CASE REPORT

Collie eye anomaly in Hokkaido dogs: case study

Keijiro Mizukami,* Hye-Sook Chang,* Mitsuharu Ota,† Akira Yabuki,* Mohammand A. Hossain,* Mohammad M. Rahman,* Mohammad M. Uddin* and Osamu Yamato*

*Laboratory of Clinical Pathology, Department of Veterinary Clinical Sciences, Faculty of Agriculture, Kagoshima University, 1-21-24 Kogirimoto, Kagoshima 890-0065, Japan; and †Animal Eye Center, 13 Ichigahora, Nagakute, Nagakute-cho, Aichi-gun 480-1131, Japan

Address communications to:
O. Yamato
Tel/Fax: +81-99-285-3560
e-mail: osam@agri.kagoshima-u.ac.jp

Abstract

Objective To describe a Hokkaido dog, one of the traditional Japanese breeds that was affected by Collie eye anomaly (CEA), and to report the genotype of this dog and the Hokkaido dog allelic frequency of the CEA-associated mutation.

Case A nine-month-old intact female Hokkaido dog without any obvious visual disturbance was diagnosed ophthalmoscopically with CEA. Severe choroidal hypoplasia was observed in the bilateral temporal area adjacent to the optic nerve head, appearing as whitish areas. Therefore, the dog was suspected of possessing the CEA-associated mutation that was previously reported as an intronic 7.8-kilo base deletion in the canine NHEJ1 gene.

Procedures SYBR Green-based real-time PCR with a melting curve analysis, conventional PCR with agarose gel electrophoresis, and direct DNA sequencing were carried out to determine the genotype of the dog. Furthermore, a preliminary genotyping survey was carried out in 17 Hokkaido dogs from three kennels using the real-time PCR method, and the pedigree relationships were analyzed using their pedigree papers.

Results The Hokkaido dog affected by CEA was proven to possess the CEA-associated mutation. Of these 17 Hokkaido dogs, 12 dogs were heterozygous carriers and five dogs were affected by this mutation. The preliminary genotyping survey and pedigree analysis demonstrated that the allelic frequency of the CEA-associated mutation is very high in Hokkaido dogs.

Conclusion These data suggest that the Hokkaido breed is highly susceptible to CEA because of the known CEA-associated mutation much like the Collie-related breeds.

Key Words: canine, choroidal hypoplasia, Collie eye anomaly, Hokkaido dog, Japanese breed, SYBR Green-based real-time polymerase chain reaction

INTRODUCTION

Collie eye anomaly (CEA) is a congenital inherited canine ocular disorder affecting the posterior segment of the eye.1–4 CEA is a pleomorphic syndrome, with variability in manifestation and severity of clinical and ophthalmologic lesions. The two main ophthalmoscopic changes are regional choroidal hypoplasia and coloboma of the optic disk or adjacent areas, which may be bilateral and are often symmetrical with equal severity. Clinically, the onset of CEA results in an ophthalmoscopically detectable window defect in the ocular fundus located temporal to the optic nerve.5 Defects of the sclera characterized by colobomatous lesions may also occur presenting as pits within or engulfing the optic nerve head or in the adjacent fundus. Mildly to moderately affected individuals appear to retain normal visual function throughout life as determined by behavioral observation and clinical electroretinography. However, severely affected dogs, particularly those with colobomas, can develop retinal detachment and intraocular hemorrhage leading to blindness, although bilateral blindness rarely occurs.

Many studies have been performed to determine the mode of inheritance for this syndrome.3,6–12 Variability in the severity and expression of the disease is well recognized in the CEA phenotype and suggests that CEA may be a complex trait with multiple genetic contributors.5 However,
recently, a mutation was demonstrated by fine-mapping study across multiple dog breeds as an intronic deletion of 7799 base pairs (bp) in the *NHEJ1* gene, enabling detection of both homozygotes and heterozygotes by a conventional polymerase chain reaction (PCR)-based diagnostic test. Furthermore, a rapid DNA assay using SYBR Green-based real-time PCR was also developed to detect this mutation.

Collie eye anomaly has historically been clinically characterized as a hereditary disorder segregated in Collie-related breeds including Australian Shepherd, Border Collie, Lancashire Heeler, Rough Collie, Shetland Sheepdog, and Smooth Collie. Molecularly defined CEA has recently been recognized in 11 breeds: Australian Shepherd; Australian Shepherd (Miniature); Border Collie; Boykin Spaniel; Collie (Rough); Collie (Smooth); Lancashire Heeler; Longhaired Whippet; Nova Scotia Duck Tolling Retriever; Shetland Sheepdog; and Silken Windhound. Notably, all of these are Collie-related breeds. Lesions that resemble CEA clinically are also observed in some dogs as a variable expression of the Merle phenotype – e.g., Catahoula Leopard Dog, Dachshund (all varieties), and Great Dane – but this phenotype is molecularly distinct from CEA. Clinical phenotypes resembling CEA have also been observed occasionally in various other non-Collie breeds including Beagle, German Shepherd, Miniature or Toy Poodle, and mixed-breed dogs, but the molecular causes of these phenotypes have not been determined.

This case study is the first report of CEA observed in one of the traditional Japanese breeds, Hokkaido dog. The affected dog was diagnosed by ophthalmoscopy and molecular methods. In addition, pedigree analysis was carried out to determine the allelic frequency in the population of Hokkaido dogs.

**CASE**

A nine-month-old intact female Hokkaido dog with a white coat was referred for entropion of the lower eyelid with associated corneal pigmentation. There were no visual deficits observed. A routine ophthalmoscopic examination revealed severe choroidal hypoplasia bilaterally and temporal to the optic nerves (Fig. 1). No other posterior segment abnormalities were noted. The rest of the physical and ophthalmic examination including intraocular pressure measurements and Schirmer tear tests was normal. After 17 days, the entropion was corrected surgically with the secondary corneal pigmentation improving as a consequence. Based on the ophthalmologic examination, this dog was diagnosed with CEA and suspected of having the CEA-associated mutation.

**MATERIALS AND METHODS**

**Collection of DNA and pedigree information**

The affected female dog was considered for breeding in a kennel (A) specializing in Hokkaido dogs. This dog had...
originated from kennel B that possessed the dam. The sire was from a different kennel (C). Saliva specimens were obtained from the affected dog and seven other dogs in kennel A, from nine dogs including the dam in kennel B, and from the sire in kennel C, using Flinders Technology Associates filter paper (FTA card; Indicating FTA Classic Card; Whatman International Ltd., Piscataway, NJ, USA) and FTA special applicator to swab saliva (Sterile Foam Tipped Applicator; Whatman International Ltd.). As a positive control specimen, saliva from a male Rough Collie that was known to have a heterozygous carrier genotype was obtained from a kennel specializing in Collie breeds. Saliva-spotted FTA cards and pedigree papers were sent directly from the kennels to our laboratory after obtaining informed consent of the breeders. DNA on FTA cards was used for all genetic analyses in this study. Pedigree analysis was carried out using pedigree papers issued from three different kennel clubs specializing in Hokkaido dogs.22,23

**Genetic tests**

The genotype of the CEA-associated mutation in the affected dog was determined using three types of genetic tests, i.e., SYBR Green-based real-time PCR with a melting curve analysis,14 conventional PCR with agarose gel electrophoresis,13,14 and direct DNA sequencing. Real-time PCR and conventional PCR were carried out according to the protocols and conditions reported previously.14 Direct DNA sequencing was carried out to confirm the DNA sequence around the 7799-bp deletion. PCR was performed using forward (F20: 5'-TGG GCT GGT GAA CAT TTG TA-3') and reverse (RM: 5'-ACC AAT CAT CCA GCC CAG CAT TTA A-3') primers. The target PCR product (279 bp) was separated on agarose gel and excised and purified using the gel extraction kit (QIAquick Gel Extraction Kit; QIAGEN, Tokyo, Japan). Direct cycle sequencing of the purified PCR product was carried out by a commercial company (Hokkaido System Science Co. Ltd., Sapporo, Japan). The genotypes of all other dogs were determined using the real-time PCR method.

**RESULTS**

The result of the SYBR Green-based real-time PCR with a melting curve analysis on the affected dog is shown in Fig. 2. Only the mutant allele was amplified, suggesting a mutant homozygote. In the melting curve analysis after PCR amplification, a single peak corresponding to the mutant product in temperature was obtained, suggesting the specific amplification without any nonspecific reactions. In the conventional PCR, only mutant product of 941 bp in length was recognized on agarose gel electrophoresis (Fig. 3), also suggesting the mutant homozygote. Furthermore, the result of the direct DNA sequence demonstrated that the genome of...
the affected dog lacked the 7799-bp region in intron 4 of the canine \( \text{NHEJ1} \) gene (Fig. 4).

Of the 17 Hokkaido dogs examined, 12 dogs including the parents of the affected dog were heterozygous carriers and the remaining five dogs were mutant homozygotes, i.e., CEA-affected dogs. Regarding this mutation, there were no normal or clear dogs. The pedigree analysis demonstrated that 13 dogs had a genetic relationship with the affected dog as shown in pedigree 1 in Fig. 5. However, the other four dogs did not have a genetic relationship with the affected dog, although they were connected with each other as shown in pedigree 2 in Fig. 5.

**DISCUSSION**

This is the first reported occurrence of CEA in the Hokkaido dog breed. The diagnosis of CEA in the affected dog was established ophthalmologically and genetically based on the CEA-specific findings of ophthalmoscopy and the results of the genetic tests. The result of direct DNA sequencing demonstrated that the mutation of the affected dog was the same as the CEA-associated mutation reported previously in Collies and other Collie-related breeds.

It is commonly thought that dogs affected with CEA belong to one of the herding breeds with Collie ancestry. In the previous surveys, the prevalence of CEA has been estimated to be 70–97% for Rough and Smooth Collies in the United States and Great Britain,1,10 68% for Rough Collies in Sweden,11 72% for Shetland Sheepdogs in Great Britain,1 13.7% for Lancashire Heelers in Great Britain,7 6% for Border Collies,15 and 4% for Australian Shepherds in Australia.24 At present, these six Collie-related breeds are recognized to be susceptible to CEA.

In the present study, a preliminary genotyping survey was carried out on 17 Hokkaido dogs from three different kennels and demonstrated that they all had at least one mutant allele, i.e., five affected dogs, 12 carriers, and no normal dogs. Furthermore, pedigree analysis suggested that affected and carrier dogs seemed to be distributed widely in the population of Hokkaido dogs, not limited to the close family related to the affected dog. The correlation between phenotype and genotype was not determined in Hokkaido dogs because none of the dogs except the affected dog underwent ophthalmoscopy to detect CEA-related findings. However, the data obtained in this study suggest that the Hokkaido breed is highly susceptible to CEA because of the known CEA-associated mutation much like those six Collie-related breeds with a predilection for CEA.

There is a question about how the CEA-associated allele became segregated in Hokkaido dogs. The possible reasons include: (i) there may have been an unsuspected historical admixture of Collie-related dogs into the Hokkaido breed, (ii) the CEA-associated mutation in Hokkaido dogs might be an independent deletion, and (iii) the CEA-associated mutation may have originated as a much more ancient event than previously suspected and have entered the Hokkaido and Collie-related gene pools from a very ancient common ancestor. The first speculation would be ruled out because of the large difference in appearance between Hokkaido and Collie-related dogs. An accidental but exactly identical deletion in the second speculation is unlikely to occur stochastically. Therefore, the last speculation is the most likely. Several reports of CEA-like disorder in non-Collie breeds

---

**Figure 4.** Electropherograms of the affected dog. The dog chromatograph shows intronic deletion of 7799 bases in the canine \( \text{NHEJ1} \) gene (dotted line).

**Figure 5.** The pedigree of Hokkaido dogs examined in this study. Genotypes were determined using SYBR Green-based real-time polymerase chain reaction assay with a melting curve analysis. *: Affected dog; A, B, and C: kennels.
might support this theory, although the findings have not yet been molecularly defined.13,19–21 This question could be resolved by careful SNP genotyping, haplotype, and linkage disequilibrium analyses.

The Hokkaido dog is one of the traditional Japanese breeds and was originally used for hunting, but is now mainly bred for show or as guard dogs, and is a protected species.22,23 Compared to other Japanese breeds, such as the Akita and Shiba Inu, the population of Hokkaido dogs is limited. Among these breeds, the Akita was described as one of the breeds with sporadic isolated colobomas in the supplementary data of the report on CEA-associated mutation,13 but this description of the Akita was not supported by genetic verification. Further work on these Japanese dog breeds is needed to elucidate the prevalence of CEA and CEA-associated mutation and to eradicate this unfavorable allele.

ACKNOWLEDGMENTS

This study was supported financially by grants (Nos. 20380173, 20-08112, and 21658109, OY) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES