Collie eye anomaly: a review

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ABSTRACT: Collie eye anomaly (CEA) is an inherited congenital visual impairment with heterogeneous signs. The first symptoms are already visible in the early embryo. Among the most affected breeds are Collies and Shetland Sheepdogs but the disease has spread to different breeds depending on the country of origin. Dogs affected with this disease share a 7.8 kb deletion in intron 4 of the NHEJ1 gene. Inheritance of this disease is autosomal recessive with incomplete penetrance. Thanks to a commercially available genetic test breeders can identify genetically affected recessive homozygotes and clinically healthy but genetic carriers of the mutation and thus select healthy parents for the next generation of dogs. However, the exact cause of the disease is not known and it is not known whether the causative mutation influences the occurrence of some other diseases (e.g. immunodeficiencies).

Keywords: hereditary; eye; disease; dog

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1. Introduction

Collie eye anomaly (CEA) is an inherited congenital visual impairment and was first described by Magrane in 1953. The disease is caused by a defective mesodermal differentiation of the fibrous and vascular tunics in the posterior polar region of the eye. The defect involves the sclera, choroid, retina and optic disc (Bedford 1982a). The genetic basis of the disease has been the subject of much speculation. Some proposed a complex genetic cause while others speculated that it might be a simple recessive trait. The latter hypothesis was definitively confirmed using genetics by Parker et al. (2007).

2. Clinical signs

CEA is a non-progressive eye abnormality which presents as a very heterogeneous disease. (Barnet and Stades 1979). The first signs to be described were observed in 35 mm long Collie embryos as a rosette-like structure near the optic disc adjacent to the optic fissure (Latshaw et al. 1969). Ophthalmoscopic examination can uncover one or more of the following abnormalities: excessive tortuosity of the retinal blood vessels; chorioretinal dysplasia (CRD); coloboma in or in the region of, the optic nerve head; retinal detachment; and intraocular haemorrhage (Donovan and Wyman 1965;
Donovan et al. 1969; Yakely 1972; Stades and Barnet 1981; Bedford 1982a; Bedford 1982b; Bjerkas 1991; Wallin-Hakanson et al. 2000). Defect of the sclera (colobomatous lesions) may also be present (Lowe et al. 2003). Chorioretinal change (CRC) can be seen as a white atrophic area near the optic disc. In affected parts there is histologically less pigment in the pigment epithelium and choroid, less choroidal blood vessels than in a healthy eye and a more fibrous choroidal layer. In more affected parts the retina appears to be less tightly attached; detachment often starts from these regions. Staphyloma is seen only with the presence of CRC and was found in about 34% of Collie eyes. A combination of staphyloma and CRC represents more serious ocular damage than CRC alone. Staphylomas vary from pits in the optic disc to large peripapillary defects (Donovan et al. 1969). In the Collie, microphthalmos and hypoplastic papillae may also be associated with CEA (Stades and Barnet 1981). Choroidal hypoplasia is a constant diagnostic feature and both typical and atypical colobomatous defects of the optic disc and the peripapillary region occur with variable frequency. The effects exerted by the various defects range from no evident visual deficiency to blindness which is related to retinal detachment or intraocular haemorrhage. The area affected by choroidal hypoplasia is normally temporal or supertemporal to the optic disc, usually at the junction of the tapetal and non-tapetal fundi. Signs of choroidal hypoplasia can include a lack of retinal or retinal and choroidal pigment, a lack of tapetal tissue, if the lesion affects tapetal fundus, and abnormality of the choroidal vasculature. In those lesions which are characterised by a lack of choroidal pigment and attenuation or absence of choroidal vessels, the sclera can be seen as a white background (Bedford 1982a). CEA is characterised by bilateral involvement but the lesions are rarely symmetrical.

The percentage of patients with defective vision is low, blindness being a function of total retinal detachment or intraocular haemorrhage and rarely occurs bilaterally. Thanks to the congenital presence of the disease, early ophthalmoscopic diagnosis is possible. The examination is both practicable and advisable at six weeks of age (Bedford 1982b). A problem in diagnosis is the “go normal” phenomenon. The fundus of a puppy’s eye seems blue on ophthalmoscopy. At about three months of age, the retina changes colour to its adult yellow or to a green appearance (Barrie et al. 1981). Chorioretinal changes of minor extent found on examination before three months of age can be masked by later retinal pigmentation. Such dogs, which then appear normal, have been classified as “go normals” (Bedford 1982b).

On ophthalmoscopy the coloboma is an excavation of the optic disc surface and in some cases the adjacent ocular fundus. Large colobomas may lead to reduced vision while smaller ones have little or no effect (Bjerkas 1991).

<table>
<thead>
<tr>
<th>Number of dogs examined</th>
<th>Breed</th>
<th>Approximately affected (%)</th>
<th>Country</th>
<th>Author</th>
<th>Year published</th>
</tr>
</thead>
<tbody>
<tr>
<td>572</td>
<td>Collie</td>
<td>79.9</td>
<td>USA</td>
<td>Donovan and Wyman</td>
<td>1965</td>
</tr>
<tr>
<td>900</td>
<td>Collie</td>
<td>87</td>
<td>USA</td>
<td>Yakely et al.</td>
<td>1968</td>
</tr>
<tr>
<td>7000</td>
<td>Collie</td>
<td>90</td>
<td>USA</td>
<td>Donovan et al.</td>
<td>1969</td>
</tr>
<tr>
<td></td>
<td>Collie</td>
<td>86.5</td>
<td>USA</td>
<td>Rubin</td>
<td>1969</td>
</tr>
<tr>
<td>120</td>
<td>Shetland Sheepdog</td>
<td>48.3</td>
<td>Netherlands</td>
<td>Barnett and Stades</td>
<td>1979</td>
</tr>
<tr>
<td>160</td>
<td>Collie</td>
<td>40.6</td>
<td>Netherlands</td>
<td>Stades and Barnett</td>
<td>1981</td>
</tr>
<tr>
<td></td>
<td>Border Collie</td>
<td>6</td>
<td>United Kingdom</td>
<td>Bedford</td>
<td>1982a</td>
</tr>
<tr>
<td>2000</td>
<td>Collie rough</td>
<td>64</td>
<td>United Kingdom</td>
<td>Bedford</td>
<td>1982b</td>
</tr>
<tr>
<td>90</td>
<td>Collie smooth</td>
<td>65</td>
<td>United Kingdom</td>
<td>Bedford</td>
<td>1982b</td>
</tr>
<tr>
<td>400</td>
<td>Shetland Sheepdog</td>
<td>72</td>
<td>USA</td>
<td>Munyard et al.</td>
<td>2007</td>
</tr>
</tbody>
</table>

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Retinal detachments can be congenital or occur particularly before two years of age (Barrie and Gelatt 1981). The detachments are usually unilateral but may sometimes be bilateral and vary in distribution from bullous to total detachment (Bjerkas 1991). Intraocular haemorrhage is not common. Bedford (1982b) found it in approximately 1% of dogs with CEA only. It may appear as unclotted blood in the anterior eye chamber or as pre-retinal bleeding and is most likely to result from bleeding from abnormal vessels in the retina. In young puppies, it may also occur from persistent hyaloid blood vessels. The incidence of CEA in various dog breeds reported to date is summarised in Table 1.

3. Genetics of the disease and affected breeds

Since the discovery of CEA as a hereditary disease there have been efforts to find out how the disease is transmitted to the next generation. With respect to the variable signs of the disease some authors proposed that it is a polygenic trait (Donovan and Wyman 1969). Others concluded from studying pedigrees of affected dogs that the syndrome is either recessive or conditioned by the merle gene that is presumed to be partially dominant but with incomplete penetrance (Roberts 1967).

The work of Yakely et al. (1968) was the first study that indicated a recessive mode of inheritance. They crossed phenotypically affected Collie bitches and phenotypically healthy male Doberman Pinschers. The F1 generation was completely free of ocular lesions. The F2 generation was produced by mating each of the two F1 males to separate F1 females; two of the 15 offspring had characteristic lesions. For the backcross generation, six F1 dogs were used to mate with phenotypically affected Collies. Twenty of the 33 pups from these matings were completely free of ocular anomalies. The remaining 13 pups had manifestations of the anomaly. Then, they examined 362 Collie dogs from several breeders and found approximately 70% of affected animals. Significant differences between sexes were not found, which indicates that the anomaly is not sex-linked.

Yakely (1972) performed a study which focused on the possibility of decreasing the prevalence of CEA by selective breeding. After test matings, which indicated a recessive mode of inheritance, it was recommended to mate only phenotypically normal dogs together or a phenotypically affected dog to a phenotypically normal one. These intercourses, however, may produce affected progeny because of hidden affected alleles in phenotypically healthy carriers. But, after a relatively short period of time there was a decrease in the prevalence of the disease from 97% to 59%. These results demonstrate the recessive mode of inheritance.

Slightly differing results were obtained by Wallin-Hakanson et al. (2000). They processed information from a CEA clinical evaluation performed in the Swedish Kennel club between 1989 and 1997 and found that the numbers of affected and clinically healthy offspring were not in concordance with the projected numbers of individual categories based on Mendelian traits. For the evaluation of results, dogs were divided into groups based on two main clinical signs of CEA – coloboma and choroidal hypoplasia (CRD). Only litters with complete CEA status for both parents and offspring were taken into account. The prevalence of coloboma in offspring from various matings (normal × normal, normal × CRD, CRD × CRD and coloboma × CRD) were not significantly different. However, the prevalence of CRD in pups from normal × normal matings (43%), normal × CRD matings (53%), and CRD × CRD matings (85%) varied significantly. The prevalence of CRD in pups from CRD × CRD matings of 85% were significantly different from 100%, which would be expected for a simple autosomal recessive trait when both parents are homozygous. In addition to the impact on eye health, coloboma may have an impact on litter size. Results show that litters from coloboma × affected matings (coloboma + CRD) were significantly smaller than those of normal × normal matings and CRD × CRD matings. Donovan et al. (1969) assumed a separate gene controlling coloboma formation, hypostatic to the normal allele of the CRD locus. Similarly, gene interactions could explain smaller litters resulting from coloboma × affected matings.

Sargan (2001) proposed that while the results of Wallin-Hakanson et al. (2000) are inconsistent with some polygenic models, they also fit an autosomal recessive model of inheritance because of possible incomplete penetrance of the causative gene. The work of Lowe et al. (2003) provided support for this idea as their segregation studies indicated that penetrance of the CEA phenotype in dogs homozygous for choroidal hypoplasia is less than 100%; they observed both incomplete
penetrance of the CEA phenotype in homozygous affected dogs and partial penetrance in some heterozygotes. In accordance with their results it appears that some of the CEA-heterozygotes can express the affected phenotype.

In 2007, a landmark study was published which significantly enhanced our understanding of CEA. Parker et al. (2007) performed an extensive clustering study based on genotypic data generated from 96 microsatellite markers. They used clustering analysis to group 132 domestic dog breeds into five groups and also to find subclusters within some of the larger groups which showed additional levels of relatedness among some breeds. They found that breeds often affected by CEA (rough and smooth Collie, Border Collie and Australian Shepherd) cluster together. Using genetic methods (fine-mapping and mutation analysis) they found a 7799 bp deletion in the fourth intron of the NHEJ1 (Non-homologous end-joining factor 1) gene. This mutation was confirmed to be causative for CEA in the above mentioned breeds and in the Shetland Sheepdog (this breed was predicted to possess the affected phenotype before, so the authors searched for a clinically affected CEA dog from this breed. Finding that the affected Boykin Spaniels were homozygous for the mutation confirmed that this breed segregates CEA as well as the mutation and haplotype.

In a traditional Japanese breed – the Hokkaido dog – one female with clinical signs similar to CEA was found. The deletion was confirmed by SYBR Green-based Real Time PCR. The authors then examined 17 Hokkaido dogs, of which 12 including the parents of the affected dog were heterozygous carriers while the remaining five dogs were mutant homozygotes, i.e. CEA-affected dogs (Mizukami et al. 2012). The large deletion which is the causative mutation, although intronic, includes several conserved elements, most notably a 124-bp segment. This stretch of DNA is highly conserved among all mammalian genomes available at the time of study, including that of the opossum, and contains binding sites for multiple regulatory proteins. It has been postulated that the reduced interaction of just such a protein with the conserved region is responsible for the CEA defect. Either NHEJ1 itself or IHH (Indian Hedgehog) could be the target gene regulated by such an interaction. IHH is a member of the family of morphogens that regulate cell proliferation, differentiation, and cell-cell communication in developing embryos, which makes it a particularly attractive candidate gene for such a scenario (Parker et al. 2007). In Drosophila, Hedgehog genes establish central patterning signals, for example, in wings and eye discs, and they also regulate several other processes, including germ-cell migration, development of the optic lamina and so on (reviewed in Ingham and McMahon 2001). In the mouse, however, the eye sizes of IHH mutants were comparable to their wild-type littermates and astrocyte precursor cells at the optic disc and in the optic nerve also developed normally (Dakubo et al. 2003). It was found that most (but not all) of the NHEJ1 proteins are essential for embryonic viability. XLF (Cernunnos, NHEJ1) was identified as one of several factors implicated in RS-(S)CID. In all human XLF patients, microcephaly and growth delay were observed (Woodbine et al. 2014).

4. Genetic testing

After the causative mutation was found, a two-step PCR test was developed for easy evaluation of the deletion state in multiple breeds. The test utilises two primer pairs placed inside and outside the deletion to quickly identify chromosomes with and without the mutation (Parker et al. 2007). Based on the previous work mentioned, Dostal et al. (2010) performed simplified analysis of the deletion without DNA isolation and Chang at al. (2010) utilised a rapid genotyping technique based on SYBR Green Real Time PCR which was applied to blood and saliva specimens on Flinders Technology Associated Filter Papers. The genetic tests are commercially available now in many countries of the world, so the breeders and owners of
afflicted breeds can have their dogs tested for the presence of the causative mutation and then selection can be made to reduce CEA in these breeds.

5. Conclusion

As the causative mutation – the deletion of 7799 bp – is located in the intronic part of the NHEJ1 gene and contains highly conserved binding sites for several transcription factors, it can have an impact on both NHEJ1 and IHH expression. CEA is congenital, so a possible explanation for disease causation is that some of the transcription factors that can bind to the deleted part are essential for embryonic development of the affected part of the eye. However, there is as yet no study focused on embryonic or postnatal expression of the mentioned proteins, so we can only speculate about the true causation of the disease. Proteins, the altered expression of which can be the cause of clinical signs seen in CEA, are involved in the non-homologous end-joining (NHEJ) or hedgehog pathway (IHH), so a postnatal influence on the individual’s health is possible when he/she is a recessive homozygote for the deletion. However, there is as yet no study reported on postnatal expression of the mentioned proteins in dogs affected by CEA in response to other pathological conditions. As dogs are mostly pet animals, their owners/breeders are not obliged to report heritable disease to any central registry. Therefore, it is not possible to verify if there is, for example, an increased incidence of cancer or immune disorders in recessive homozygotes. For the future it would be advisable to verify if CEA-affected animals tend to suffer from any other health problems. For a better understanding of the disease it is necessary to determine how the deletion is involved in the development of the clinical signs of CEA. Thanks to modern methods available now, it is possible to develop a transgenic model to study diseases. Due to the high conservation of the genomic region surrounding the deletion, a mouse model (with short generation interval) may be suitable for experimental work focused on expression analysis of the mentioned proteins during the embryonic development of the eye (and other tissues) as well as postnatal development of recessive homozygotes for CEA deletion, and to investigate, if these homozygotes suffer from additional health problems.

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7. REFERENCES


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