

Introduction to Research Behind the PLN-Associated DNA Test

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Soft-coated Wheaten Terriers are genetically predisposed to adult onset protein-losing nephropathy (average onset, 6.3 ± 2.0 years). We performed a genome-wide association study (GWAS), using the most recently available “SNP chip”; “SNP” stands for single nucleotide polymorphism. The specific chip used was the Illumina CanineHD BeadChip with 177,000 validated SNPs.

A GWAS looks at many specific positions that are known to have DNA variations comparing affected dogs and control/normal dogs; different DNA bases have been observed in different individuals at these chromosomal positions. For example, at DNA position 11,078,912 on dog chromosome 14, the DNA bases G and T have both been observed in various dogs. These alternative forms are referred to as alleles.

The SNP chip determines what alleles are found at 177,000 different chromosomal positions, and the data from many dogs are examined to see if there are any SNPs where one allele is found in the affected dogs, and the other allele is found in the control dogs more often than would be expected by chance. Our study revealed several SNPs in one chromosomal region that had alleles associated with PLN-affected dogs, and these results were statistically significant (p value 2.22×10^{-7}) when comparing DNA samples from affected and geriatric (≥ 14 years) unaffected Wheatens, telling us that this chromosomal region contained a gene or genes that have alleles that predispose Wheatens to PLN.

Gene sequencing of candidate genes in the region revealed a single nucleotide change in each of two adjacent genes, NPHS1 and KIRREL2, which encode the proteins nephrin and Neph3/filtrin, respectively, which are found in the slit diaphragms between the podocyte foot processes of the glomeruli of the kidney. Mutations in nephrin and decreased expression of filtrin are associated with protein-losing nephropathy in humans. The alleles that predispose the dogs to PLN have an arginine amino acid (amino acids are the protein building blocks) instead of a glycine in nephrin and an arginine in place of a proline in an evolutionarily conserved proline-rich region in filtrin. These novel mutations are not described in other species, nor found in 747 dogs of 114 other breeds, except in 3 dogs, including 2 Airedales, 1 of which is doubly homozygous/affected and 1 is doubly heterozygous, and 1 Bloodhound, heterozygous for the filtrin mutation only. Risk for nephropathy is highest in dogs homozygous for the mutations. Inheritance appears complex, with variable expression, possibly additional genes

and/or environmental triggers modifying the phenotype. This is the first canine inherited podocytopathy (kidney disease involving the podocyte cells of the kidney) described.

In 145 Wheatens studied for both PLN-predisposing alleles, whenever one gene has the predisposing base change, the other gene also has the predisposing allele, so we don't know if one or the other, or both, are responsible for PLN. For now, it is easiest to consider them together. Each dog gets one copy from each parent, so if they have 2 copies of the predisposing alleles we call them homozygous affected (positive), if they have 1 copy they are called heterozygous, and if they have no copies they are called homozygous normal/clear (negative).

The statistics comparing the phenotypes (PLN affected vs. geriatric/normal dogs) and their distribution for genotypes (homozygous affected, heterozygous, and homozygous normal) showed a very strong statistically significant association for PLN in homozygous affected dogs compared with heterozygous or homozygous normal dogs. Among 145 Wheatens there were only 2 dogs that had PLN but did not have any copies of the mutations. These dogs probably had PLN due to infectious, inflammatory, or immune-mediated disease, which can be seen in any breed dog. Since we asked owners of dogs with PLN to send in their DNA samples, our sample population is biased and we were bound to get some of these acquired type PLN cases. What's more interesting is that there were 16 geriatric control dogs with 2 copies of both mutations (homozygous affected) that lived long lives, so having the mutations doesn't always lead to illness.

To try to put a numeric value on the association of the alleles (genotype) with occurrence of the disease, the odds ratios (rather than the relative risk or the absolute percentage risk) were calculated. Due to the retrospective case-control design, we can't estimate the risk of PLN in the population. We would like to know what the proportions of the homozygous affected, heterozygous and homozygous normal/clear are in the Wheaten populations, but cannot deduce that from our data. The odds ratios showed that homozygous affected dogs (2 copies) had the highest risk for PLN, heterozygous dogs (1 copy) had intermediate risk, and homozygous normal dogs (no copies) had the least risk for developing PLN.