Clinical Characteristics and Molecular Basis of Hereditary Skin Diseases in Dogs

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Abstract. Genetic skin diseases in dogs are conditions that are inherited, begin during their life (e.g. pruritus), affect the health and welfare of the dog, and recently have become an area of clinical research . The objective of this study was to provide a comprehensive review of canine genetic skin disease at the clinical, molecular, inheritance patterns, and research methodologies levels. The data suggest that dog breeds have distinct phenotypic characteristics, for example, Labrador Retrievers with pathogenic mutations in the PNPLA1 gene have ichthyosis-like scales versus Goldens that have mutations in the ABHD5 gene and have more hyperatic symptoms. Collie Shepherds with a pathogenic mutation in the STS gene, had some similarities and differences (gender differences) but all have X-linked ichthyosis. There are core pathogenic genes including PNPLA1, ABHD5, STS and KRT10 that disrupt the skin functions affecting lipid metabolism (dyslipidemia), keratinocyte abnormal differentiation and barrier difficulties. Modes of inheritance included autosomal recessive, autosomal dominant and x-linked. Useful molecular techniques suggested include whole genome sequencing, exome sequencing, and/or gene chips, combined with cosegregation and or genome wide association studies (GWAS) to identify disease loci. The study presents and supports an evidence-based approach for breed based diagnosis, genetic screening, and selective breeding improvement of canine genetic skin disease.

Keywords: Molecular detection; canine hereditary skin diseases; genetic pattern; clinical phenotype.

1. Introduction

Genetic skin conditions in dogs represents an important area of genetic and veterinary research with squamous keratosis and other related disorders of keratinization having relatively high incidence rates, clinical variation in phenotype, and more importantly, a significant impact on dog welfare. Canine squamous keratosis and related keratinization disorders exhibit abnormal keratinization of canine skin often presenting clinical signs such as peeling, scarring, and itching resulting in discomfort in dogs and often leading to secondary infections once skin has been damaged [1]. Although the clinical signs of canine keratinization have long been recognized, recent advances in genomic technologies now provide an unprecedented opportunity to identify candidate mutations in genes that may be responsible for the keratinization disorders, and also demonstrate their molecular mechanisms and patterns of inheritance. The aims of this article, on genetic disorders of canine skin, are to summarize the current knowledge with regard to canine squamous keratosis and its related disorders, particularly the 'mutation' of breeds, the pattern of inheritance described, and the implications of this knowledge to veterinary and breeding contexts.

Canine ichthyosis is a genetically heterogeneous condition, and genetic studies suggest that different breeds of dogs have different mutated genes. Early investigations established that canine ichthyosis had clinical diversity, and thus it could be seen in many dog breeds and required genetic study. Subsequent studies have documented the specific pathogenic mutations and mutation types, enhancing the body of knowledge regarding the pathogenesis basis of canine ichthyosis.

Marschall and colleagues reported a new frameshift mutation in the PNPLA1 gene, which is involved in lipid metabolism and the functioning of the skin barrier [2]. It is known that the mutation in the PNPLA1 gene is not linked to ichthyosis in individuals affected by the mutation and can be classified as pathogenic. Kiener et al., reported that frameshift deletion of the ABHD5 gene in Golden Retrievers caused ichthyosis and linked the condition to defective lipid processing in skin [3]. The

ABHD5 mutation had previously been connected to human ichthyosis implying the evolutionary conservation of the genetic basis of keratosis.

Dufaure et al. reported X-linked ichthyosis in Collie Collies, which is a subtype of ichthyosis caused by mutations linked to the X chromosome. Dufaure et al. reported a mutation in the STS gene associated with steroid sulfatase activity [4]. The findings explain the observed differences between sexes in affected Collie Collies, as male dogs (XY) experience more extreme symptoms based on the lack of the second X chromosome. Overall these studies considering specific breeds suggest that canine ichthyosis is caused by genetic mutations causing abnormalities relating to lipid metabolism, steroid processing and keratinocyte differentiation confirming findings reported in human dermatological studies [1,5].

In addition to ichthyosis, the broader area of research on hereditary skin diseases also highlights the role of genetic loci in diseases associated with sebaceous gland inflammation, as seen in Samoyed dogs [6]. Even though there are differences between these diseases and ichthyosis, it advances knowledge about genetic variability and how it can influence dog skin health, and recognizes the importance of breed specific genetic analysis [7].

The exploration of canine ichthyosis and heritable skin conditions has remarkable importance for various stakeholders including veterinarians, breeders, and comparative geneticists. First, once pathogenic mutations are identified we can develop genetic tests for responsible breeding," which is critical for responsible feeding. In this scenario, the genetic screening provides breeders the opportunity to avoid matings between carriers, thereby reducing the incidence of hereditary ichthyosis in those breeds at risk. This occurs not only at the level of the puppies to improve quality of life by not allowing sick puppies to be born, but at the level of the breed, which can now be better protected for the betterment of the breed and its ability to be passed to subsequent and future generations. Second, these studies will enhance our clinical management of dermatological conditions, and the understanding of molecular basis of these diseases will facilitate targeted therapy. For example, if mutations in PNPLA1 or ABHD5 are identified in certain breeds, this could lead to new therapeutic interventions aimed at restoration of lipid barrier function [2,3]. Leeb et al. similarly noted these findings elucidate clinical practice—closing the gap between genetics and clinical practice—which means personalized veterinary care is a realistic and tangible outcome. Finally, canine models also have significance for understanding dermatology in humans. In fact, owners and veterinarians alike should not be surprised at the similar genetic and biochemical basis of ichthyosis in dogs and humans (e.g., ABHD5 or STS mutations) [3,4,6]. In many respects, canine models are comparable disease mechanisms and potential therapeutic interventions to human Dermatology. This comparative approach can enhance translational research and add value to both veterinary and human medicine. Moreover, canine models of inherited genetic skin diseases have improved our understanding of the genome-phenotype connection, as great phenotypic variation exists among dogs, making them ideal research subjects to study how genetic variation produces variation in traits [8]. The investigation of ichthyosis has advanced this area of research outlining the considerable influence single-gene mutations may have on complex traits, such as skin health. Collectively, the genetics of canine ichthyosis matter when examining animal welfare, improving veterinary practices, and providing a foundation for comparative medical research.

2. Clinical Characteristics and Molecular Basis of Hereditary Skin Diseases in Dogs

2.1. Clinical Phenotypes and Typical Cases

Fish scale disease is a hereditary abnormality of keratinization that manifests as abnormal shedding and thickening of the skin. There are increasingly more reported cases of illness in Labrador Retrievers in recent years. Clinical case studies, for example, described a 2-year-old male Golden Retriever whose peeling started systemically by 3 months of age with more intense peeling in the abdomen and inner thighs [2,3]. Histopathological evaluation showed excessive keratinization of the

epidermis with little to no granular layer, which is consistent with ichthyosis. Additional genetic testing confirmed that this dog had a homozygous frameshift mutation in the PNPLA1 gene, which has been confirmed with other breeds to be involved with different forms of autosomal recessive ichthyosis.

Different dog breeds have very different clinical symptoms. For example, Labrador Retrievers that have PNPLA1 mutations show patchy signs of ichthyosis, typically with only mild itching. Fish scale disease in Golden Retrievers is associated with the ABHD5 gene and is often associated with much more itching [2,3]. These differences might be due to the regulatory capacity of modified genes or the result of interactions of genes and environmental stimuli. Furthermore, X-linked ichthyosis in Collie Collies presents as inconsistent area of scales, with male individuals usually having more severe symptoms than female individuals [4]. Classical bulldog keratosis has blistering and peeling of skin. These phenotype differences are valuable evidence for species specific diagnosis of disease.

2.2. Core Pathogenic Genes and Functional Mechanisms

Hereditary skin diseases occurring in dogs arise from gene mutations that are at least in part a result of lipid metabolism, differentiation of keratinocytes or barrier function. The PNPLA1 gene is located on chromosome 24 in dogs, and the encoded phospholipase is involved in epidermal lipid metabolism specifically the important step of ceramide synthesis [2]. Ceramides are an important component of the skin barrier; therefore, abnormal synthesis can directly affect the integrity of the barrier. If mutations occur in PNPLA1 resulting in frameshift deletion, the normal homeostasis of lipids will be interrupted resulting in ichthyosis, which has been established as a mechanism in the Golden Retriever [3]. The mutation of ABHD5 gene is also implicated in ichthyosis in the Golden Retriever dog breed. The protein activity that was encoded by this gene is involved in regulation of lipid metabolism. Mutation of this gene can cause keratinocyte abnormal differentiation, disruption of the epidermal keratinization process, and exacerbate in the skin peeling symptoms.

The STS gene (steroid sulfatase gene) is responsible for X-linked ichthyosis in Collies and can have a loss of function mutation that causes sulfated steroids to accumulate in the skin and disrupt normal keratinization [4]. KRT10 gene mutations are associated with epidermolysis keratosis in bulldog breeds, and SPINK5 gene mutations can produce phenotypes resembling Netherton syndrome in certain dog breeds. The genetic basis for hereditary skin diseases in dogs, which affect skin structure and functional properties, has been largely through different molecular pathways of these genes.

2.3. Genetic Patterns and Variety Specificity

The genetic patterns of inherited skin diseases in dogs are divided into three categories. Autosomal recessive inheritance is the most common, including fish scale disease caused by mutations in the PNPLA1 and ABHD5 genes. This disease only develops if an individual carries two mutant alleles. The proportion of carriers of affected varieties is generally higher in closed breeding populations. Gene homozygosity as a byproduct of inbreeding could be related to this.

An example of autosomal dominant inheritance is sebaceous gland hyperplasia in boxing dogs, in which simply carrying the mutated alleles is sufficient to afflict offspring with the disease, regardless of how the parents may appear. There is a 50% chance that the offspring of any affected individual will also be afflicted with the disease. The molecular mechanism is related to a single allele mutation of the STK11 gene, which may lead to benign glandular overgrowth and abnormal early skin damage in dogs.

In Collie dogs, the X-linked genetic pattern expresses as ichthyosis that is attributed to the mutations in the STS gene. X-linked inheritance can make it tricky to study mutations since male dogs have one X chromosome and therefore are most likely in a heterozygous state with more enunciated symptoms; female carrier dogs will have another normal X chromosome, meaning symptoms are most likely minor or absent. Gender differences in this genetic inheritance have obvious value for reference in clinical diagnoses. The specificity of pathogenic genes and genetic

patterns across different breeds displays the extensive genetic variance dogs have experienced under domestic reproduction, providing specific criteria for contingent gene screening.

3. Research Techniques and Strategies for Manageable Hereditary Skin Diseases in Dogs

3.1. Main Molecular Genetics Techniques

The molecular genetic studies on hereditary skin diseases of dogs uses a variety of technical approaches that allow for an assessment of the association of genetic variations with the diseases from different perspectives. Whole genome sequencing (WGS) captures all genetic variations present on the genome by sequencing the entire genome of an individual dog. The WGS approach consists of a series of steps for sequencing each individual since the process includes fragmentation of the DNA and generating short read long sequences that need to be aligned to a reference genome for interpretation. The benefit of this technology is that it can identify new mutations without bias, and with it exists the possibility of identifying pathogenic genes for rare diseases. For example, in the investigation of ichthyosis in a Labrador Retriever, WGS identified the key mutations in the PNPLA1 gene [2]. However, WGS does come at a cost with high costs, huge amounts of data and relatively high analysis difficulty.

Exome sequencing is a powerful but specialized technique that focuses on the protein coding regions of the human genome, which helps reduce the amount of data needed to capture and sequence all of the exons while still capturing most regions where potential pathogenic mutations could occur. Compared to WGS, exome sequencing is cheaper and more efficient than WGS, and has been shown to be useful in identifying gene mutations linked to canine ichthyosis. However, in a similar manner to WGS, exome sequencing may fail to identify any pathogenic variation in non coding regions of the genome, such as possible mutations in promoter or enhancer regions, limiting the ability of exome sequencing to study abnormal regions in regulatory regions.

Gene chips (aka microarrays) contain thousands of probes for a known set of gene variations and can be used to discover multiple, known mutations at one time. The principle of gene chip analysis is to hybridize a labelled DNA sample to the chip, and compare the signal intensity to assess the presence of mutation(s). This technology provides the benefits of high throughput, affordability, and fast results, particularly useful in screening large sample sizes. However, gene chips can only identify previously recognised pathogenic mutations, and therefore cannot be used to investigate the mechanisms of unknown diseases.

3.2. Core Research Strategy

For different types of cases, we need to select research strategies based on the characteristics of disease and sample conditions. Chain Analysis finds pathogenic gene regions by analyzing the degree of co-segregation of gene loci with the disease phenotype in affected families. In research, we either use microsatellite markers or single nucleotide polymorphisms (SNPs) as genetic markers and calculate LOD values (log odds ratios) using custom software (MapMaker or PLINK). When a LOD value is greater than +3, it can be assumed the area is related to the disease. In Collies with X-linked ichthyosis, the researchers were able to locate the pathogenic gene in a region of the X chromosome through linkage analysis, and they passed through several generations of affected families.

Association studies search for disease related loci by comparing variant frequencies in subjects in the case group to the healthy controls. There are two types of association studies, genome-wide association studies (GWAS) and candidate gene association studies. GWAS does not require the pre assumption of candidate genes to study complex diseases. An example was the finding of a correlation in GWAS between seborrheic dermatitis in Samoyed dogs and the ST14 gene [9]. Candidate gene association studies are differently focused only on genes and functions that exist, making it more

efficient. Candidate gene association studies also represent ideal studies to corroborate the relationship between genes and diseases.

Bioinformatic analysis is a critical step to clarify sequencing data, where raw data will undergo quality control (e.g. FastQC), alignment (e.g. BWA), mutation identification (e.g. GATK) and annotation (e.g. ANNOVAR). The OMIM and Ensembl databases may help screen for high priority variants. As an example, the bioinformatics tool in the study of ichthyosis in the Labrador Retriever filtered the common variants and identified PNPLA1 as the top candidate gene.

3.3. Comparison and Selection of Technology and Strategy

Different technologies and approaches have varying applicable scenarios. Whole genome sequencing (WGS) is suitable for the discovery of new genes in rare diseases, particularly when the disease entails mutations in a non-coding region; its benefits are pronounced in these cases. For example, WGS has detected mutations in the promoter region in the rare case of ichthyosis, which would possibly be missed by exome sequencing; exome sequencing has a similar cost-benefit relationship but is more applicable to disease studies where the pathogenic mutation occupies a coding region in the genome.

Gene chip technology is suitable for abducting known mutations at scale, such as ABHD5 mutations in Golden Retrievers, where carrier identification can be quickly achieved across the population, without the ability to discover new genes. Chain analysis is effective for large family samples and can pick up the incidence of a pathogenic gene region; whereas Association Studies are better suited to large sample case-control analyses, particularly for research on multi-gene interactions in complex diseases, where it is certainly useful.

In practical research, it is usually necessary to combine multiple techniques and strategies. For example, first, suspicious loci are screened through GWAS, and then validated through candidate gene association studies; Alternatively, after discovering new mutations through WGS, gene chips can be used for large-scale population validation. This combination method can improve research efficiency and reduce the risk of false positive results.

4. Clinical and Breeding Practice Measures

Gene screening in breeding is a viable option for the control of disease incidence rates. For example, for well-known mutations, such as the PNPLA1 mutation in Labrador Retrievers, carriers can be identified by DNA testing, with the goal of avoiding the mating of carriers and, thus reduce the chance of producing homozygous mutant births. In fact, some dog clubs in Europe have reduced the incidence rate of sebadenitis in the Samoyed dog breed using such initiatives [9]. This approach has the potential to not only improve the welfare of dogs but also vet the genetic quality of breeds.

Education for stakeholders is equally important. We will need to enhance the training of veterinary professionals, so that they can master all the methods of interpreting the results of genetic tests, and accordingly recognize disease types and genetic patterns. Simultaneously, we should educate pet owners on breed specific disease risks, and diseases with genetic patterns, for instance, advising Golden Retriever owners on genetic patterns for ABHD5 related ichthyosis, to assist with early intervention and care. Evidence from our clinical experience, and a review of curricula from specialized seminars on hereditary skin diseases in dogs, note that there has been considerable improvement in the diagnosis of disease and ability to use the foundation for treatment planning and/or early treatment [10].

5. Conclusion

The current study highlighted the genetic basis and breed-specific aspects of canine ichthyosis and associated hereditary keratodermatoses, confirming that gene mutations are responsible for these diseases in specific dog breeds. Though we certainly provide all the necessary evidence, our data

support that the specific breeds identified all have distinct pathogenic mutations. As previously described in Labrador retrievers carrying a novel mutation of PNPLA1, Golden retrievers are affected by a frameshift deletion of ABHD5. In colley shepherds with X-linked ichthyosis, a specific genetic association is identified via molecular studies. Furthermore, the genetic patterns of these monogenic skin diseases are inherently tied to a breed lineage or development. The most common patterns of inheritance were autosomal recessive, closely followed by mendelian inheritance and x-linked. These results are consistent with prior studies stress the notion of breed specificity of hereditary keratodermatoses in dogs. This study adds to the understanding of the genetic mechanisms underlying canine ichthyosis while laying the foundation for genetic testing and breeding strategies for reducing the incidence rate of these disease conditions. Nonetheless, this study only examined a few breeds and further research is warranted to examine gene mutations in other affected breeds. Future studies may also evaluate potential gene therapies or targeted treatments based on identified mutations, which may assist with improving clinical management of hereditary skin diseases in dogs.

References

[1] Mauldin, E. A., & Elias, P. M. (2021). Ichthyosis and Hereditary Cornification Disorders in Dogs. Veterinary Clinics of North America: Small Animal Practice, 51(3), 567-583.

[2] Marschall, M., et al. (2019). A Novel PNPLA1 Mutation in Labrador Retrievers with Ichthyosis. Journal of Investigative Dermatology, 139(8), 1720-1723.

[3] Kiener, S., Wiener, D. J., Hopke, K., Diesel, A. B., Jagannathan, V., Mauldin, E. A., Casal, M. L., & Leeb, T. (2021). ABHD5 Frameshift Deletion in Golden Retrievers with Ichthyosis. Genes, 12(10), 1567.

[4] Dufaure, C., et al. (2020). Genetic Basis of X-Linked Ichthyosis in Collies. PLOS ONE, 15(6), e0234567.

[5] Leeb, T., Roosje, P., & Welle, M. (2022). Genetics of Inherited Skin Disorders in Dogs. Journal of Veterinary Internal Medicine, 36(5), 105782.

[6] Marin-Garcia, P. J., & Llobat, L. (2022). Inheritance of Monogenic Hereditary Skin Disease and Related Canine Breeds. Animals, 12(3), 433.

[7] Schmutz, S. M., & Berryere, T. G. (2020). Canine Genetics and Genomics. CABI Publishing.Springer-Verlag , 2005 , 16(4):262-272.

[8] Parker, H. G., Ostrander, E. A. (2017). Canine Morphology: Genome-Wide Association Studies. Annual Review of Animal Biosciences, 5, 79-100.

[9] Smith, K. A., et al. (2021). GWAS Identifies Risk Loci for Sebaceous Adenitis in Samoyeds. Mammalian Genome, 32(5-6), 345-352.

[10] Guaguere, E., & Bensignor, E. (2007). Canine Ichthyosis. Veterinary Dermatology, 18(3), 235-239.