Questions About the PLN Research

Dr. Meryl Littman and Dr. Paula Henthorn, University of Pennsylvania School of Veterinary Medicine very kindly answered these questions for us. We want to thank them for their help.

1. Has the mode of inheritance been determined?

We know that the mode of inheritance is not a straightforward Mendelian inheritance, for instance, it is not simply recessive or simply dominant. The information we have from the Wheagle colony at NCSU suggested a dominant mode of inheritance, yet the Open Registry and field experience suggested a recessive mode of inheritance, supporting the notion of complex inheritance. Complex inheritance can be the product of predisposing alleles of multiple genes (at least 2) acting together, with or without environmental triggers. We also know that there is variable severity of disease with some dogs markedly affected at a young age, and some only mildly affected later in life. There appears to be no age limit for onset of illness. So this disease has been difficult to study.

2. Was PLN found to be polygenic?

Our genome-wide association study (GWAS) showed a region of one chromosome that was strongly associated with PLN. Two candidate genes within that region encoding 2 slit diaphragm proteins, nephrin and filtrin, were found to have mutations associated with PLN. The alleles at these loci do not perfectly predict which dogs will or will not be affected. (This is NOT a surprise, since we knew before we started the GWAS that the disease does not show a simple, single-gene mode of inheritance). There may be more genes involved with or without the presence of environmental triggers.

3. Can you determine whether PLN in a particular dog is genetic or acquired?

There are many things that can cause PLN in any breed dog, and these factors may be additive stressors or environmental triggers for dogs with underlying genetic risk, such as infectious diseases, inflammatory diseases, allergic or immune-mediated diseases, and neoplasia. It's hard to discount that there may be an intersection of genetic and acquired factors in a particular individual.
The Open Registry and PLE/PLN Research

4. Will there be a need for the OR to continue after introduction of genetic tests for either PLN/PLE?

The OR is an important tool to study PLN, IBD, PLE, Addison’s disease, renal dysplasia. There are no genetic tests for most of these. Even with the genetic PLN testing, we need to continue surveillance of the incidence of PLN in heterozygous vs. homozygous dogs so we can guide breeders with their decision-making. The OR helps lead researchers to study SCWT and their diseases and helps educate owners, breeders, and veterinarians about all these diseases.

5. Should PLN dogs still be reported to the OR?

Yes, I recommend the OR be continued.

6. Will new PLN dogs be accepted without the PLN DNA Test?

Yes.

7. Will the test be run on dogs currently on the list for which there is DNA?

Yes, if submitted, confidentially, or if the owners want it listed, the results can be put on the OR, but that’s up to them and perhaps the committee that decides what goes on the OR. We did not test every sample that we have in the Bank. We “only” tested 186 samples from Wheatens and that was enough to show strong statistical significance that the variant alleles are associated with PLN. We also tested more than 750 samples from non-Wheatens to be sure the variant alleles are not common or generally found in dogs. It sounds like a great study to do all of the samples we have from Wheatens in the Bank that have documented phenotypes (not all do), but that will be expensive and the techniques are more difficult and time consuming to get quality samples of DNA from old frozen tissue samples. It is easier to do the test on cheek brush or blood samples, so if the dog is still alive, we prefer those types of samples. If the dog is deceased and we only have frozen tissue, and if we haven’t tested it yet, these can be done on a case by case basis if the owner fills out a submission form and we receive payment to help subsidize the costs (it costs more to do than the $125 we’re charging for cheek swabs or blood).

8. If a dog currently on the OR is negative on the test, will he be removed?

If a dog meets the criteria for having PLN, he should be listed. If the DNA testing is done, if the owner gives permission for the DNA results to be listed, I think it would be helpful to share that information with the community. From what we know now, we would suspect he may not have the genetic PLN, but since more genes may be found in the future, we can’t be absolutely sure. I think any dog that meets the criteria for having PLN should be listed and then we can keep track of how
commonly we are seeing dogs with PLN without any DNA markers. It would appear from our studies so far that this scenario would not be that common.

9. How will the PLN DNA Test findings relate to the Canine Phenome Project at Missouri or to the NIH study?

If we receive samples in good condition, they can tested. If the owner wants samples to be sent to us and get results back, they would need to ask the people that have the sample to see if it’s in good enough condition and if they can send it to our lab just like any other submission. We will test samples from wherever they come if there is a submission form filled out with payment. It’s possible in the future a large amount of samples would be tested from the other banks but this would be for an anonymous prevalence study and not to give people test results back.

10. What is the status of the PLE research?

The first set of SNP’s from the original Genome Wide Association Study (GWAS) did not show a statistically significant region for PLE study. We then did a second set of SNP chips and are studying the merged data from the two sets. We are looking for an area of interest for PLE to focus in on for candidate genes (as we had done before for PLN). Hopefully with more numbers of cases in the GWA studies we will find mutations associated with PLE. So far it looks more complicated than PLN.

Timeline

11. When will the test be publicly available and how can I get my dog tested?

Now! May 1, 2012 we began to distribute cytology brushes at dog shows in order to collect anonymous cheek swab samples to study the prevalence of the PLN-associated DNA markers in the current population of show dogs and breeding stock. For those owners wishing to receive results, submission forms and instructions are now available. Please see enclosed forms and instructions for how to have cheek swabs sent out. To receive cytology brushes, send a self-addressed, stamped, business envelope FOR EACH DOG to:

Michael Raducha/ PLN Test  
Ryan Veterinary Hospital, Rm. 4022  
University of Pennsylvania School of Veterinary Medicine  
3900 Delancey St.  
Philadelphia, PA 19104-6010

There are other questions the Club may want answered, for instance, what is the prevalence of these mutations in the general population of Wheatens in North America? In other countries?
Questions About The Test

12. How is the test done?

We are using standard methods for genotyping, that are in the paper we are submitting.

13. Blood sample or swab?

We can use blood or cheek swabs. Semen can also be used. Blood samples should be sent in purple/lavender top tubes (EDTA) with an ice pack, by overnight mail or 2\textsuperscript{nd} day delivery, with a call-in to arrange a submission date. Brushes can be sent by regular mail without ice or any need to call first. Frozen tissue may be sent but requires ice packs, a styrofoam box, overnight mail, and a call-in to arrange a specific submission date so that the sample will not thaw. We prefer cheek swabs, blood, or semen rather than tissues.

14. At what age can the test be done?

Any age, but if a puppy has not been weaned, it needs to be separated from the dam overnight so that there is no chance of contaminating the brush with the dam’s milk (which contains her cells and her DNA). For now we are not accepting puppy tails/dewclaws.

15. Who takes the sample…me or my vet?

Blood – vet; swabs – anybody.

16. Where will the test be run…my vet’s office, Penn, a commercial lab?

At Penn.

17. What will the cost be? What is the time line to receive results?

It is $125 per dog. The results will be reported in 2-4 weeks, possibly longer if we are inundated with samples, which may occur when the test is first available.

18. Will there be a “litter discount”?

Yes, $100 each if more than 3 pups from a litter are tested, with a maximum of $500 per litter, regardless of the number of pups.
19. **Will the test be made available outside the US?**

Yes. It's probably easiest to send cheek brushes from other countries. Brushes are generally not available from vets at this time. SCWT clubs in other countries may want to organize PLN DNA Kit distribution using the system set up for distributing the cheek brushes in the US.

20. **I have frozen semen on my deceased dog. Can it be tested?**

Yes, but it may take longer to get the results. It can be sent frozen, but we only need a few drops of semen, so a straw could be thawed (for potential use, you can’t refreeze it) and only a few drops are needed for the DNA sample. At this time, please contact Dr. Littman for additional information.

21. **I have provided samples to (Penn, Missouri, etc). Can I have those tested?**

If the sample is sent to us and is in good condition, we can test it.

22. **Will the genetic test results specify that the dog has one or both mutated genes?**

Yes. (We think it is more accurate to refer to them as predisposing alleles....)

23. **Is the test a linkage test or direct gene(s) test? Please explain the difference.**

We consider this a direct gene test, however, we have not yet proven that one or the other of the amino acid changes found in the predisposing alleles actually causes PLN. The fact that different mutations in the nephrin protein cause a very severe PLN in humans is extremely strong evidence, but not direct evidence. A linkage test shows if there’s a change close to the problem, but not the problem itself (it’s not as good). Our data supports the assumption that we are looking exactly on the problem, which we found by tedious fine mapping (gene sequencing) of genes that caused similar problems in humans. The predisposing alleles cause changes in the proteins they encode, namely nephrin and filtrin, which are proteins in the slit diaphragm of the glomerulus, which normally is not permeable enough to allow protein to leak into the urine.

24. **How will test efficacy be determined?**

We are testing both genes, and if they don’t agree with one another, we will retest to make sure we did not make a mistake. If they still differ, we will ask the owner to stay in touch so we can follow the dog’s health. We will be getting more stats over time and updating the odds ratios as necessary. The odds ratios in the paper show a very strong association for PLN occurring in homozygous positive.
25. If my dog’s samples were used as part of the research, will you provide me with the results?

To be decided.

26. Will permission to publically disclose anonymous outcomes be required for test eligibility?

No.

27. If not, will de-identified results be aggregated to further research and understanding of prevalence?

Yes, and we plan to aggregate non-identified information and disseminate our results concerning prevalence and distribution of carrier status in the Wheaten community in order to help with breeding decisions.

Test Results

28. What is the rate of false positives? False negatives?

Since there are 2 tests being done on each dog, we have an internal control checking for repeatability. False positives or negatives would most likely be caused my misidentification of the dog’s sample at one end or the other. Cheek swabs may be contaminated by DNA from another dog via food (milk from the dam), feces (coprophagia), etc. Blood samples are less often contaminated or misidentified.

29. If my dog is positive for both mutations, will he definitely get PLN?

No but he is at much higher risk, based on our studies. He should be monitored closely so that interventions can be started as early as necessary to help him. We need to study the factors that may alter the expression of the phenotype, either by protecting a dog with 2 copies from getting sick or the factors that may cause a dog with only 1 copy to get sick. For instance, does also having food allergies, IBD, or PLE bring on PLN even if the dog only has 1 copy? Are there “protective genes” that help dogs with 2 copies keep from getting sick? Does avoiding environmental triggers (allergies or infections) help?

30. If my dog is positive for one mutation, negative for the other, will he get PLN?

We don’t know. The 2 genes are very close together, so if your dog is positive for one mutation, it is probably positive for the other. However, we will follow any dogs that differ in the two genes very closely. But, perhaps the more important question is, if my dog is heterozygous for both predisposing alleles, will he get PLN? You will
see the odds ratios in the paper when it comes out and see that there is a much lower risk for heterozygotes compared with homozygous positives, but there is still some risk over homozygous normals.

31. If my dog is negative for both mutations, can he get PLN?

Yes, because any dog of any breed can get PLN. Also, it could be that there are other genes involved in PLN in SCWT, so time will tell as we learn more. But if your dog is homozygous negative, he would have much less risk of having PLN compared with dogs that are homozygous positive and probably less than the heterozygotes too. Your dog would be very popular because everybody would want to breed with him, but we need to be careful about losing diversity in the breed by using popular sires. We must consider all the traits that we want to keep in our dogs.

32. If my dog tests negative, do I have to continue to have annual blood testing and urinalysis?

Yes, I would continue to do screening tests because there is no genetic test for PLE yet, so it would be good to continue to check serum albumin and globulin. And a urinalysis is recommended now for all dogs (any breed) for good annual preventive care. Also, I would want to be careful until we get more statistics not to discount PLN. We have only had money to test 145 SCWT so far for both mutations and 747 other dogs. With the money we get from testing, we will be able to test more samples we have in our Bank, to do prevalence studies on the current generation of dogs (including many anonymous samples) and continue studies concerning PLE.

33. Will the PLN DNA Test results specify if the dog is a carrier?

Yes. It will tell us if the dog is homozygous positive (2 copies of both predisposing alleles), heterozygous (1 copy of each predisposing allele), or homozygous normal (no predisposing alleles).

Breeding Impact

34. If PLN was found to be polygenic, does the test differentiate the specific genes such that careful selection of carriers can be bred?

All SCWT studied so far had both of the 2 genes working in tandem – either mutated or normal, because these genes are very close together on the same chromosome (and they are both important in the glomerular filtration barrier). If we find dogs with just one of the genes mutated, we will be able to discern if one of the gene predisposing alleles is more important than the other in causing PLN.
35. Please detail possible breeding combinations.

For now, since both of these genes are close together on one chromosome and seemingly always carried together in Wheatens (in linkage disequilibrium), it is easiest to consider them together as if they were one gene in our “lingo” of homozygous, heterozygous, etc. We have listed the genotypes of homozygous positive (2 copies of the predisposing alleles at both genes, which means it got 1 copy from its sire and 1 copy from its dam), heterozygous (1 copy of the predisposing alleles that it got from either its sire or its dam), and homozygous normal (no copies). Whether a dog is homozygous positive or heterozygous depends on how many copies of the predisposing alleles it has.

36. What will be the result of breeding the following:

a. Both parents are homozygous negative: GREAT! All pups should be homozygous negative! If they aren’t, a traveling salesman got into the yard!

b. Both parents are homozygous positive (for both mutations): Oh no! All pups will be homozygous positive, which means they are at the highest risk for developing PLN. This breeding is not recommended.

c. One parent is homozygous normal, the other is homozygous positive. All pups will be heterozygous for both mutations. These pups appear to be at intermediate risk, although the statistics indicate they are at less risk than homozygous positive dogs.

d. One parent is heterozygous and the other is homozygous positive: Each pup has a 50% risk for being heterozygous and a 50% risk for being homozygous positive. This breeding is not recommended.

e. Both parents are heterozygous: Each pup has a 25% risk for being homozygous positive, 50% risk for being heterozygous, and a 25% chance for being homozygous normal. This breeding is not recommended because we get 25% with high risk.

f. One parent is heterozygous and the other is homozygous normal: Each pup has a 50% risk for being heterozygous and a 50% chance for being homozygous normal.

**IMPORTANT NOTE:** While we have discouraged several different kinds of breedings, we are going to have to be very cautious about decisions to throw out carriers or we will lose diversity in the breed and maybe reveal other genetic problems (especially if inbreeding is increased). **We will need to be very careful.** It is one of our top priorities to gather and distribute as much useful information as possible to facilitate making breeding decisions.
Health Impact For The Individual Dog

37. So the test results are back and you want to know what to do if your dog is:

1. A HOMOZYGOUS NORMAL dog (with no copies of the predisposing alleles associated with PLN) - This dog will be very popular for breeding! If it has other great qualities you want to perpetuate, go for it! However, realize that we don’t have genetic tests yet for allergies, inflammatory bowel disease, PLE, Addison’s, renal dysplasia, etc, so breed carefully away from these problems. We recommend annual screening (blood and urine tests) for all Wheatens. If this dog shows proteinuria, we need to get that feedback from you, and we will want to study these dogs more to find out why they have proteinuria despite not having the predisposing alleles that affect the structure of the slit diaphragm.

2. A HETEROZYGOTE dog (with 1 copy of the predisposing alleles) - This dog is at intermediate risk for developing PLN. It is at much lower risk than a dog with 2 copies, but it is at somewhat higher risk than a dog with no copies. Realize that the Wheagles at NCSU were not all normal, and they had 1 copy of the predisposing alleles. It’s possible that having other genetic problems such as allergies or environmental infections or inflammatory disease can provoke these dogs to get sick with PLN. It is also possible that some dogs have modifier genes that protect them, because many heterozygote dogs lived into their geriatric years. A heterozygote dog should be monitored carefully throughout its life for proteinuria, and have annual screening tests done (blood and urine).

3. A HOMOZYGOUS POSITIVE dog (with 2 copies of the predisposing alleles) – This dog has the highest risk for developing PLN itself. It should be monitored closely all its life for proteinuria (some people would test the urine 2-3 times per year beginning at age 4 years or so) in addition to the annual screening tests (blood and urine). Early intervention and treatment for proteinuria and any other problems such as allergies, infections, and inflammatory diseases may help these dogs live good quality lives for years. They may even live into their geriatric years, and we want to know about them! It’s possible that some dogs have modifier genes that protect them from getting sick.

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