1 – samples in the study breakdown.

Total dogs	**995**
Total of Informative dogs	**606**
Potential cases	***141***
Bilateral	90
Unilateral or very Asymmetric*	51
Controls	***465***
Too young to be properly assessed for study inclusion at this time	294
Total of Excluded dogs	**389**

*Defined as: Cataract appears in one eye first; 2 or more years must past since the formation of the first cataract when the second one appears

Additionally, many owners contributed with vital updated eye exams for their dogs, which aided in choosing if a given case or control dog was appropriate for a Genome-Wide Association Study. In fact, the last series of analyses used a total of 97 cases and 99 controls within the whole population, classified in varied sub-types and combinations. A very thorough re-assessment of phenotypes was carried out by our group to be certain of the sample quality level.

Total genotyped	**219**
Total cases	**97**
Bilateral	49
Asymmetrical	48
Early onset	57
Late onset	40
Total controls	***99***
Total of Excluded	**23**

Table 2 – genotyped ACS samples breakdown.

The most significant population sub-division is among those cataracts appearing in the 2-5 years of age range and progressing, as well as the ones with a later (5-8 years of age) onset and progression. Since our last report, we observed that inherited cataracts within the 5–8-year-old population are more likely to be bilateral (>75%) than asymmetrical (<25%) in appearance. This was also reinforced by data from our current GWAS, which supports a genomic region and associated variants that would optimistically explain a very significant number of affected dogs in this category.

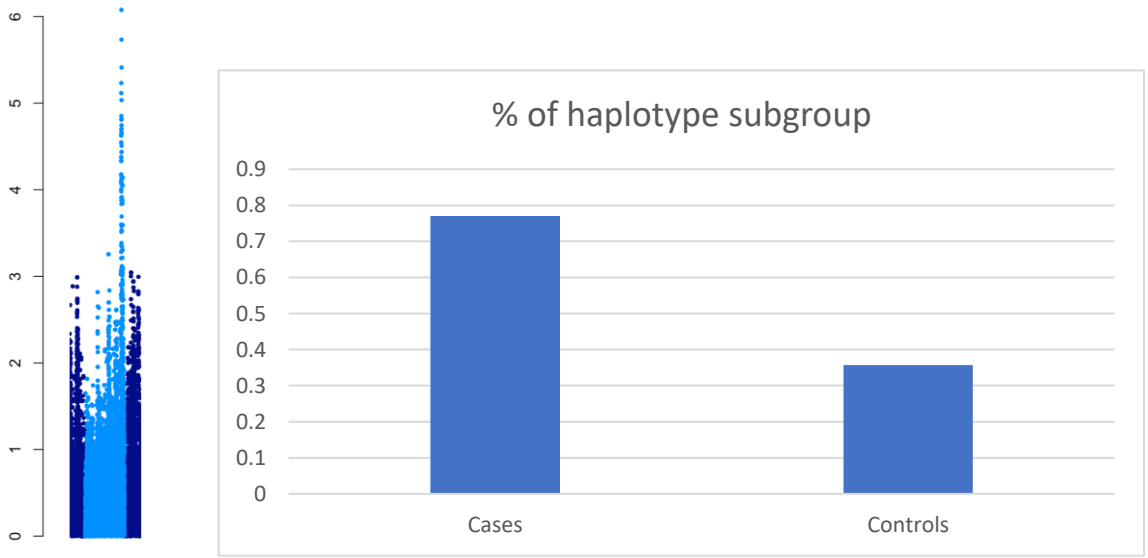

Figure 1 – sub-group (cataract cases 5-8 yrs of age) GWAS peak and haplotype distribution.

Exploring the region in the whole-genome sequencing data, we obtained >9,000 variants that we

checked against public and in-house databases. We suspect that due to the nature of the region and the most plausible candidate variants if the association remains consistent (when a larger population is tested), the mechanism associated could be regulatory. Genotyping is ongoing in the affected dogs and relevant controls.

The recent thorough reassessment of phenotypes and additional genotyped dogs also helped in the efforts concerning the hunt of a general locus (all cataract categories).

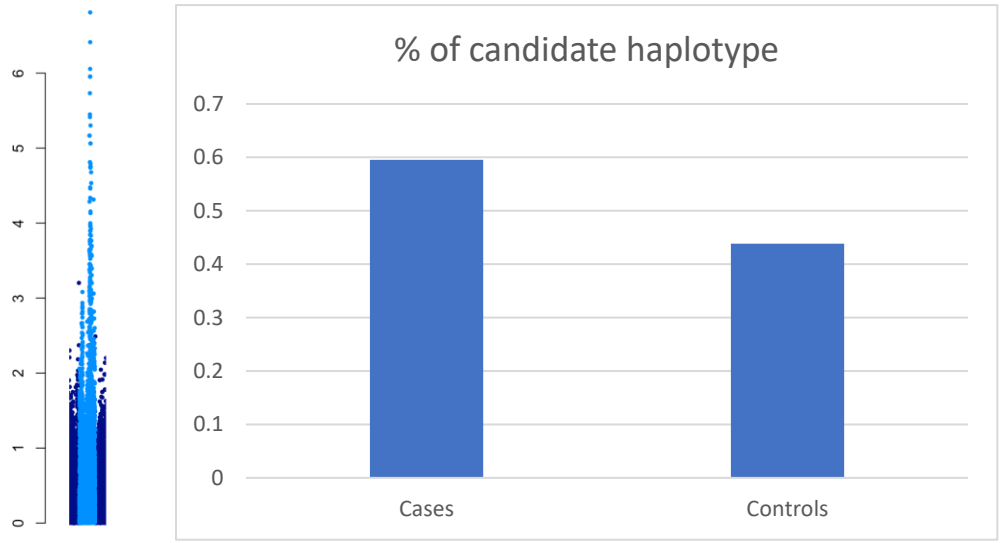

Figure 2 - All-cases (all ages and includes bilateral and asymmetric) GWAS peak and haplotype distribution. Note the high frequency in the control.

Exploring this second region in the whole-genome sequencing data, we obtained >5,000 variants that we checked against public and in-house databases. In this case, the nature of the most plausible candidate variants vary, the mechanism associated could be regulatory but also affecting directly lens function. Genotyping is ongoing in the affected dogs and relevant controls, but at a reduced emphasis given that so many controls have same haplotype.

One significant delay in our effort is due to the fact that different iterations of samples lead to a loss of signal in candidate areas. For that reason, it occurs our genotyping effort must be redirected once new samples are added to the study. Although this is in part expected, it remains a problem.

However, in order to address this issue, we are undertaking a new strategy. Since the cost of Whole-Genome Sequencing (WGS) is significantly decreasing over time the analysis of a

single genome dropped between 1/5th -1/10th of its price (depending on the specific type used) in the last 10 years. This opened the door to studies previously economically unfeasible. WGS is especially crucial for genetic problems in which the mapping difficulty is due to the complexity of the inheritance. For this reason, we elaborated a WGS-GWAS strategy that can be implemented along with, and in parallel with the increase of sample pool number.

Thus, our strategy consists in employing an alternative WGS method that is sufficiently accurate for our purposes but has a significant decrease in its cost. We plan to genotype 10-15 American cocker cataract cases and controls (each) and integrate such data to the dogs already sequenced. The plan is to use this dataset primarily for a GWAS that uses millions more markers than its standard version. The data available would also make candidate marker discovery easier by "killing two birds with one stone" and accelerating toward the conclusion of the project. This analysis will be integrated into the ongoing standard sample collection and GWAS studies.

On the breeders' part, we once again stress the critical importance of updates and new samples. Lack of updates endangers the quality of the dataset and therefore the results. Similar studies can reach double or triple in the number of genotyped dogs. High numbers are essential. In the upcoming days, we will contact owners whose dogs are in the study but have not provided us with updated exam information yet. We also ask any owner of older (>9 yrs) non-affected dogs to send samples for the study. We think that due to the nature and inheritance of the condition, eye-normal controls are vital. Positive feedback and large numbers are the keys to success.