Canine Collie Eye Anomaly (CEA) underlying genetic causes and future medical/therapeutic implications

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Abstract

Collie eye anomaly (CEA) is a recessive genetic disorder that brings about serious ocular effects, including retinal abnormalities and even blindness. This disease affects many dog breeds, the most common being collies. The causation of CEA is the deletion of 7,799 bp located in the intronic, part of the NHEJ1 (non-homologous end-joining factor 1) gene on chromosome 37. The mechanism of action of how the genotype is correlated to the phenotypic expression is still unknown in canines. PCR is currently used to diagnose CEA. Newer studies have shown that select high CEA frequency breeds express the disease's phenotype without the NHEJ1 mutation. This suggests the existence of multiple genotypes responsible for CEA yet to be discovered. Observing analogous NHEJ1 gene mutations within humans with similar ocular defects can contribute to a better understanding of the mechanism of action in canines. Human medicine studies have identified that the intronic segment of NHEJ1 gene that is deleted in genetic ocular defects is also an enhancer for another gene necessary for normal eye development, the Indian Hedgehog gene (IHH). I theorize that this mechanism of action is conserved between species explaining how the deletion of the NHEJ1 gene segment can cause CEA. It is also further theorized that dogs with CEA phenotypic expression lacking the NHEJ1 mutation could possess mutations to other enhancers to the IHH gene or direct mutations within the IHH gene itself. Further research is needed and could lead to future preventive genetic testing or gene therapies for CEA for dogs.

1. Introduction

1.1. Background of Collie Eye Anomaly

Choroidal hypoplasia, more oftenly referred to as collie eye anomaly (CEA), is a hereditary, congenital disorder that brings about visual impairments among canines (Palanova 2015). Collie eye anomaly is important as it can be a common genetic disorder that occurs with high frequency for collies. Within the United States alone, certain breeds of collies can have a genotypic incidence of CEA as high as 95% and phenotypic incidence as high as 85% (Gilger 2006). Dog owners whose pets have been affected by CEA often find out the diagnosis once irregularities in the retina or even blindness has been developed at around two years of age. Defects brought about by CEA can range from no visual defects to complete blindness (The Humane Society Veterinary Medical Association [HSVMA] 2011).

1.2. CEA Symptom Affected Breeds

Today, CEA is known to affect a variety of canine breeds. Namely, collies are among some of the most heavily affected, including border, rough, and smooth collies. Other breeds have historically been diagnosed with collie eye anomaly, including: Nova Scotia duck tolling retrievers; Shetland sheepdogs; and different shepherd varieties, such as the American shepherd, English shepherd, and Australian shepherd. Additionally, many Australian-bred breeds have been known to possess collie eye anomaly including the Australian cattle dog, Australian Kelpie, Australian Koolie, Australian Stumpy Tail Cattle Dog, and Australian Working Kelpie (HSVMA 2011).

1.3. Research Timeline: Collie Eye Anomaly

This defect was first denoted in 1953 by veterinary ophthalmologist, William G. Magrane, in his journal article “Congenital anomaly of the optic nerve in collies” (Magrane 1953). Following Magrane’s initial observation of CEA, many other scientists and researchers have followed suit in discovering more of the intricacies of the disease. In 1965, scientists Donovan and Wyman, in one of the earliest studies on CEA following Magrane, learned of CEA’s autosomal recessive inheritance pattern; in other words, the trait must be passed down from both parents for the offspring to express it. CEA’s mode of inheritance specifically had sparked much debate among such researchers. A study by Wallin-Håkanson et al. in 2000 suggested CEA to be a polygenic autosomal disease in which multiple genes may take part in the inheritance of CEA from parents to their offspring. Other studies have supported other key features of the disease. For example, studies from Yakely, et al. in 1968, also suggests that CEA is not sex-linked, as its frequency in males and females were similar. It was not until 2007, in a study by Parker et al., that a causative agent of CEA was found.

2. Genetics

2.1. Currently Known Etiology of Collie Eye Anomaly

Following revolutionizing studies that better refined researchers’ and veterinarians’ knowledge of CEA, Parker et. al.’s 2007 study concluded a more definitive genetic explanation behind CEA. With the advancement of genetic testing and genome technologies such as fine-mapping and mutation analysis and the performance of their study through genotypic data, generated from 96 microsatellite markers in which 132 breeds were categorized into 5, was used to find the specific mutation. The deletion of the 7,799 bp sequence – is located in the intronic, or non coding section of the RNA transcript, part of the NHEJ1 (non-homologous end-joining factor 1) gene on chromosome 37 (National Cancer Institute 2012).

2.2. Unknown Potential Etiologies

Recent studies have also been able to identify that the ophthalmic phenotypes brought about by CEA are not always correlated with the NHEJ1 gene mutation. An assessment by Brown et al. genetically analyzed Nova Scotia Duck Tolling Retrievers (NSDTR), a breed commonly affected by CEA (Brown 2017). The observed NSDTRs presented with colobomas of their optic nerve head (ONH), a way in which CEA can phenotypically be expressed, yet lacked the NHEJ1 intronic deletion that is associated with CEA (National Eye Institute, 2023b). While this study could not conclusively explain the etiology of the ONH coloboma due to high genomic inflation and no significant regions of the genome correlated with the ONH coloboma found, it greatly suggests the existence of multiple different genotypes responsible for CEA that have yet to be discovered. This is especially odd considering that NSDTRs with CEA are homozygous for the deletion (Larsen 2015). Other breeds, such as Berger des Pyrenees and Soft Coated Wheaten Terriers, have also been met with genetic inconsistencies, where they present CEA symptoms but are not homozygous for the deletion (Larsen 2015).

2.3. PCR Testing: Identification of Collie Eye Anomaly

CEA is most commonly identified with the use of a worldwide, commercially available PCR test, which in recent years has been greatly improved upon. This two-step PCR test works by recognizing mutation-affected and non-affected chromosomes and placing two primer pairs inside and outside the deletion of the 7,799 bp region. Other genetic studies on CEA, such as Dostal et al.’s in 2010, have simplified this process by not requiring the isolation of DNA in order

to observe the canine's deletion of the 7,799 bp region (Dostal et. al 2010). A different study in 2010 focused their research around finding a novel and rapid genotyping technique to identify CEA in which they used Flinders Technology Associates filter paper (FTA card) as DNA templates with blood and saliva samples, a process that was based on SYBR Green Real Time PCR (Chang et al. 2010).

2.4. NHEJ1 Gene's Correlation with Eye Development

Within humans, analogous mutations of the human NHEJ1 gene, more specifically, a 0.5 Mb disease-associated chromosome 2q35 locus resulting in an intronic variant of the NHEJ1 gene, have been found to have caused complications with eye development without affecting the function of the gene itself (Wormser 2023). A study found that this NHEJ1 intronic variant has been correlated with at-birth eye defects among Jewish Iranian families including: human microphthalmia, the absence of an eye(s) at birth; anophthalmia, when an eye(s) is smaller than normal at-birth due to not being fully developed; and ocular coloboma with the use of chicken and mice animal models (National Eye Institute, 2023a; Verma 2007). Through this study in conjunction with studies of the genetic basis of CEA, mutations of the NHEJ1 gene is not only correlated with CEA but also the effect of mammalian eye development, including humans, mice, and chickens. With the same mutation of the NHEJ1 gene resulting in the same ocular defects, the study concludes the NHEJ1 gene to be conserved between species and can be an explanation as to why clinical signs of CEA are similar to the eye defective symptoms within humans and other mammals. These similarly documented ocular defects to CEA in association with an intronic deletion of the NHEJ1 gene in humans could greatly contribute to a better understanding of the intronic deletion of the same gene for canines regarding CEA. The same study has also been able to identify another gene, the Indian Hedgehog gene (IHH), that leads to similar eye defects and developmental issues associated with NHEJ1 gene's intronic segment. Using mice and chicken animal models, they identified that a variant of the NHEJ1 gene's intron is an enhancer for the IHH gene. Similarly to the NHEJ1 gene itself, the IHH gene with the variant of the NHEJ1 gene's intron as an enhancer presented within humans could be a potential etiology for CEA within canines. In other words, canines who present the phenotypic expression commonly associated with CEA but without the mutation of the NHEJ1 gene may have a mutation of enhancers to the IHH gene or even a direct mutation with the IHH gene itself.

3. Medical Knowledge of Collie Eye Anomaly

3.1. Diagnosis and Clinical Signs

With increased modernized technology, the confirmation of CEA today is relatively easy and accurate. With direct-to-consumer genetic and PCR tests being available to dog owners and breeders, it is now possible to know whether their canines are potential carriers or expressers of the trait (Dostal et. al 2010). However, early diagnosis can be difficult and is often diagnosed after the dog's eye and vision is affected (Williams and Downing). As a result, a reference and eye examination by a veterinary ophthalmologist is recommended to commonly affected breeds as early as six weeks of age. The evaluation primarily consists of viewing the fundus, or opposite of the pupil, with an indirect ophthalmoscope to have a greater field of view, and observing for anomalies with the higher magnification power of the direct ophthalmoscope (Larsen 2015). Veterinary ophthalmologists can often view for several different abnormalities including chorioretinal change (CRC), staphylomas, microphthalmos and hypoplastic papillae, and other colobomatous defects. CRC is when less retinal and choroidal pigment is present; the

choroidal layer becomes more fibrous and visually can be noticed as a white atrophic area under the optic disc (Palanova 2015). Compared to a healthy eye, more choroidal blood vessels should be present; thus, the presence of less pigment. Staphylomas, detachments or less tightened parts of the retina, are also a sign of CEA and present with CRC. Staphylomas are particularly jarring, as they can affect the severity of the visual impairments of the dogs affected: ranging from pits in the optic disc to large peripapillary defects. The presence of both CRC and staphylomas are known to present more serious ocular damage versus possession of just CRC alone. Microphthalmia and hypoplastic papillae are both conditions where the eye(s) of the dog present to be smaller (China National Center for Bioinformation 2024). These visual defects can vary from no visual problems to blindness. While congenital, defects such as retinal detachments can worsen, so CEA is described to be somewhat progressive (Yu-Speight 2021). Overall, veterinarians and researchers on CEA have yet to find a cure for CEA.

3.2. Current Preventatives and Medical Management of Collie Eye Anomaly

Due to a current lack of cure for CEA, the recommended preventative is genetically testing dogs for CEA genotypes and effectively, not breeding dogs together that would likely reproduce offspring with a high chance of CEA. Owners with CEA-prone dog breeds, as aforementioned in the introduction, should have their dogs' DNA tested for CEA for early detection in an effort to minimize worser symptoms, visual impairments, and other developmental effects (Larsen 2015). If caught early there are certain services, such as laser surgery, that can be performed to attempt retina reattachment but that is the extent of current preventative therapies. Retina reattachment therapies have been met with relatively great success with some studies indicating 98% anatomical surgical success and 92% of dogs regaining visual ability after surgery. (Spatola, Steel) In general, canines who develop CEA and go blind are definitely able to have a good quality of life (Texas A&M University Veterinary Medicine and Biomedical Sciences 2016). Owners are encouraged to set a comforting and consistent environment for the animal, allowing them to become attuned to the layout of their homes. With this in mind, often blind dogs have proven to still have an excellent quality of life and even be more attached to their owners (Texas A&M University Veterinary Medicine and Biomedical Sciences 2016). Overall, sightless dogs have more similarities than differences to sighted dogs.

4. Future Prospects

4.1. Current Research Endeavors

There are several veterinary genetic professionals further researching CEA and bettering detection, particularly by the advancement of PCR technologies. Optimization of PCR for detection and better differentiating healthy and affected canines are one of the current states of research regarding CEA (Holeckova et al., 2022). Researchers in Thailand hope to better determine the frequency of CEA from NHEJ1 genotyping assay and better identify NHEJ1 genotypes with development of a new multiplex PCR assay (Chommanad and Nuch 2021).

4.2. Suggested Research Areas

Theorized from the previous studies discussed, future canine research on CEA should focus on finding the mechanisms of action behind the NHEJ1 and the IHH gene to better understand the correlation of the different genotypes with the CEA phenotype. These increased efforts can contribute to the discovery of additional etiologies behind CEA beyond the intronic

deletion of the NHEJ1 gene. Human medicine studies can also contribute to the understanding of CEA and the mechanisms of action behind the involved genes. Researching further on the IHH gene and other genes responsible for eye development are also productive directions to take. Through understanding the mechanisms of action and other etiologies, finding a cure or at least preventatives and different gene therapies would greatly benefit veterinary treatment of CEA.

5. Conclusion

CEA, a common autosomal recessive disorder, is prevalent among collies and other related canine breeds. CEA is especially of concern due to the serious and long term ocular defects the disease can provoke, in some rare cases, complete blindness. While many canines can still have a decent quality of life in spite of their vision problems, continuing to understand the pathogenesis of CEA is still significant, as many of the affected breeds are highly valued for work and sport and visual problems can hinder their participation in these activities. CEA is a disease that should continue to be greatly studied and explored. Improvements in the disorder's genetic understanding, medical antidote, and enhanced identification technologies are advised. While many advancements in PCR testing have been made, more effective PCR testing can further prevent and address challenges of early detection of CEA. Most importantly, the emphasis on understanding the mechanisms of action of involved genes that are also associated with CEA phenotypic expression in order to have better preventatives and gene therapies. Suggested areas include understanding the mechanism of action behind the NHEJ1 gene, and the conformation of the IHH gene with the variant of the NHEJ1 gene's intron as an enhancer as another potential etiology of the disease. Exploring the mechanism of action of the NHEJ1 gene's intronic deletion and experimentally validating the mutation as a deletion of an IHH enhancer as another cause of CEA would immensely contribute to the much needed genetic understanding of CEA. With the extensive tools and abilities of higher education individuals with knowledge of veterinary medicine and research can greatly support the need for the additional research to ultimately ameliorate canine ophthalmic health.

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