The Science of Alopecia X:

Shedding the Myths

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Dogs may experience temporary partial coat loss as a result of normal shedding periods, infection, parasite infestation, allergic reactions, hormonal or other environmental causes including poor nutrition or a variety of underlying health concerns (1, 2). There are also variants of 'normal' near or complete lack of coat in such breeds as the Chinese Crested, which is hairless on the trunk of the body but possesses long hair on the legs, tail and head in its hairless variety. A long, thick covering of hair on the entire body is characteristic of the coated variety. Conversely Poodles and many double-coated Nordic breeds including Keeshounds, the Chow Chow, Elkhound, Pomeranian and others may suffer a symmetric bilateral coat loss phenotype affecting primarily the neck, trunk and tail (Figure 1), of unknown etiology, variable time of onset ranging from 9 months to >10 years that while may be punctuated by periods of re-growth, generally is permanent without intervention.

This condition has been described by a variety of names, including growth hormone responsive alopecia, castration responsive alopecia, post-clipping alopecia, follicular dysplasia, adrenal hyperplasia-like syndrome, pseudo-Cushing's syndrome, black skin disease (BSD) or others (1-3). Alopecia X is a term that has been coined to emphasize that the cause is unknown and this "disease" may in fact be the result of a variety of causes and conditions rather than being a single, well defined syndrome.

Alopecia X typically presents as an apparent defect in the hair follicle cycle not caused by hypercortisolemia typical of Cushing's syndrome, or by hypothyroidism. The normal hair follicle cycle consists of three distinct phases of variable length. Anagen is the phase of active growth of the hair strand, telogen is a resting phase associated with a lack of significant hair lengthening, and categen involves changes to transition from the anagen growth phase to the quiescent telogen phase (1, 4). In dogs of defined coat length (dogs that do not grow hair requiring regular trimming), the coat will be in prolonged periods of the quiescent telogen phase for months or longer without new hair growth being initiated. Typically the telogen hairs are healthy and well-anchored, and thus dogs in

telogen phase are properly and fully coated.

In alopecic Pomeranians, the hair cycle is thought to have a defect in which it becomes arrested in the telogenic phase where new hair growth is unable to initiate. Hair loss typically begins by age 1.5-3 years, though its onset is variable may occur earlier or much later in life. Generally the outer guard hairs are lost first, leaving the soft undercoat and giving the appearance of a fluffy puppy coat (2). It should be noted that while the breed standard indicates that an ideal Pomeranian should possess a harsh outer coat with a soft undercoat, some variation exists from specimen to specimen in the harshness of the outer coat and the quality of the undercoat. A specimen that appears to possess primarily a softer coat does not in itself indicate a dog progressing to alopecic hairlessness. Additionally, many dogs are excessively trimmed and sculpted for aesthetic reasons. This trimming generally cuts more of the outer guard hairs and may give a similar appearance to the loss guard hairs. As the condition progresses, the soft undercoat is continually lost on the main trunk of the body, the neck and often the tail, without significant re-growth. The final result is a dog that generally appears normally coated in the head, all four legs and occasionally the tail, but is completely bald or simply possesses fine wisps of diffuse hair on the trunk of the body (Figure 1). The exposure of the skin to the sun can result in damage and hyper-pigmentation, leading to the common description of "Black Skin Disease".

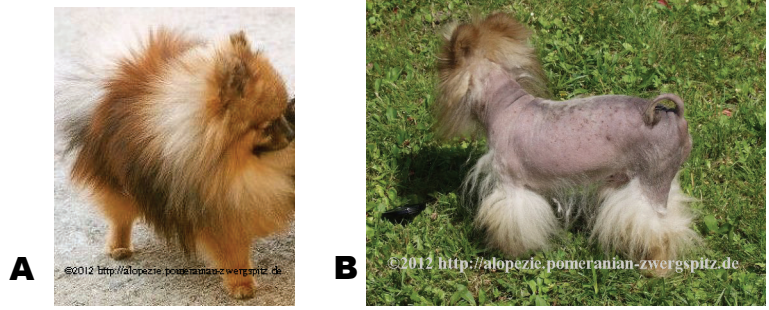

Figure 1. A) Pomeranian before coat loss due to Alopecia X. B) Same Pomeranian after Alopecic loss of coat. Note the pattern of hair loss across the body with retained coat on the head and legs. Photo courtesy of Brigitte Sovonja ©2012 http://alopezie.pomeranian-zwergspitz.de used with permission.

Cause of Alopecia X

There are few sources of data concerning the occurrence of Alopecia X in the Pomeranian, or data that indicate whether the incidence is increasing (anecdotal accounts aside), decreasing or remaining steady. According to self-reported survey results (http://www.offa.org/surveys/_survey_pom.html [accessed May 14, 2012]), roughly 16% of dogs entered suffer Alopecia, with an equal proportion of males and females over five years of age suffering Alopecia, though a higher incidence of males than females under two years and between two and five years of age appear to display the characteristic hair loss. This is in agreement with the belief that the incidence is typically higher in males. However these results may or may not all be true cases of Alopecia X as they are simply unconfirmed and self-reported. Additionally, a self-reported health survey may be anticipated to emphasize Pomeranians with health issues, as the impetus to report problems is likely greater than to report the lack of a health concern. Reporting by our breeders of the health status of all dogs owned or produced (alive or deceased, healthy or otherwise) in this survey would help to provide an accurate picture of the health of our breed, both from an Alopecia X perspective and otherwise, though such data alone would not give an indication of whether the incidence is increasing versus 20 years ago. Long-time breeders whose lines are affected by Alopecia X and who force their lines further and further inbred over time will undoubtedly claim that the rate of "BSD" is increasing, because it will do so in the highly inbred dogs they continue to produce. This is unless perhaps after they have made a conscious effort to eliminate it by ending breedings to dogs known to produce Alopecic dogs and to breed their lines to outside dogs thought not to be "carriers" and where the incidence in the general population that they begin breeding to is somewhat lower than their "BSD"-producing lines.

There have been many theories—some reasonable and others outrageous—concerning the cause of Alopecia X. There have been suggestions that poor food quality and vaccinations, toxins in the environment or

other factors are the culprit (5). Most breeders and researchers suspect that there is a strong genetic component to the disease (1, 4, 6-9), for a variety of reasons, including: 1) there appear to be "carriers" who are not affected but frequently produce offspring that are affected and other dogs who are "not carriers" and never produce it; and 2) some dogs are affected while others raised in the same environment, exposed to the same food, medications and other conditions do not. It is suspected that there may be a genetic defect in one of many components of the normal hair cycle that at some point arrests the process in the telogenic phase. Normal hair growth can occur in Alopecic poms or these dogs would be born bald and remain so throughout their lives. However, at some point this growth is arrested. Alopecia X appears to be influenced by hormonal signals, and hormones are intimately involved in the control of the hair follicle cycle (1, 3, 4, 6, 7, 10). Cascades of hormonal signals vary through the life cycle of all mammals, including the Pomeranian. Castration and treatments with melatonin or a variety of hormones has produced temporary or apparently permanent coat re-growth in some specimens (1, 3), and the internet is littered with fallacious "BSD cure regimens" or reports of secret formulas to restore coats. However, the condition is highly variable in the age of onset, the response to castration and such re-growth regimens, suggesting that the disease may not be due solely to a single full penetrance genetic defect.

Natural selection essentially no longer exists in the breed as it would for wild animals such as a wolves or coyotes fending for themselves. Our artificial selection of the dogs that will produce the next generation could in fact contribute to the incidence of Alopecia X. Some may suggest that dogs are "carriers" of the disease or in fact sufferers before full onset may possess particular attractive attributes, such as fuller coats that we desire in show specimens and thus we could unknowingly be selecting the Alopecic dogs to breed. There has been no scientifically sound, statistically significant data to support or disprove said theory. Any anecdotal information that indicates a direct link of Alopecia X to specific coat development characteristics and progression that has been deduced in a single, highly inbred population where a multitude of genetic factors unassociated with Alopecia X would be fixed, would likely not hold statistical relevance to the broader, diverse Pomeranian population. A properly controlled scientific study may support or disprove such a theory.

Alopecia X: a genetic disease—?

Examination of pedigrees of affected dogs shows how difficult it is to make solid conclusions about a causative gene. The status of some dogs is unknown, age of onset is variable and occasionally late in life, and a reluctance to disclose the status of dogs for fear of being attacked or ostracized by fellow breeders means that there are usually gaps in the record or direct misinformation. However, Alopecia X does not appear to be a dominant characteristic, as a dominant trait in an offspring will appear somewhere in each generation behind it, but need only appear in one parent (Figure 2). There are many examples of seemingly unaffected dogs producing affected offspring which is inconsistent with a dominant, full penetrance trait. Some have suggested that Alopecia X is an X-linked recessive trait due to the higher incidence in male offspring than female offspring. Each male possesses a single X chromosome while each female possesses two. A male will be affected if their single copy of such a gene were to be defective. The dog could not be an unaffected carrier. A female would only be affected if it received a defective copy from each parent but if one parent had the defective trait, the female could be an unaffected carrier producing both affected male and carrier female progeny. There appear to be unaffected male carriers of the disease which is inconsistent with an X-linked recessive trait (Figure 2).

Taking these and other data into account, it is likely that Alopecia X is in fact 1) a genetic disease due to a defect in the gene for one single protein product involved in the hair follicle cycle (1, 4) or an associated hormone signaling pathway (4, 6, 7), with some genetic or environmental influence to account for the varied age of onset and response to therapy, or 2) that it is a genetic disease and the variability is due to the interplay of multiple genetic defects in one or more of several associated genes which occur in any one dog to produce the disease and the variability observed. If 1) is the cause of the disease, the variable age of onset and response could be due to hormonal signals related to some unknown external environmental factor. Equally likely, the genetic makeup of the dog may influence the progression of Alopecia X due to unknown gene modifiers. Cystic Fibrosis is the most

common genetic disease in humans (reviewed in (11)). It results from a mutation in a single gene that is inherited in a recessive manner and is entirely the cause of the disease (12). The mutation produces a defect in lung hydration that results in lung infection, damage, and ultimately death. However disease progression and severity vary from patient to patient, among those suffering an identical mutation. In addition to differing environmental factors, including quality of treatment, variations in other genes in the patient have been identified by genome-wide association studies and appear to influence to some extent how the disease progresses (13). All patients, if left untreated, will suffer lung damage and ultimately death, but this complement of other genes has a significant influence on how quickly the disease advances. Alopecia X onset and progression may occur analogously, being caused by a single recessive genetic change but modified by the genetic complement in each particular dog. Related dogs would likely progress similarly while unrelated Alopecic dogs would be expected to progress differently, such as with a different age of onset or response to castration.

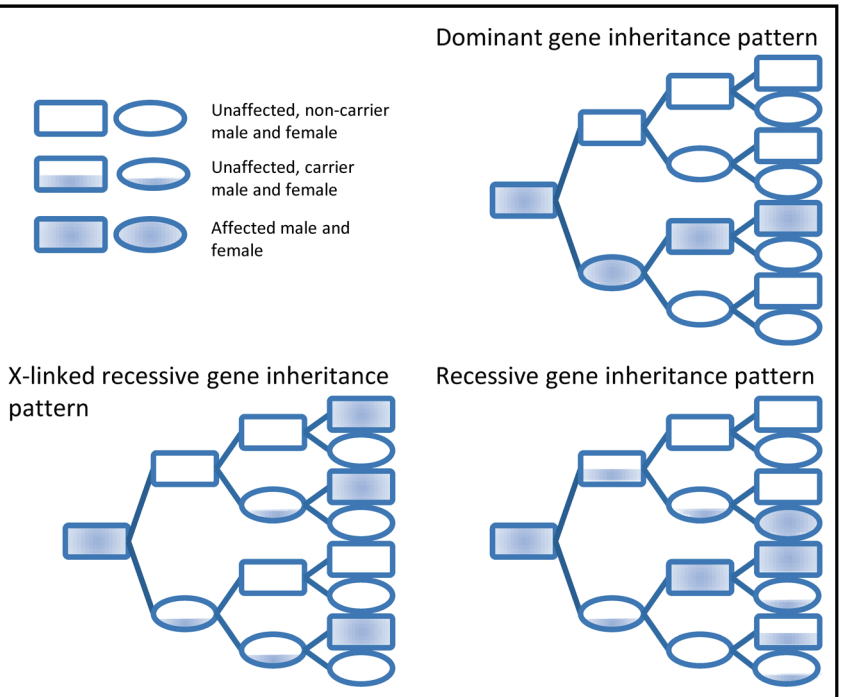

Figure 2. Potential modes of inheritance of a defective Alopecia X gene. Under situations where a gene of interest follows a dominant pattern of inheritance, each affected offspring receives a copy of the gene from an affected parent. Affected dogs do not skip a generation. In a recessive inheritance pattern, either the mother or father can be affected or a carrier. Note that the parents of an affected dog are either carriers or affected, but in some generations there may be carriers who show no signs of the disease. In X-linked recessive inheritance, the gene is present on the X chromosome. Male dogs only have one X chromosome inherited from the mother. Therefore a male cannot inherit the disease from the father, but can by a carrier mother and will always inherit it from an affected mother. There are no male carriers, only female carriers and affected males and females, but affected males are far more common. The inheritance pattern of Alopecia X appears to be most like a recessive (non-X-linked) gene.

Efforts to identify a defective gene in Alopecia X

To date there has been considerable research effort in the study of the genetic cause of Alopecia X. In an attempt to identify the associated gene, and for a lack of other clues to the location of the genetic defect, researchers have used comparisons to similar conditions in other animals as a starting point. As described earlier, "hairless" dogs exist that are considered normal for their breed, where an obvious example is the Chinese Crested. In fact these dogs suffer Canine Ectodermal Dysplasia (CED), which presents as both a lack of coat and defects of the teeth. This condition is a monogenic, autosomal semi-dominant trait, meaning that it is caused by a defect in a single non-X-linked gene that produces hairlessness in any dog that inherits a single copy from either parent, but the disease is lethal in the embryonic stage for dogs inheriting the trait from both parents. Hairless Chinese Crested dogs are born lacking hair in their typical hairless regions rather than losing hair at a later time. The Leeb group (14) used a technique called genome-wide association mapping to identify a short duplicated region in the sequence in hairless dogs of a gene called *FOXI3*, which is thought to be involved in regulating development. The duplicated sequence likely disrupts the gene and alters hair and tooth development. The characteristics of hairlessness in the Chinese Crested clearly differ significantly from Alopecia X of the Pomeranian. However, the same research group is currently conducting studies to identify the Alopecia X defect using similar techniques.

Mouse models exist for Alopecia where there is a lack of the gene *Ctsl*. These mice lose their coats around the age of sexual maturity, which is similar to the age of coat loss in at least some Alopecic Pomeranians. The Leeb group (9) examined the *Ctsl2* gene (equivalent of the *Ctsl* mouse gene) in Pomeranians and found that this gene was not altered in Alopecic dogs versus control normally coated dogs, suggesting that this is not the defective gene responsible for the disease. Another mouse model of Alopecia results from the elimination of the *Ptch2* gene. Mausberg et al (8) examined this gene in normally coated and affected Pomeranians as well as normally coated dogs of other breeds. They found that there was no mutation in the gene that was associated with the affected Pomeranians.

Alopecia X in the molecular era

Research is continuing by a few groups to identify the Alopecia X-associated gene. One method to identify the gene is to search for mutations in other genes, or other genetic markers called SNPs (single nucleotide polymorphisms) that are passed from parent to offspring in Alopecic dogs. Genes are generally considered to independently assort from a parent to their offspring. However if genes or SNPs are located close to an Alopecia X-causing gene on a particular chromosome, there will be a greater likelihood that the genetic marker will also be passed along to the offspring. If the location on the chromosome is known for such a genetic marker and it can be identified to be associated with Alopecia X inheritance, while not directly related to the disease, it could be used as a starting point to search for the gene. Researchers can begin sequencing the DNA in the region near to the genetic marker and compare sequences for affected dogs, and normally coated dogs. Differences that are consistent between affected dogs but not observed in normal dogs would be a good indication that this particular region is the one affected. This type of identification method has been used to identify the genetic causes of a variety of diseases in humans in the past.

For human diseases, we have entered the molecular era of the study of genetics. After a 13 year, $3 billion effort, the entire human genome has been sequenced. It is routine to sequence regions of DNA in patients affected by a genetic disease and comparison to the known sequences in the database to identify the cause of a disease. The gene defective in CF was identified by an exhaustive search over a decade before the genome was fully sequenced (12). Modifier genes for the disease, however, are easily being identified now by comparing the sequences in multiple patients and asking where there are common differences versus what is expected in the human genome using genome-wide association studies as described above (13). The cost to sequence the human exome: the complement of all of the genes in the genome, has dropped from billions to little more than $ 1000 in the last several years, making routine genetic studies on individual human patients within the realm of possibility.

Sequencing is being done or has been done for a variety of animals. There is an aggregate genome sequence available for the dog, and it is possible to sequence the genome of the Pomeranian breed as well. This effort requires funding contributed by the Pomeranian community, but not *billions* or *millions* of dollars as some claim, but likely *thousands* to *tens-of-thousands* of easily-achievable dollars. If the genome of an Alopecic dog can be sequenced, it can be compared directly to the aggregate dog genome or to specific breeds unaffected by Alopecia X. This will highlight differences that make the Pomeranian unique and give it its characteristic shape, size, and other features. It is expected to also highlight any genetic changes that cause Alopecia X. Researchers won't be able to directly identify which changes are responsible for Alopecia X, but may be able to make educated guesses if changes occur in hormonal regulation genes or genes involved in the hair follicle cycle.

The next step would be to focus on our best-guess genetic differences. Because the sequences in the area around the changes would be known, it would be an easy step to sequence these small regions in multiple Alopecic and normally coated dogs. It should be straightforward to identify which changes are due to Alopecia X. Alternately, we could sequence the entire genomes of other affected and non-affected dogs to see directly which changes are specific for Alopecia X. As the sequence would already be known, it would be simple to develop a genetic test to differentiate between a normal gene and an Alopecia X gene. Such simple and economical (typically $ 50-75 per dog) tests exist for a host of genetic diseases in dogs and other species. This is the ultimate goal in the study of

Alopecia X.

Toward the future

It is unlikely a "cure" will ever be developed for Alopecia X as the process of identifying a small molecule that can correct the defect and having that drug approved for use is literally a multi-billion dollar effort. There simply isn't sufficient pay-off for a drug company to engage in such an effort. If a drug used for another condition in dogs or humans were identified that showed efficacy in treating the defect, it could however be adopted for Alopecia X. Most probably, a genetic test will be the sole tool for our use to deal with the condition. It would allow us to know the status of all dogs before breeding and avoid matings that would produce bald dogs. Care would be needed in the use of the information from such a genetic test. The desire might be for the community to "eliminate" all affected AND carrier dogs from the breeding pool. This could devastate the breed, depending on the true prevalence in the Pomeranian population. We could lose entire unique lines and much of the diversity that keeps a breed healthy. The loss of genetic diversity could in fact bring other genetic diseases into greater prevalence due to the limited remaining breeding stock, if one or a few possess a particular defect.

There must be a concerted effort moving forward to protect genetic diversity in the breed and this should form the basis of policy at the breed club level.

However, until an Alopecia X test is developed, the main concerns are 2 fold: 1) to understand how to proceed protecting the breed but producing as few bald dogs as possible, and 2) raising the funds as a community necessary to identify the gene and get the test developed that we so badly need.

There are a few tenants that breeders concerned about Alopecia X may follow:

- Try to be as aware as possible about the incidence of Alopecia X in the dogs in your pedigrees, and also in what they have produced in other breedings. This is not always an easy task.
- Monitor closely your dogs to see if you can see any patterns that lead you to suspect dogs who will develop the disease. Such evidence would not necessarily hold for other dogs from different lines.
- Have your bald dogs properly diagnosed to eliminate other causes of hair loss.
- Share information, but realize that what you see in your dogs may not be a universal trait and only a properly-controlled scientific study may tell us what characteristics are truly common before complete coat loss.
- Try to minimize early breedings before Alopecia X would become apparent.
- Do every breeding with a purpose, and be aware of the risks, not only for Alopecia X but other diseases as well. Does this breeding make more sense and produce less risk than another breeding?
- Minimize the use of dogs suspected of being carriers and certainly those affected.
- Inbreed and line breed carefully and with a purpose, being doubly sure of the risks versus benefits.
- Use judicious breedings to outside dogs that you know haven't produced Alopecia X, particularly when you feel the incidence of Alopecia X in the general population is lower than what you are seeing in your lines.
- Breeding to "unknown" outside dogs could be just as risky, or even more so than using a known suspect dog. Foreign dogs don't necessarily have a lower incidence—what you don't know *can* hurt you.
- Donate information and time as well as DNA samples and pedigree information to legitimate scientific studies examining Alopecia X.
- Preserve diversity in your lines and in the population. There are rare instances where certain groups of dogs could be lost by simply eliminating all suspected carriers of Alopecia X or other diseases from the breeding pool. This would be a greater disservice to the breed moving forward. Instead in these situations, do carefully controlled breedings to dogs that have never produced Alopecia X in many breedings over a number of years.

Finally, we as the Pomeranian community need to raise funds to support the scientific research being

done. We are at a point now where an Alopecia X-related gene likely can be identified at a lower cost than ever and a simple genetic test should be possible. We all must contribute to the development of this vital tool. It is up to the members of the PCOC, APC, etc. to join together and raise the funds necessary to save our breed. If we don't care enough to do it, no one else will. Last year 790 purebred Pomeranians were registered with the Canadian Kennel Club. I advocate making a donation for each Pomeranian produced or sold by each of our members to contribute to the genome sequencing effort or to Alopecia X research in general. Even a donation of 1% of the sale price of each dog sold will add up quickly. If the average dog is sold for just $ 2000, that means a small contribution of $ 20. But this adds up to $ 16 000 from the registered dogs produced last year alone. However, not all dogs registered in Canada were produced by PCOC members and not all members will care enough to contribute, which is why we need to work extra hard and donate even more. With matching funds when we donate and our APC neighbours contributing as well, we can really raise the needed funds quickly. Many of the PCOC members have already donated and are continuing to do so. Whether you have or haven't donated in the past, please visit:

http://www.american pomeranianclub.org/health.htm
now and please donate!

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