Early Onset Adult Deafness (EOAD) in Rhodesian Ridgebacks

As previously reported, Dr. Mark Neff carried out the full genome sequence for an affected Ridgeback and on the basis of that original analysis was able to narrow the location of the likely mutation responsible for EOAD from the 2.4 billion letters in the full genome to a section containing "only" 3.5 million letters. Analysis of this stretch of DNA narrowed the search to 125 candidate mutations.

Last year Dr. Neff sequenced the full genome of another relatively unrelated EOAD Ridgeback and found that of the 125 candidate mutations, only 41 were homozygous in the second affected dog.

In the next step of the project, Dr. Neff and his colleague Allson Ruhe at projectDOG genotyped these 41 mutations against 45 additional RRs; 5 affected, 3 carriers (parents of affected) and 37 non-affected population dogs). This study identified 24 mutations that fit the expected pattern for a recessive causal mutation:

- Homozygous[1] in affected dogs
- Heterozygous[2] in known carriers (sire or dam of an affected dog).
- Not present at all in non-affected and non-carrier dogs.

Of these 24 mutations, based on scientific reasoning that I will not try to explain, they identified five "most interesting" mutations.

In the final step to date, Allison Ruhe at projectDOG has genotyped 3-5 of these mutations against a total of 405 Ridgeback samples, some collected as long as ten years ago when Dr. Neff was still at UC Davis, and many submitted in the last few years since he moved to the Van Andel Institute. These markers are all very highly correlative with the known profile of the disease, as follows:

- Of 24 EOAD-affected dogs, 21 were homozygous in all tested mutations.
- Of 14 known carriers, <u>all</u> were heterozygous in all tested mutations.
- Of 69 family dogs (related to deaf dogs but not sire or dam), 25 had one copy of each of the mutations and are therefore carriers. The other 44 had no copies of the mutations and are therefore clear.
- Of the remaining 298 dogs of unknown status, 17 had one copy of each of the tested mutations and are therefore carriers, presumably not known to the submitters. The other 281 had no copies of the mutations and are therefore clear.

As reported above, three dogs of the 405 dogs tested to date are outliers, submitted as affected but found to not have the DNA mutations. There are various possible explanations for these false negatives, including sample mixup or possible mis-diagnosis of the dog. Unfortunately, these samples were all submitted more than 10 years ago and the dogs are all deceased, so it is impossible to obtain new samples. Therefore, for the time being, these three results are being considered as “false negatives”, and related dogs will continue to inform on their status. However, it should be recognized that all of the remaining 21 affected dog samples submitted in the last ten years fit the pattern of being homozygous in all tested mutations.

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[1] Homozygous means has two copies of each of the mutations.
[2] Heterozygous means has one copy of each of the mutations.

One or more of these five highly correlated mutations will almost certainly turn out to code for a defective protein that is the actual cause of EOAD. However, to breeders, identifying the actual causal mutation is less important since the markers being evaluated appear to be highly predictive. Of the 405 dogs screened with the candidate mutations, there have been no false positives (dogs with two copies of the mutations but who are nevertheless not affected or dogs with no copies of the mutations but who are sire or dam of an affected) and there are only three false negatives (dogs with no copies of the mutations but who were reported to have had the disease). Therefore, the predictive power of the markers being studied is very good.

The project is now entering a new phase in which it will be of great benefit to test another 500 dogs, ideally from families with suspect pedigrees and mutually benefit owners by accelerating access to dog disease status. In this research phase of the project, all submitted dogs will be tested with multiple candidate mutations with the aim to reduce the number of correlative markers. Eventually, the goal is to exclude all of the markers until the single causal mutation is discovered. This is considered an extension of the research project since each DNA sample submitted will be screened with multiple markers (in some cases, as many as five screens for each dog tested). It is recommended that submitters contribute a modest donation of $15 each for the first 500 dogs tested in this phase to help offset the high costs of testing multiple markers.

Samples can be submitted by online registration at projectDOG. The web address is www.projectdog.org/ From the options at the top of the page, select “DISCOVERY TESTING”. These samples will be assayed with multiple candidate mutations and the test results will be reported to you as one of four possible outcomes:

1. **High confidence clear** (all tested markers are homozygous for the normal/reference allele, dog most likely unaffected, clear of disorder).
2. **High confidence carrier** (all tested markers are heterozygous, one normal and one mutation allele - normal presentation, may transmit either a normal gene or an affected gene to offspring. gene or an affected gene to offspring).
3. **High confidence affected** (all tested markers are homozygous mutant); if homozygous and non-affected, these become 'outliers' and warrant further testing. Owners will be contacted individually for followup.
4. **Exceptionally Informative** (reserved interpretation); results suggest a recombination event among the tested markers. Such results are highly informative because they may help exclude some of markers currently being used and allow the identification of the single mutation responsible for the disorder in the breed. Owners will be contacted individually for followup studies.

If you wish to participate in this phase of the project, the passcode for the first 500 samples submitted will be "liondog". When results are available, you will be able to access them directly through the projectDOG website. When results are available, you will be able to access them directly through the projectDOG website.

Dr. Neff and his team are very grateful to all of the owners and breeders for contributing samples over the course of this study. If you previously submitted a sample, they would like to provide you with results, if available. Many samples were used in the early phases of the study and may have not been tested for the currently targeted markers, or are depleted, or are archived at UC Davis

and not readily available to projectDog. Therefore, for samples submitted in 2012 or earlier it is encouraged that you resubmit a fresh sample. If your dog is deceased and you would still like to follow up with a result, please contact me (heathcock@berkeley.edu). I will maintain a list and the projectDOG staff will subsequently submit a formal request of UCD to provide archived samples for testing. Our experience is that it can take a few months to obtain an archived sample from UCD.

If you have submitted a sample more recently, please email projectDog (ridge@projectdog.org) and include the following information:

1. Submitter name
2. Dog name
3. Approximate date the sample was provided
4. Type of sample submitted (i.e. blood sample, buccal swab, saliva swab) if known.

If individual results are available, a test report will be provided as soon as possible.

Going forward, all samples submitted through the projectDOG online portal will be tested with the currently targeted markers and results will appear automatically on your myDOG page on the projectDog website.

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