Different allelic frequency of progressive rod-cone degeneration in two populations of Labrador Retrievers in Japan

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ABSTRACT. Progressive rod-cone degeneration (PRCD) is an autosomal recessive disease caused by c.5G>A mutation of the PRCD exon 2. This mutation has been identified in various breeds, including Labrador Retriever. The present study aimed to examine the allelic frequency of PRCD in Labrador Retrievers in Japan. A domestic and a guide dog population were genotyped for PRCD using polymerase chain reaction-restriction fragment length polymorphism. The allelic frequency of c.5G>A in domestic and guide dog populations (0.114 and 0.026, respectively) differed significantly. The allele with c.5G>A mutation appeared to spread widely in the domestic population as compared to that in the guide dog population. This might be the result of mating control for PRCD in the guide dog population.

KEY WORDS: allelic frequency, Labrador Retriever, mutation, progressive rod-cone degeneration
UltraPower™ DNA Dye solution (Gelex International, Tokyo, Japan).

Allele-specific PCR was performed in five dogs (two wild-type dogs, two heterozygous and one homozygous mutant-type), whose genotypes were identified by PCR-RFLP. Reverse primers specific for the wild-type allele (G) and the mutant allele (A) were designed. The primer for the allele G corresponded to the wild-type sequence, except for an artificial artifact introduced at the third codon from 3‘ end, indicated in lower-case (PRCD-G: 5′-GCTGAGTAGGAAGAGGGTGGTca-3′). The primer specific for the mutant allele (A) corresponded to the mutant sequence, except for an artificial artifact introduced at the third codon from 3’ end (PRCD-A: 5′-GCTGAGTAGGAAGAGGGTGGTta−3′). The second codon from 3‘ end in each primer was matched to the site where the point mutation occurred. These primers were used with the forward primer used for PCR-RFLP to amplify 173-bp products. Two reactions were set up for each sample, one with the primer specific to allele G, and the other specific to allele A; these primers were used in combination with the common forward primer. PCR was carried out as described above. The following thermocycling conditions were used: initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 65°C for 20 sec, and extension at 72°C for 20 sec, and the amplicons were visualized as described above.

Allelic frequencies in each dog population were calculated based on genotype frequencies. Genotype data were analyzed by chi-square test for deviation of genotype and allele frequencies from the predictions of Hardy-Weinberg equilibrium [8]. Fisher’s exact test was used to compare the allelic frequencies of the domestic and guide dog populations [3].

The genotype frequencies of PRCD obtained with PCR-RFLP agreed with Hardy-Weinberg equilibrium in both the domestic and guide dog populations (Table 1). The frequency of allele A in the domestic population was significantly higher than that in the guide dog population (P<0.01). Based on these results, expected incidence rate of PRCD in domestic and guide dog populations was estimated to be 1.3 and 0.07%, respectively.

Labrador Retrievers were divided into domestic and guide dog populations. As breeding stocks of these two populations are different, incidence of genetic diseases may differ [7]. In the present study, the genotypic frequency of PRCD and allelic frequency of c.5G>A were examined in each population. The allelic frequency of c.5G>A in the domestic population was significantly higher than that in the guide dog population (P<0.01). Based on these results, expected incidence rate of PRCD in domestic and guide dog populations was estimated to be 1.3 and 0.07%, respectively.

In allele specific PCR, the G allele-specific primer yielded an amplicon in dogs with GG or GA genotypes, but not in AA genotype (Fig. 1). However, the A allele-specific primer yielded an amplicon in dogs with GA or AA genotypes, but not in the GG genotype. The results of an allele-specific PCR were consistent with those of PCR-RFLP.

Labrador Retrievers were divided into domestic and guide dog populations. As breeding stocks of these two populations are different, incidence of genetic diseases may differ [7]. In the present study, the genotypic frequency of PRCD and allelic frequency of c.5G>A were examined in each population. The allelic frequency of c.5G>A in the domestic population was significantly higher than that in the guide dog population. Mating control to prevent the spread of PRCD has been introduced in the guide dog population several years ago. This might be the reason for low frequency of c.5G>A in this population (0.07%), indicating that only one affected dog is present in 1,400. Since there are 2,000 individuals in the guide dog population in Japan, no dogs affected by PRCD might be present, suggesting almost complete exclusion of c.5G>A from this population.

The allelic frequency of c.5G>A in domestic population is 0.114 and incidence of PRCD is predicted to be 1.3%. The allelic frequency in Toy poodle and Chihuahua in Japan has been reported to be 0.088 and 0.019, respectively [4], indicating that PRCD incidence is 0.77 and 0.036%, respectively. Incidence of PRCD in domestic Labrador Retrievers is higher than that in Toy poodle or Chihuahua, suggesting that c.5G>A mutation is spreading widely in this population before people realize this disease. As evident from the guide dog population, the frequency of the mutant allele can be decreased by controlled mating. Therefore, if genotype-based mating is performed in the domestic dog population, incidence of PRCD might decrease. Since PRCD develops after middle age of dogs, sometimes the disease is not detected by general eye examination in the age group used for mating. Therefore, genotyping might be a superior method for the diagnosis of PRCD-affected dogs than ophthalmic examination.

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**Table 1.** Genotypic and allelic frequencies of Labrador Retrievers

| Genotype | N  | GG (%) | GA (%) | AA (%) | A allele frequency |
|----------|----|--------|--------|--------|-------------------|
| Domestic | 114| 89 (78.1)| 24 (21.0)| 1 (0.9)| 0.114 |
| Guide dog | 170| 161 (94.7)| 9 (5.3)| 0 (0)| 0.026 |

a) AA, homozygous for c.5G>A.

**Fig. 1.** Allele-specific PCR of PRCD. Genotype of each individual using specific primer for G and A alleles are shown. The G allele-specific primer yields an amplicon in the genotypes GG and GA, but not in AA. The primer specific to the A allele yields an amplicon in the genotypes GA and AA, but not GG.
In the allele-specific PCR analysis of five dogs, which had been genotyped by PCR-RFLP, the GG, GA and AA genotypes could be clearly identified. Real-time PCR has been developed for genotyping of PRCD previously [2, 4, 9]. However, real-time PCR is more expensive than conventional PCR, despite being an excellent method. Nevertheless, allele-specific PCR described in the present study is a cost-effective method and thus may be another option for genotyping PRCD.

The present study revealed significant difference in the allelic frequency of c.5G>A of PRCD exon 2 in domestic and guide dog populations of Labrador Retriever in Japan. Incidence of this disease in the domestic population is relatively high, whereas that in the guide dog population is low. These results suggested that controlled breeding based on genotyping could reduce the incidence of PRCD in Labrador Retrievers.

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