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# Discrepancy in compliance between the clinical and genetic diagnosis of choroidal hypoplasia in Danish Rough Collies and Shetland Sheepdogs

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**Summary**

Collie eye anomaly (CEA) is a congenital, inherited ocular disorder which is widespread in herding breeds. Clinically, the two major lesions associated with CEA are choroidal hypoplasia (CH) and coloboma, and both lesions are diagnosed based on ophthalmological examination. A 7.8-kb intronic deletion in the gene encoding non-homologous end-joining factor 1 (*NHEJ1*) has been reported to be the causative mutation underlying CH when present in the homozygous state. In this study, we have investigated the compliance between the clinical and genetic diagnosis of CH in the Danish Rough Collie and Shetland Sheepdog populations. Our results show that the deletion in *NHEJ1* is not predictive for CH in the Danish Rough Collie population, whereas the clinical and genetic diagnosis is in accordance with each other in the Shetland Sheepdog population. Based on these results, it can be concluded that the intronic deletion in *NHEJ1* is not the causative mutation but, rather, a marker linked to the locus underlying the trait in some populations but linked to both the wild-type and CH-causing locus in most dogs in the Danish Rough Collie population.

**Keywords** choroidal hypoplasia, Rough Collies, Shetland Sheepdogs

Collie eye anomaly (CEA) is a congenital, inherited ocular disorder which has primarily been demonstrated in herding breeds. The disease is widespread in Collies and Shetland Sheepdog with a prevalence of, for instance, 64% and 72% in the UK (Bedford 1982) and 48% and 41% in the Netherlands (Barnett & Stades 1979; Stades & Barnett 1981) in the two breeds respectively. Clinically, the two major lesions associated with CEA are choroidal hypoplasia (CH) and coloboma. CH affects the choroid temporal or superotemporal to the optic disk resulting in vascular abnormalities with wider and fewer vessels with abnormal, non-radial arrangement. Pigment depletion in the retinal pigment epithelium and absence of tapetal tissue allows visualization of these areas as pale areas. Coloboma is characterized by excavations within or adjacent to the optic disk. Both the clinical expression and the degree of visual affliction vary to a great extent among individuals (Barnett 1979). The proportion of CEA patients with defective vision is small, and total blindness is related to retinal detachment or intra-ocular hemorrhage (Bedford 1982). It has been debated whether CH and coloboma are parts of the same trait (Yakely *et al.* 1968) or separate traits (Donovan *et al.* 1969; Wallin-Håkanson *et al.* 2000); however clinically, coloboma is a more severe condition than is CH.

In order to get an accurate clinical diagnosis of CH, ophthalmological examination within the first 10 weeks of the puppy's life is paramount. After this period, CH changes might be masked due to tapetal development and choroidal pigmentation, the so-called go-normal effect (Bedford 1982; Bjerkås 1991).

Using linkage mapping in families phenotyped with respect to CH at an early age (Lowe *et al.* 2003) combined with a multibreed approach for fine-mapping (Parker *et al.* 2007), a 7.8-kb intronic deletion in the gene encoding non-homologous end-joining factor 1 (*NHEJ1*) has been reported to be the causative mutation underlying CH when present in the homozygous state.

In order to test the compliance between the clinical and genetic diagnosis of CH in the Danish Rough Collie and Shetland Sheepdog populations, cheek swabs from 45 and 56 dogs, respectively, unrelated at the parental level, were collected for genotyping. All Collies except two (one unaffected and one affected) had a clinical diagnosis

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established between 5 and 7 weeks of age, and all Shetland Sheepdogs had a clinical diagnosis established before 10 weeks of age by veterinarians certified as ECVO eye scheme examiners. The dogs were examined according to the ECVO scheme (http://www.ecvo.org/inherited-eye-diseases) with indirect ophthalmoscopy using appropriate lenses (at least a 30- and a 20-diopter lens) after treatment with a mydriatic. Pale areas and/or areas with abnormal vasculature in the typical position for CH were considered diagnostic. In particular, in color-diluted animals, irregularity with fewer, wider and non-radial arrangement was taken into consideration as diagnostic for CH. The clinical status of the 45 Collies was 34 affected and 11 unaffected, and the clinical status of the 56 Shetland Sheepdogs was 28 affected and 28 unaffected. Because we aimed at collecting both affected and unaffected dogs in both breeds rather than sampling at random, the clinical status within our test populations does not reflect the true prevalence within the respective breeds in Denmark. However, the skewed distribution in the Collie samples confirms the general perception that the prevalence is high in this breed in Denmark.

Cheek samples were collected on Watman® FTA cards using buccal swabs (VWR). DNA was isolated using the QiaAmp DNA extraction kit following the manufacturer's recommendations (QIAGEN). Genotyping was performed using the two-step PCR protocol described by Parker *et al.* (2007) using the primers NHEJ1-F17: 5′-TCTCACAGGC AGAAAGCTCA-3′ and NHEJ1-R17: 5′-CCATTCATTCCTTT GCCAGT-3′ to amplify 603 bp representing the wild-type allele (wt), in which the intron is not deleted, and NHEJ1-F20: 5′-TGGGCTGGTGAACATTTGTA-3′ and NHEJ1-R23: 5′-CCTTTTTGTTTGCCCTCAGA-3′ to amplify 872 bp across the allele with the 7.8-kb intron deletion (del). Moreover, a primer pair (NHEJ1int_F: TGGCCTTCTCTCACTCTGGT/ NHEJ1int_R: GCAATTGCTCAGGTTTTGGT), amplifying a 352-bp fragment within the intron reported to be deleted in the affected dogs, was used to confirm the presence of the deletion. The PCR products were run on a 1.5% agarose gel, and fragment sizes were assessed using a 100-bp DNA ladder.

Among the 45 Collies, 44 dogs were genotyped to be homozygous for the deletion (del/del) and one dog was heterozygous (wt/del). Among the 56 Shetland Sheepdogs, 25 were genotyped to be homozygous for the deletion (del/del), 16 were heterozygous (wt/del) and 15 were homozygous for the wild-type allele (wt/wt). The compliance between the clinical diagnosis and the DNA diagnosis appears in Table 1. Within the population of Collies investigated in this study, it is apparent that the compliance between the clinical diagnosis and the DNA test was poor, whereas it was good for the Shetland Shepherd population. The chi-square test for a $2 \times 2$ contingency table using the results from the Collie population shows that chi-square = 3.16 (df = 1), which is lower than 3.84, indicating that there is more than a 95% chance of independent segregation between a hypothetical locus underlying the clinical symptoms and the *NHEJ1* locus in this population. Conversely, calculating the chi-square based on the results from the Shetland Sheepdog population shows that chi-square = 38.22 (df = 1), and thus, there is less than 0.1% chance of independent segregation between the two loci in this population. Interestingly, the four Shetland Sheepdogs that were classified as affected by the clinical diagnostic test and classified as unaffected by genotyping (one wt/wt and three wt/del) were all of the Blue Merle coat color—a coat color in dogs for which it is generally recognized by veterinarians that the ophthalmological examination is hampered because the ocular tapetum is poorly developed or absent. The presence of the deletion both within the affected and unaffected Collies was confirmed using the NHEJ1int primer pair, showing that the discordance within this breed cannot be explained by a polymorphism in the primer binding sites of the diagnostic primers; that is, the NHEJ1int failed to amplify both in clinically affected and unaffected dogs, whereas the 352-bp fragment was confirmed to be present in the unaffected Shetland Sheepdogs.

**Table 1** Compliance between clinical and genetic diagnosis.

| | Genotypes | | | | | | |
|---|---|---|---|---|---|---|---|
| | Clinically unaffected | | | Clinically affected | | | |
| Breed | wt/wt | wt/del | del/del | wt/wt | wt/del | del/del | Total |
| Shetland Sheepdog | 14 | 13 | 1 | 1 | 3 | 24 | 56 |
| Rough Collie | 0 | 1 | 10 | 0 | 0 | 34 | 45 |

wt, wild-type allele; del, allele with the 7.8-kb intron deletion in *NHEJ1*.

We contacted the owners to follow up on the health status of 21 Collies (18 with a clinical CH diagnosis and three diagnosed as clinically unaffected before 10 weeks of age) and 14 Shetland Sheepdogs (seven with a clinical CH diagnosis and seven diagnosed as clinically unaffected before 10 weeks of age). According to the owners, only one of these dogs had impaired vision. These results support the notion that the proportion of CEA patients with defective vision is small.

In conclusion, it appears that the deletion in *NHEJ1* is not predictive for CH in the Danish Rough Collie population. Thus, it can be concluded that the intronic deletion in the gene is not the causative mutation but, rather, a marker linked to the locus underlying the trait in some populations but apparently linked to both the wild-type and CH-causing locus in other populations. *NHEJ1* is located in a fairly gene-rich region on dog chromosome 37, and many of the genes in the region are not well annotated at the functional level. Thus, the mutation underlying CH might either be situated in a regulatory region affecting *NHEJ1* expression or in a closely linked gene. Given that the proportion of CEA

patients with defective vision is quite small, it appears that a diagnostic test directed toward identifying dogs at risk for developing coloboma would potentially be of greater clinical importance in the breeds suffering from CEA compared with a test diagnosing CH.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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