Indications for presence of a major gene for thyroid cancer in German Longhaired Pointers

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ABSTRACT: In 2010 an abnormally large number of German Longhaired Pointers (GLP) was offered for treatment of thyroid cancer. Pedigree analysis revealed a potential monogenic background. Histopathological examination revealed a variety of sub-types of thyroid cancer. In total 20 cases, ranging from 4.5 to 13 years old, and 28 controls were genotyped with the >170K CanineHD BeadChip. A GWAS resulted in a number of, and not in a single, genome wide suggestive peaks. This lead to the hypothesis that there are two types of thyroid cancer in the GLP of which only one is highly heritable. When only affected dogs younger than 7 years were included in the GWAS, a single region with 5 SNP in complete LD between cases and controls was detected. This result suggests the presence of a major gene that explains the onset of thyroid cancer in the German Longhaired Pointer.

Keywords: Thyroid cancer; Dogs; Genetic

Introduction

Thyroid cancer is a rare type of cancer in dogs but the Golden Retriever, Beagle, and Siberian Husky are predisposed compared to other breeds (Wucherer and Wilke, 2010). Affected dogs are usually in the age of 9 to 10.4 years (Withrow and Vail, 2007). In 2010 an abnormally large number of German Longhaired Pointers (GLP) with thyroid cancer was referred to the Veterinary Oncology Referral Centre ‘De Ottenhorst’. This prompted contact with Wageningen University to request help with researching a potential genetic background.

The breeder of many of these dogs, originating from a single bitch, was contacted and that particular bitch appeared to have died of thyroid cancer. The breeder agreed to contact all the owners of offspring of this bitch, and they all agreed to collaborate. Those dogs were clinically checked and more appeared to have (until then, undiagnosed) thyroid cancer. A call in the journal of the breed club and the Dutch Journal for Veterinary Medicine revealed additional GLP dogs that have (or had) thyroid cancer. Most of the dogs were closely related, but not all. A pedigree analysis revealed that common ancestors of the parents of all affected dogs could only be found in the 1950’s and 1960’s. This was not sufficient evidence for a genetic background, as the GLP is not a common breed and popular working dogs were responsible for a large proportion of the offspring. Therefore, most dogs of that breed are related. Another potential problem is that GLP are hunting dogs, and their owners do not always tend to take their sick dogs to a veterinarian. In addition, thyroid cancer can easily be misdiagnosed as a hematoma or cyst because puncture often yields hemorrhagic fluid due to the hemorrhage and necrosis commonly found within these tumors. On top of that, breeders tend not to go public with the health problems of their breeding dogs and bitches. However, the fact that large proportions of related litters were affected by thyroid cancer still indicated a genetic predisposition.

The aim of the research presented in this paper was 1. To test the hypotheses that there is a heritable background to this disease, and 2. To determine the mode of inheritance using a case-control design. In this paper we present the first results of this research.

Materials and Methods

Data. The phenotype of the dogs was defined as whether the dog had developed thyroid cancer or not. In total 62 dogs were diagnosed with thyroid cancer, either at the Veterinary Oncology Referral Centre ‘De Ottenhorst’ or at another veterinary clinic. Blood was collected from 43 of those dogs, and from 60 dogs without thyroid cancer. It would have been ideal to have siblings as controls to the cases with thyroid cancer. However, the onset of thyroid cancer in GLP can be at an age as old as 13 years. To be certain that a dog would not develop thyroid cancer it was necessary to use dogs of at least 13 years old as controls. Two controls were half sib of two cases. However, most controls were only slightly (a ≤ 0.016) related to the cases. The controls were checked for absence of thyroid cancer by experienced staff of ‘De Ottenhorst’ by palpating the thyroid area.

There are several sub-types of thyroid cancer in dogs, very similar to the ones described in humans. Thyroid samples, obtained from 31 GLP dogs after surgical removal, were histo-pathologically examined. From those results it became clear that there was no uniformity in the type of thyroid cancer. The most common was the follicular type that occurred in 14 dogs, and in an additional 10 dogs it occurred in combination with either the compact type (4), the papillary type (4), or a combination of these types (2). Four dogs only showed the compact type, and three dogs only the papillary type. In one dog it was most extreme: in the left lobe was the compact-follicular type and in the right the papillary type. This large diversity, in combination with the common ancestors of the parents of affected dogs, resulted in the first hypothesis of a single, recessive, major gene that determines the actual onset of thyroid cancer.
Other genes no doubt are involved in determining what subtype of thyroid cancer will develop.

For financial reasons DNA was extracted and genotyped using the >170 K CanineHD BeadChip (Illumina Inc) for only 20 cases and 28 controls. To get a good cross-section of the cases in the population, the age of affected dogs in the analyses ranged from 4.5 to 13 years of age.

**Statistical analyses.** A genome-wide association study was performed with a case-control test for allele frequency differences and no additional pedigree connections. The GenABEL package (Aulchenko et al, 2007) was used for the analyses. Only SNP’s with a call-rate of at least 0.90 and a minor allele frequency of at least 0.01 were included in the analyses. No SNP’s were excluded because of deviation from HW equilibrium because the data was a selected sample. Initially there were 171760 SNP, and after removing non-informative SNP and SNP that did not meet the selection criteria, 111770 remained for further analyses. The data was of insufficient size to obtain a false discovery rate. Therefore, to identify genome-wide significance levels, a Bonferroni correction was applied using an alpha of 0.01, resulting in a \(-\log10(P)\) value of 7.05.

**Results and Discussion**

To test the hypothesis that the occurrence of thyroid cancer in GLP is determined by only a single major gene, the first analysis was performed using all data and making no distinction between the sub-types of cancer. Results showed a number of genome-wide significant peaks, but the related SNP were not in complete LD in cases compared to controls. It is very likely that there will be multiple genes involved in determining the sub-type of thyroid cancer, as is described in other types of cancer in dogs (Karlsson et al., 2013). However, our pedigree analysis suggested that a single major gene could explain the actual onset of the development of thyroid cancer, although that was not apparent from our GWAS results.

One explanation of this mismatch between results and hypothesis could be that we were dealing with phenotypes with different causal backgrounds. In other breeds, dogs developed thyroid cancer only at older age (Wucherer and Wilke, 2010), while in the GLP some of the dogs already developed it at much younger age. Therefore, the next step was to look into this effect of age and analyze only the young dogs as a separate group of cases. Seven years of age was used as threshold for the young group, resulting in eight cases with genotypes. Those were included in the second GWAS analysis as cases, while still using all 28 controls. The result was 5 SNP in a 20 cM region with a very high genome-wide significance level (GW P<0.01). These five SNP were in complete LD in the cases compared to the controls. There were other, less significant, SNP in other regions, but those SNP were not in complete LD in cases compared to controls. This combination of results suggests that our hypothesis of a single major gene influencing the onset of thyroid cancer could be true.

In the region of the 5 SNP in complete LD are a number of genes. Two of those are described in relation to cancer in human, though not thyroid cancer. This makes them interesting candidate genes. Next step we are going to take is to sequence the area in search for the causal mutation(s).

**Conclusion**

Results thus far suggest that there is a single major gene influencing the initiation of thyroid cancer development in the GLP already before dogs are seven years of age.

**Literature Cited**


