Chapter

Genomics Underlying Familial Thyroid Carcinoma in Dogs

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Abstract

Thyroid cancer is the most common endocrine neoplasm occurring in dogs. We reported familial thyroid follicular cell carcinomas (FCCs) in 54 Dutch German longhaired pointer (GLP) dogs. We investigated the genetics of the FCC in these dogs, including the germline risk mutations and somatic driver mutations. We identified the germline risk factor locating in the *TPO* gene for these hereditary FCCs through a combination of genome-wide association study (GWAS) and homozygosity mapping analyses using SNP array genotype data and whole-genome sequencing data. We further investigated the somatic mutation landscape of these FCCs using high-depth whole-genome sequencing technology of the tumors. A recurrent missense mutation in the *GNAS* gene was identified as a very promising driver mutation. We validated this somatic mutation using Sanger sequencing and revealed a prevalence of 62.5% among thyroid tumors identified in the Dutch GLPs. In addition, we can also review the findings in genetics of other canine thyroid tumors in recent years.

Keywords: thyroid carcinoma, dog, animal, germline risk factor, somatic mutation

1. Introduction

Dogs have a pair of thyroid glands that are located on each side of windpipe in the neck. Dogs, like humans, develop thyroid cancers in these glands. Thyroid cancer is the most common endocrine neoplasm, accounting for 1%–4% of all canine neoplasms [1]. Thyroid neoplasms can be classified as adenomas or carcinomas. Adenoma is a benign neoplasm, and carcinoma is malignant. Carcinomas are distinguished from adenomas by capsular and/or vascular invasion. Approximately 60%–90% of canine thyroid neoplasms are carcinomas [1]. The thyroid cancer in dogs can be unilateral or bilateral, which account for 67%–75% and 25%–35%, respectively [2]. The typical clinical sign of a thyroid tumor in dogs is the palpable mass in the neck. Other clinical complaints related to thyroid carcinoma include intermittent cough, alopecia, polyuria, polydipsia, weight loss [3].

A canine thyroid tumor can originate from either follicular cells or C-cells (parafollicular cells) in thyroid gland. Thyroid tumors in dogs consist of mainly eight histological subtypes according to a classification of World Health Organization [4], and they are highly similar to corresponding types in humans in histological growth pattern. There are seven subtypes of thyroid tumor originating from follicular cells, including follicular thyroid carcinoma (FTC), papillary thyroid carcinoma (PTC), compact

thyroid carcinoma (CTC), follicular-compact thyroid carcinoma (FCTC), poorly differentiated thyroid carcinoma, undifferentiated (anaplastic) thyroid carcinoma, and carcinosarcoma. The thyroid cancer originating from C-cells is called medullary thyroid carcinoma (MTC). Most of canine thyroid tumor originates from follicular cells, accounting for 64%–71%, which is also called non-medullary thyroid carcinoma (NMTC). Thyroglobulin is a protein made by the follicular cells of the thyroid gland, and calcitonin is made by C-cells [5, 6]. Thyroglobulin and calcitonin immunohistochemistry experiments are useful to differentiate thyroid cancer originating from follicular cells or C-cells. Thyroid carcinoma originating from follicular cells exhibits positive immunolabeling for thyroglobulin, and MTC exhibits strong immunoreactivity for calcitonin [1].

Thyroid neoplasms in dogs are usually non-functional. Clinical or biochemical evidence of hyperthyroidism can be observed in less than 25% of affected dogs. Meanwhile, hypothyroidism is also possible because of destruction of normal thyroid tissue, suppression of thyroid-stimulating hormone (TSH) secretion, and subsequent atrophy of normal thyroid tissue [2].

Metastasis is common in canine thyroid carcinoma. Approximately one out of three dog patients have metastasis by the time of diagnosis [1, 7], and 65%–90% of untreated dogs are diagnosed with regional or distant metastasis at necropsy [2]. The lung and regional lymph nodes are the most common organs where canine thyroid carcinoma metastasizes to. Metastasis to other organs is occasionally seen, such as the adrenal glands, liver, heart, brain, kidneys, and bone [1]. In dogs, risk of thyroid tumor increases with age. The average age at diagnosis is between 9 and 10 years. Dogs at age > 10 years have a significantly higher risk of thyroid cancer than younger dogs [1, 8].

Canine thyroid tumors show also some differences if compared with humans. For instance, in humans, females have approximately 2–3 times higher risk for non-medullary thyroid cancer than males [9]. While, in dogs, most studies reported equal incidence of thyroid carcinoma in both sexes [3, 8]. The mechanism underlying the sex bias in incidence of thyroid cancer in humans is still unclear. Another difference is that the most prevalent subtype of thyroid cancer is different between humans and dogs. In humans, PTC is the most frequently diagnosed thyroid cancer, accounting for approximately 85%–90% [10]. However, in dogs, PTC is relatively rare, and FTC is the most common type of thyroid carcinoma [1].

2. A familial canine thyroid carcinoma

Reports of hereditary thyroid carcinoma in dogs are limited. One report presented a hereditary canine MTC with a potential dominant inheritance of autosomal or X-linked inheritance in a family of a mixed dog breed with Alaskan malamute as a major influence [11]. We reported a hereditary thyroid follicular cell carcinoma in a large number of Dutch German longhaired pointer (GLP) dogs [3]. Over the past ~20 years, thyroid tumor was identified in 84 Dutch GLPs. Among those affected GLPs, 54 had histologically diagnosed thyroid follicular cell carcinomas (FCCs), 29 were suspected cases solely based on clinical diagnosis (such as palpable mass in the neck), and 1 had thyroid adenoma. The identified histology subtypes of the FCC include FTC, CTC, FCTC, PTC, and carcinosarcoma. The same as findings in other studies about canine thyroid tumor [1], FTC is the most common among all these subtypes, accounting for 46%. Meanwhile, no sex predisposition was observed in those affected GLPs. Canine FTC

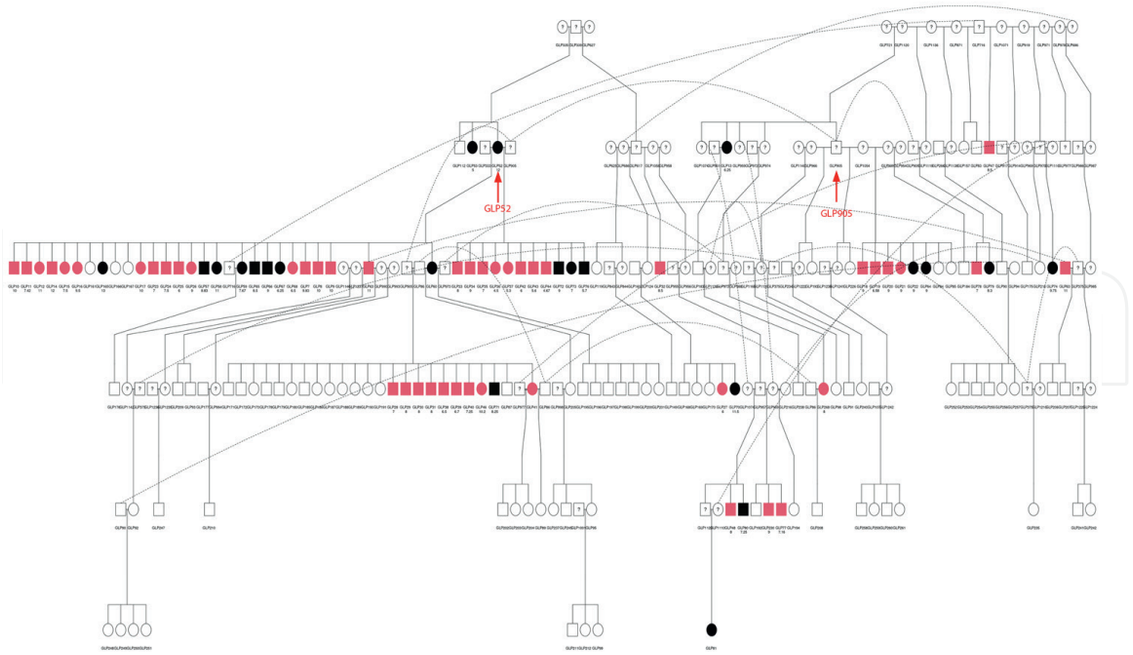

Figure 1.
Pedigree of dogs related to two dogs (pointed by red arrows) that were intensively used in breeding [3]. Forty-five histopathologically confirmed affected dogs are closely related to these two dogs. Circles represent females, and squares represent males. Dot line shows identical dogs. Affected dogs with histological diagnosis are highlighted in red, and suspected affected dogs (without histopathology diagnosis) are in black, whereas unaffected dogs remain white. A question mark represents the dogs with unknown status.

incidence increases with age where approximately 57% of cases were diagnosed at ages between 10 and 15 years. However, FCCs in these GLPs were diagnosed earlier where 76% of cases were diagnosed before 10 years of age.

These affected GLPs are very closely related in terms of genetic relationship, where many of them are first-degree relatives, suggesting that these FCCs belong to a familial form of thyroid carcinoma (**Figure 1**). In humans, a hereditary thyroid cancer is diagnosed when there are two or more first-degree relatives affected [12]. In dogs, according to authors' knowledge, there is no definition of a hereditary thyroid cancer yet. The definition of hereditary thyroid carcinoma in humans can be borrowed. A striking high incidence of the FCC in offspring of two GLPs was seen (**Figure 1**). Most of identified affected dogs are related to these two GLPs in pedigree. In the past, in dog breeding, some prominent dogs were used intensively including breeding with relatives, which resulted in the introduction of genetic defects and spread unwanted diseases in the population. The familial FCC here is a good example.

3. Germline risk factor of the familial FCC

Cancer is a disease caused by one or multiple mutation(s) in the genome of cells. A familial cancer has one or multiple germline causal mutations that are inherited from parents of the individual. Identification of germline causal variants has important value for both cancer prevention factors of human thyroid cancer. GWAS is a statistical method to identify genomic regions that are associated with targeted traits/disease taking advantage of linkage disequilibrium between genomic variants.

Linkage disequilibrium is the non-random association of alleles at different genomic loci in a given population. It makes it possible to identify the genomic region associated with the investigated trait/disease even when the causal loci are not genotyped. After a GWAS analysis, fine-mapping can be performed to identify the causal variants in the targeted genomic region. Whole-genome sequencing data are usually used in fine-mapping, which can identify all the variants when compared with the reference genome. In order to confirm the causal variants and underlying molecular mechanism, further *in vitro* or *in vivo* experiments are needed., diagnosis, and novel drug development. Next-generation sequencing technologies make it easy to obtain whole-genome sequencing data of an individual. However, identification of germline causal mutations for a disease/cancer is still challenging. In the past decade, many genome-wide association studies (GWASs) have been performed to identify germline risk.

Identification of germline risk factor of a hereditary form of thyroid cancer is still challenging. In humans, only approximately 5% of a form of familial non-medullary thyroid cancers have well-documented germline risk factors [13]. We performed a series of analyses based on a combination of SNP array genotype data and whole-genome sequencing data to identify the germline risk mutation that confers a higher risk for thyroid carcinoma in Dutch GLPs [14]. We combined a GWAS analysis and a homozygosity mapping to identify the genomic region that is associated with the FCC. This combined strategy was used because clear population stratification was observed between the genotyped affected and unaffected dogs. In the GWAS analysis, to correct over false-positive discoveries, genomic relationship matrix estimated based on genotype data was incorporated as a random effect. Homozygosity mapping was also used because the FCC in these dogs has very likely an autosomal recessive inheritance pattern according to pedigree. Homozygosity mapping used in that study is based on runs of homozygosity (ROH)-based approach, which is a powerful method to identify genomic region that is associated with a recessive disease. A common genomic region was identified by both the GWAS and homozygosity mapping analyses. Next, we performed fine-mapping using WGS data of 11 affected and 11 unaffected GLPs to identify the germline mutations that are in the targeted region. A series of stringent filtering was performed on the variants identified in the targeted

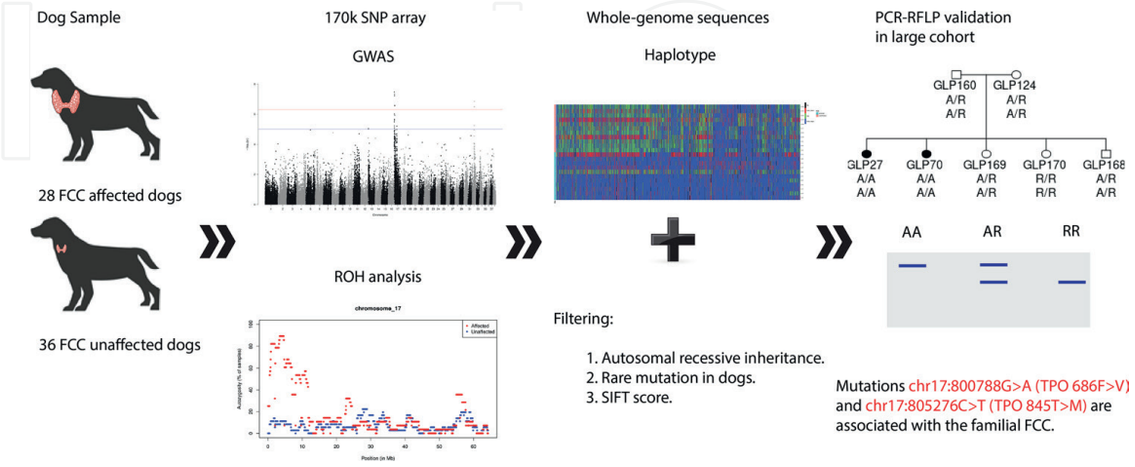

Figure 2.
Graphic abstract of identification of the germline risk mutations in the familial FCC [14]. 170K SNP array genotype data were obtained from 36 healthy German longhaired pointer (GLP) dogs and 28 GLPs affected by the familial thyroid follicular cell carcinoma. The genotype data were used in the genome-wide association analysis and homozygosity mapping to identify genomic region associated with the familial thyroid follicular cell carcinoma.

genomic region based on WGS data: 1) must be deleterious predicted by pathogenicity prediction tools; 2) must fit an autosomal recessive inheritance pattern; 3) must be rare in general dogs; 4) must be conserved across species. At the end, we identified two deleterious mutations, chr17:800788G>A (p.686F>V) and chr17:805276C>T (p.845T>M), in the *TPO* gene. We further genotyped these two variants in 186 GLPs (59 affected and 127 unaffected) using PCR-RFLP experiment and revealed 16.94 and 16.64 of the relative risk of homozygous recessive genotypes compared with homozygous genotypes for the reference allele (**Figure 2**).

The genetic cause of general canine thyroid cancer is still poorly studied. There is no study that investigated the genetic causes of canine thyroid cancer at a genome-wide scale, except for our study. Genetic causes of canine thyroid cancer need to be revealed since thyroid tumor is the most frequent endocrine neoplasm in dogs.

4. Somatic mutations in canine thyroid cancer

Most of thyroid carcinomas in dogs are sporadic, the same as it is in humans. Sporadic cancers are caused by somatic mutations that occur in somatic cells, which are different from germline causal mutations in hereditary cancers. These somatic mutations are not inherited from parents of the individual, but are acquired by random DNA replication error during cell divisions that occurred by chance or due to exogenous or endogenous carcinogens that can increase the risk for the cancer. These exogenous carcinogens include smoking and X-ray. Endogenous carcinogen includes reactive oxygen species produced during metabolism [15]. When these mutations occur in proto-oncogene or tumor-suppressor gene, then a cancerous cell may arise.

Identification of somatic mutation at a genome-wide scale becomes possible with the development of next-generation sequencing technologies. The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) projects have profiled tens of thousands of human cancers of different types and origins [16, 17]. Normally, whole-genome or whole-exome sequences of tumor tissue and matched normal tissue (blood or healthy tissue adjacent to tumor tissue) are generated and compared to identify those somatic mutations that are unique to tumor cells. Among somatic mutations, according to their role in tumorigenesis, driver and passenger mutations are defined. Driver mutations are somatic mutations that are important to the tumor initiation and growth, and passenger mutations are those neutral mutations that do not contribute to tumorigenesis. Identification of driver mutation is one of the major tasks in oncogenic research. Identification of driver mutations sheds light on molecular mechanisms underlying tumor initiation and development. Those driver mutations have potential value to be used to develop targeted treatment to kill cancerous cells.

Somatic mutations of human thyroid carcinoma have been extensively investigated at a genome-wide scale. Somatic mutations of canine thyroid carcinoma are still poorly studied. A somatic mutation in *P53* gene has been identified in canine FTC [18]. We profiled the somatic mutations of the hereditary FCC identified in Dutch GLPs at a genome-wide scale [19]. As far as we know, there was no genome-wide profile of somatic mutations in canine thyroid carcinoma before our study. In our study, a missense somatic mutation in the *GNAS* gene, p.A204D, stands up where it was identified in four of seven FCC samples that were whole-genome sequenced and validated in 20 out of the 32 affected dogs' thyroid tumor samples [19]. This

high prevalence of the somatic mutation is a strong evidence of the driver role of this mutation in these canine thyroid carcinomas.

The *GNAS* gene encodes the alpha-subunit of stimulatory G-protein (Gαs) that can activate adenylyl cyclase downstream of G-protein-coupled receptors (GPCRs). Activated adenylyl cyclase increases cellular cyclic adenosine monophosphate (cAMP). cAMP is an important second messenger that can upregulate many downstream molecular signaling cascades, including pathways involved in cell proliferation, such as the PKA signaling pathway [20].

The *GNAS* gene is a known proto-oncogene. Somatic mutations in the *GNAS* gene have been identified in many different types of tumors in humans. It is known that activating mutations in the *GNAS* gene can result in increased cell division in humans. The most common activating mutations in the *GNAS* identified in human tumors are p.R201C/H/S and p.Q227R/L [21]. According to an investigation in 274,694 human tumors, appendiceal adenocarcinoma has highest frequency of *GNAS* activating mutation (35.9%). Ovarian carcinosarcoma, rectum adenocarcinoma, gastroesophageal junction adenocarcinoma, stomach adenocarcinoma diffuse type, small intestine adenocarcinoma, stomach adenocarcinoma, esophagus adenocarcinoma, breast carcinoma, colon adenocarcinoma, breast invasive ductal carcinoma, and duodenal adenocarcinoma have prevalence of *GNAS* somatic mutation in the range between 5% and 7% [21]. However, prevalence of somatic mutation in the *GNAS* gene in human thyroid cancer seems to be low where only 13 of 1,837 human thyroid neoplasms capture *GNAS* somatic mutations. Likewise, somatic mutations in the *GNAS* gene were identified in only two out of 496 PTC samples that were included in the TCGA project [22].

Besides our genome-wide study, Campos et al. investigated somatic mutation landscape of 43 canine FCCs and 16 canine MTCs by targeted sequencing of some driver genes identified in human thyroid carcinoma [23]. Those genes include *HRAS*, *KRAS*, *PIK3CA*, *BRAF*, *RET*, and *PTEN* genes. However, they only identified two missense mutations in the *KRAS* gene that are homologous to mutations identified in human thyroid carcinoma. No somatic mutation in other genes under investigation was identified. This seems to suggest that canine thyroid carcinoma uses different driver mutations compared with human thyroid carcinoma. In our study, *GNAS* p.A204D somatic mutation was observed in FCC neoplasms of 62.5% of affected GLPs. However, in human PTCs, *GNAS* somatic mutation is rarely observed. This suggests the potential difference in driver events of thyroid carcinoma between humans and GLPs. We suggest that the prevalence of the *GNAS* somatic mutation in more canine thyroid tumors should be investigated because it might be a major driver mutation of canine thyroid tumor according to our study. Meanwhile, we also suggest investigating driver mutations in sporadic canine thyroid carcinomas at a genome-wide scale to elucidate the molecular mechanisms underlying canine sporadic thyroid tumor initiation and development and to investigate the potential value of dogs with sporadic thyroid carcinoma to be used as disease models.

5. Pathways involved in thyroid carcinoma in dogs

In humans, molecular signaling pathways that are involved in thyroid carcinoma are extensively investigated. The most dominant molecular signaling pathways are the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K)/Akt pathways [24]. Activation of these pathways as a result of activating mutations

in proto-oncogenes (such as *BRAF* and *RAS* genes) involved in the pathway leads to cancerous cells. In dogs, PI3K/AKT pathway is also involved in the pathogenesis of thyroid carcinoma with the evidence of increased expression of several genes associated with the pathway [23]. However, the involvement of MAPK pathway in canine thyroid carcinoma development needs to be investigated. Besides the evidence of increased expression of genes, to confirm the role of these pathways in the canine thyroid carcinoma development, somatic mutations in those genes should also be further investigated.

6. Inbreeding and disease incidence

Inbreeding is the production of offspring from mating or breeding of individuals or organisms that are closely related [25]. It can be technically defined as mating of a pair of animals with relationship that is closer than the average level within the breed or population studied. In domesticated animals, inbreeding can decrease the performance of animals, such as decrease in milk yield, which is termed as inbreeding depression. The genetic basis of inbreeding depression is that increased homozygosity with inbreeding increases the frequency of unfavorable genotypes within the population [25]. Inbreeding can increase the incidence of inherited diseases in the population, especially those with a recessive inheritance pattern. In our studies, we estimated inbreeding coefficients using either pedigree or genome-wide genotype data. We observed significantly higher inbreeding levels in affected GLPs (average pedigree-based inbreeding: 0.23, average genotype-based inbreeding: 0.51) in comparison to those unaffected GLPs (average pedigree-based inbreeding: 0.14, average genotype-based inbreeding: 0.48). This suggests the importance of inbreeding control in preventing inherited diseases, including cancer, in pedigree dog breeding.

7. The usage of identified germline and somatic mutations

Identification of germline risk mutations can be valuable for cancer prevention. For hereditary cancer with a simple inheritance pattern, the identified germline risk mutation can be used to identify those individuals that have a higher risk for the cancer through a genetic test. Those animals then can be removed from the breeding program to eradicate the inherited cancer from the population. Without a genetic test, it is hard to completely remove a disease with an autosomal recessive inheritance pattern from the population because of difficulty in identifying those heterozygous animals. For a cancer with a complex inheritance pattern, a polygenic risk score can be calculated based on all identified germline risk mutations to predict the risk of the cancer development for an individual. For those people with a high risk score for certain disease, early prevention, such as healthy dietaries and life styles, can be taken to diminish the risk. Frequent examination can also be arranged to detect the disease earlier and cure easier.

We developed a genetic test based on one of the germline risk mutation (chr17:800788G>A) in the *TPO* gene to identify GLPs that have a higher risk for the FCC. This genetic test is now commercially available for GLP breeders and owners after testing 142 GLPs at the Animal Breeding and Genomics laboratory. To date, this genetic test has been performed on more than 150 GLPs from a few countries. The frequency of germline risk allele is 25.4% in those tested GLPs. This frequency is

rather high and indicates that the risk allele is hard to be completely eradicated from the population by conventional breeding strategy. The genetic test can be especially valuable for GLP breeders to breed healthy dogs. It enables breeders to find those dogs at a high risk for the FCC before any signs of the disease and then those dogs can be excluded from the breeding program.

8. Germline and somatic mutation interaction

TPO gene encodes an enzyme, thyroid peroxidase, which plays an important role in production of thyroid hormones. There are seven key steps in the thyroid hormone synthesis: 1) iodine uptake into thyroid follicular cells by the sodium/iodide symporter (NIS); 2) synthesis of two key proteins, thyroid peroxidase (TPO) and thyroglobulin (TG), and secretion of TG into the follicular lumen; 3) iodide transport into the follicular lumen; 4) iodide oxidation to form iodine by TPO; 5) iodination of TG tyrosine residues to generate monoiodotyrosine (MIT) and diiodotyrosine (DIT) by TPO; 6) coupling of iodotyrosines to form thyroxine (T4) and triiodothyronine (T3) by TPO; 7) endocytosis of TG-thyroid hormone complex and T3 and T4 cleaved from it by proteases in the lysosomes [26, 27]. TPO is involved in steps 4, 5, and 6. Meanwhile, hydrogen peroxide (H_2O_2) is needed in those reactions catalyzed by the TPO. We suspect that germline mutations identified in the *TPO* gene may impair the activity of the TPO enzyme and result in less consumption of H_2O_2, therefore increased level of H_2O_2. This assumption needs to be validated in future using, for instance, cell experiments. However, hydrogen peroxide is a type of reactive oxygen species that can induce DNA damages. Elevated H_2O_2 probably induces many somatic mutations occurring in the thyroid follicular cells and finally a cancerous cell form when a driver mutation occurs. In those familial FCCs, one of driver mutations is the recurrent somatic mutation identified in the *GNAS* gene.

9. Medullary thyroid cancer in dogs

Regarding MTC, both spontaneous and hereditary forms have been reported in dogs [7, 11, 28, 29]. Up to 20%–30% of human hereditary MTC is caused by activating mutations in the *RET* proto-oncogene [30]. In the hereditary MTCs that were studied by Lee et al., the authors sequenced the *RET* gene but identified no mutation in that gene [11]. The germline genetic causes of canine MTC including the somatic driver mutations are still not clear. Canine familial MTC is similar to human familial MTC in clinical symptoms and morphology of histology, suggesting their value to be used as a disease model. However, unraveling the genetic basis of canine MTC is needed for that purpose.

10. Thyroid cancer in other species

Besides the occurrence in dogs, thyroid tumor has also been reported in many other species, such as guinea pig [31], cat [32], horse [33], cattle [34, 35], barred owl [36], rat [37], and ferret [38, 39]. Thyroid hyperplasia was seen in fish over one hundred years ago, and thyroid neoplasms were also reported in fish [40]. Thyroid carcinoma has been induced in mice using transgenic technology for the study of

pathogenesis [41]. Genetic causes of thyroid carcinoma in these species (except for mice) are generally not known. Mapping of the genetic causes of thyroid carcinoma in these species is needed to elucidate the molecular mechanism of tumorigenesis. However, it is challenging due to the difficulty in identifying sufficient amount of cases for causal mutation mapping in those species.

11. Dogs as cancer models

An animal model is an indispensable model to investigate the pathogenesis, molecular mechanisms of tumor initiation and development and to test novel treatments. Scientists have increasing interest in dog disease models in recent years. Dogs may have many advantages to be used as disease models compared with the most popular model species such as mouse and rat [42]. Dogs are the most popular companion animals globally and are the second in medical surveillance and preventative health care after humans [42]. Dogs are more similar to humans such as in genetics, immune systems, body size. Meanwhile, pet dogs share living environment with their owners, thus receiving similar environment factors. There is, for example, a gap between mouse model and chimpanzee models in clinical trials. There is no doubt that mice contribute a lot to novel drug development. However, it is also true that many drugs work during trials on mice but fail in human clinical trials because of difference in physiology between these two species.

Breed dogs have in general low genetic diversity due to two bottleneck events in history: one is domestication from grey wolf around 15,000 years ago, and another one is strong artificial selection in order to fix certain traits of breeds during the breed formation in the past ~200 years [43]. This low genetic diversity results in many genetic diseases/disorders, such as osteosarcoma in Rottweiler, elbow dysplasia in Labrador Retriever, and medial patellar luxation in Chihuahua [44, 45]. According to a study of Farrell et al., there are 396 hereditary disorders identified in 215 officially recognized dog breeds in the United Kingdom [46]. Many of these disorders also occur in humans, which makes those dogs potentially valuable disease models to investigate the pathogenesis of those disorders. Low genetic diversity within each dog breed makes mapping of causal variants of those diseases easier in dogs than that in humans. Much smaller number of SNPs are needed to identify the genomic region that is associated with an inherited disease in dogs in comparison to humans because of the low genetic diversity and large haplotype blocks [42]. This facilitates unraveling the genetic basis of inherited disease.

In rodents, thyroid carcinoma usually has a follicular architecture but does not have the morphological or cytological characteristics used in the diagnosis of papillary carcinomas in humans [37]. Canine thyroid carcinoma is more similar to human thyroid carcinoma in morphology of histology in comparison to thyroid carcinoma in rodents. GLPs with familial FCCs can be a valuable disease model to elucidate the molecular mechanism underlying tumor initiation and development with driver mutations in the *GNAS* gene.

12. Conclusion

We summarized our research regarding a familial thyroid follicular cell carcinoma in Dutch German longhaired pointer dogs. We identified two deleterious mutations

in the *TPO* gene that confer great relative risk in homozygous status. A genetic test was developed and made commercially available for breeders and owners of GLPs. We expect to greatly diminish the incidence of the familial FCC in GLPs by using this genetic test and will help to eradicate the familial FCC from the GLP population. Furthermore, we identified a recurrent somatic mutation, the *GNAS* p.A204D, in the familial FCCs with a prevalence of 62.5%. Identification of this somatic mutation implicates the role of GPCR-mediated molecular signaling pathways in the FCC initiation and development. With a high prevalence of the *GNAS* p.A204D somatic mutation, those affected dogs might serve as a good disease model to understand the pathogenesis of tumors associated with *GNAS* somatic mutations and to test novel treatments that targets on tumors with *GNAS* somatic mutations.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Hassan BB, Altstadt LA, Dirksen WP, Elshafae SM, Rosol TJ. Canine thyroid cancer: Molecular characterization and cell line growth in nude mice. Veterinary Pathology. 2020;**57**(2):227-240

[2] Liptak JM. Canine thyroid carcinoma. Clinical Techniques in Small Animal Practice. 2007;**22**(2):75-81

[3] Yu Y, Krupa A, Keesler RI, Grinwis GCM, de Ruijsscher M, de Vos J, et al. Familial follicular cell thyroid carcinomas in a large number of Dutch German longhaired pointers. Veterinary and Comparative Oncology. 2022;**20**(1):227-234

[4] Kiupel M, Capen C, Miller M, Smedley R. Histological Classification of Tumors of the Endocrine System of Domestic Animals. Armed Forces Inst. of Pathology; 2008

[5] Peiris AN, Medlock D, Gavin M. Thyroglobulin for monitoring for thyroid cancer recurrence. JAMA. 2019;**321**(12):1228

[6] Garrett JE, Tamir H, Kifor O, Simin RT, Rogers KV, Mithal A, et al. Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. Endocrinology. 1995;**136**(11):5202-5211

[7] Campos M, Ducatelle R, Kooistra HS, Rutteman G, Duchateau L, Polis I, et al. Immunohistochemical Expression of Potential Therapeutic Targets in Canine Thyroid Carcinoma. Journal of Veterinary Internal Medicine. 2014;**28**(2):564-570

[8] Wucherer KL, Wilke V. Thyroid cancer in dogs: An update based on 638 cases (1995-2005). Journal of the American Animal Hospital Association. 2010;**46**(4):249-254

[9] Patel S, Pappoppula L, Guddati AK, Annamaraju P. Analysis of race and gender disparities in incidence-based mortality in patients diagnosed with thyroid cancer from 2000 to 2016. International Journal of General Medicine. 2020;**13**:1589-1594

[10] Pusztaszeri M, Auger M. Update on the cytologic features of papillary thyroid carcinoma variants. Diagnostic Cytopathology. 2017;**45**(8):714-730

[11] Lee J-J, Larsson C, Lui W-O, Höög A, Von Euler H. A dog pedigree with familial medullary thyroid cancer. International Journal of Oncology. 2006;**29**(5):1173-1182

[12] Nosé V. Familial thyroid cancer: A review. Modern Pathology. 2011;**24**:S19

[13] Guilmette J, Nosé V. Hereditary and familial thyroid tumours. Histopathology. 2018;**72**(1):70-81

[14] Yu Y, Bovenhuis H, Wu Z, Laport K, Groenen MAM, Crooijmans RPMA. Deleterious mutations in the TPO gene associated with familial thyroid follicular cell carcinoma in dutch german longhaired pointers. Genes. 2021;**12**(7):997

[15] Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. Science. 2015;**349**(6255):1483-1489

[16] Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, et al. The cancer genome Atlas Pan-cancer analysis project. Nature Genetics. 2013;**45**(10):1113-1120

[17] Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, Bernabé RR, et al. International network of cancer genome projects. Nature. 2010;**464**(7291):993-998

[18] Devilee P, Van Leeuwen IS, Voesten A, Rutteman GR, Vos JH, Cornelisse CJ. The canine p53 gene is subject to somatic mutations in thypoid carcinoma. Anticancer Research. 1994;**14**(5a):2039-2046

[19] Yu Y, Manders F, Grinwis GCM, Groenen MAM, Crooijmans RPMA. A recurrent somatic missense mutation in GNAS gene identified in familial thyroid follicular cell carcinomas in German longhaired pointer dogs. BMC Genomics. 2022;**23**(1):669

[20] Turan S, Bastepe M. GNAS spectrum of disorders. Current Osteoporosis Reports. 2015;**13**(3):146-158

[21] Tirosh A, Jin DX, De Marco L, Laitman Y, Friedman E. Activating genomic alterations in the Gs alpha gene (GNAS) in 274 694 tumors. Genes, Chromosomes & Cancer. 2020;**59**(9):503-516

[22] Agrawal N, Akbani R, Aksoy BA, Ally A, Arachchi H, Asa Sylvia L, et al. Integrated genomic characterization of papillary thyroid carcinoma. Cell. 2014;**159**(3):676-690

[23] Campos M, Kool MMJ, Daminet S, Ducatelle R, Rutteman G, Kooistra HS, et al. Upregulation of the PI3K/Akt pathway in the tumorigenesis of canine thyroid carcinoma. Journal of Veterinary Internal Medicine. 2014;**28**(6):1814-1823

[24] Zaballos MA, Santisteban P. Key signaling pathways in thyroid cancer. The Journal of Endocrinology. 2017;**235**(2):R43-R61

[25] Doekes HP, Veerkamp RF, Bijma P, de Jong G, Hiemstra SJ, Windig JJ. Inbreeding depression due to recent and ancient inbreeding in Dutch Holstein–Friesian dairy cattle. Genetics, Selection, Evolution. 2019;**51**(1):54

[26] Rousset B, Dupuy C, Miot F, Dumont J. Chapter 2 Thyroid hormone synthesis and secretion. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc, 2000

[27] Koibuchi N. Molecular mechanisms of thyroid hormone synthesis and secretion. Nihon Rinsho. 2012;**70**(11):1844-1848

[28] Arias ES, Castillo V, Trigo R, Aristarain MC. Multiple endocrine neoplasia similar to human subtype 2A in a dog: Medullary thyroid carcinoma, bilateral pheochromocytoma and parathyroid adenoma. Open Veterinary Journal. 2016;**6**(3):165-171

[29] Piñeyro P, Vieson MD, Ramos-Vara JA, Moon-Larson M, Saunders G. Histopathological and immunohistochemical findings of primary and metastatic medullary thyroid carcinoma in a young dog. JVS. 2014;**15**(3):449-453

[30] Schmid KW. Histopathology of C cells and medullary thyroid carcinoma. In: Raue F, editor. Medullary Thyroid Carcinoma: Biology–Management–Treatment. Cham: Springer International Publishing; 2015. pp. 41-60

[31] Gibbons PM, Garner MM, Kiupel M. Morphological and immunohistochemical characterization of spontaneous thyroid gland neoplasms in guinea pigs (*Cavia porcellus*). Veterinary Pathology. 2012;**50**(2):334-342

[32] Iwamoto Y, Kassai T. Two cases of feline thyroid carcinoma. Journal of Animal Clinical Medicine. 2017;**26**(2):76-80

[33] Saulez MN, Viljoen A, Kafka U, Rubio-Martinez L, van Wilpe E, Steyl J. The use of nuclear imaging for a mixed C cell microfollicular carcinoma of the thyroid gland in a mature horse. Equine Veterinary Education. 2013;**25**(3):118-125

[34] Vítovec J. Epithelial thyroid tumors in cows. Veterinary Pathology. 1976;**13**(6):401-408

[35] Harmon BG, Kelley LC. Immunohistochemistry of ultimobranchial thyroid carcinomas in seven slaughtered cows and one bull. Journal of Veterinary Diagnostic Investigation. 2001;**13**(2):101-105

[36] Brandão J, Manickam B, Blas-Machado U, Cohen E, Mejia-Fava J, Divers S, et al. Productive thyroid follicular carcinoma in a wild barred owl (Strix varia). Journal of Veterinary Diagnostic Investigation. 2012;**24**(6):1145-1150

[37] Bartsch R, Brinkmann B, Jahnke G, Laube B, Lohmann R, Michaelsen S, et al. Human relevance of follicular thyroid tumors in rodents caused by non-genotoxic substances. Regulatory Toxicology and Pharmacology. 2018;**98**:199-208

[38] Wills TB, Bohn AA, Finch NP, Harris SP, Caplazi P. Thyroid follicular adenocarcinoma in a ferret. Veterinary Clinical Pathology. 2005;**34**(4):405-408

[39] Fox JG, Dangler CA, Snyder SB, Richard MJ, Thilsted JP. C-cell carcinoma (medullary thyroid carcinoma) associated with multiple endocrine neoplasms in a ferret (*Mustela putorius*). Veterinary Pathology. 2000;**37**(3):278-282

[40] Fournie JW, Wolfe MJ, Wolf JC, Courtney LA, Johnson RD, Hawkins WE. Diagnostic criteria for proliferative thyroid lesions in bony fishes. Toxicologic Pathology. 2005;**33**(5):540-551

[41] Shimamura M, Shibusawa N, KurashigeT,MussazhanovaZ,MatsuzakiH, Nakashima M, et al. Mouse models of sporadic thyroid cancer derived from BRAFV600E alone or in combination with PTEN haploinsufficiency under physiologic TSH levels. PLoS One. 2018;**13**(8):e0201365

[42] Davis BW, Ostrander EA. Domestic dogs and cancer research: A breed-based genomics approach. ILAR Journal. 2014;**55**(1):59-68

[43] Frantz LAF, Bradley DG, Larson G, Orlando L. Animal domestication in the era of ancient genomics. Nature Reviews. Genetics. 2020;**21**(8):449-460

[44] Boge GS, Moldal ER, Dimopoulou M, Skjerve E, Bergström A. Breed susceptibility for common surgically treated orthopaedic diseases in 12 dog breeds. Acta Veterinaria Scandinavica. 2019;**61**(1):19

[45] Edmunds GL, Smalley MJ, Beck S, Errington RJ, Gould S, Winter H, et al. Dog breeds and body conformations with predisposition to osteosarcoma in the UK: A case-control study. Canine Medicine and Genetics. 2021;**8**(1):2

[46] Farrell LL, Schoenebeck JJ, Wiener P, Clements DN, Summers KM. The challenges of pedigree dog health: Approaches to combating inherited disease. Canine Genetics and Epidemiology. 2015;**2**(1):3