Mutation in the *RPE65* gene causing hereditary retinal dystrophy in the Briard dogs: application of a new detection method

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ABSTRACT: Inherited eye diseases are widespread in most of the pure dog breeds and they show a severe impact on canine health, welfare and working ability. Congenital stationary night blindness (CSNB) was originally described in Briards. CSNB is slow progressive retinal degeneration with very early onset of clinical symptoms and is inherited in an autosomal recessive manner. The causative mutation (Y16567.1: c.487_490delAAGA) for CSNB was identified in exon 5 of the *RPE65* gene. This deletion results in a frameshift and leads to a premature stop codon and expression of a non-functional protein. To date, only expensive, laborious or unpractical methods have been used for detection of the mutation in the canine *RPE65* gene. The main goals of this study were to develop a new method for routine genotyping of the causative mutation and to assess its occurrence in the Czech population of Briards. The method of electrophoresis in the gel Spraedex EL600 can be widely used for genotyping of the *RPE65* gene as a basis of proper genetic counselling and an improvement of genetic health in the Briard populations. In the studied population, the following frequencies of alleles + (wild) and – (mutant) were observed – 0.939 and 0.061, respectively.

Keywords: *RPE65* gene; CSNB; dog; Briard; electrophoresis

Inherited eye diseases are widespread in most of the pure dog breeds and they show a severe impact on canine health, welfare and working ability. Therefore, the efforts of veterinarians, geneticists and breeders are focused on disclosure of their aetiology, genetic background and therapy. Congenital stationary night blindness (CSNB) was originally described by Narfström et al. (1989) in Briards as a specific form of retinal dystrophy. CSNB is slow progressive retinal degeneration with very early onset of clinical symptoms and it is inherited in an autosomal recessive manner. Aguirre et al. (1998) and Veske et al. (1999) identified a mutation (Y16567.1:c.487_490delAAGA) in exon 5 of the *RPE65* gene that is causative for CSNB in Briards. This deletion results in a frameshift and leads to a premature stop codon and expression of a non-functional protein. *RPE65* protein with its robust isomerohydrolase activity is responsible for regeneration of 11-*cis* retinol in the visual cycle (Moiseyev et al., 2005). The *RPE65* gene is fully conserved in mammals and is expressed exclusively in the retinal pigment epithelium (RPE) (Nicoletti et al., 1995).

Supported by the Academy of Sciences of the Czech Republic (Grant No. 1QS500450578) and Czech Science Foundation (Grant No. 523/03/H076) and IRP IAPG (Grant No. AVOZ 50450515).

In humans, several mutations in the *RPE65* gene are recognised or presumed as causes of inherited eye disorders such as retinitis pigmentosa and Leber's congenital amaurosis, the homology disease to canine CSNB (Gu et al., 1997; Marlhens et al., 1997; Morimura et al., 1998; Kondo et al., 2004).

Dekomien and Epplen (2003) screened the *RPE65* gene in individuals of 25 canine breeds affected with progressive retinal atrophy. Despite of the identification of a series of polymorphisms, none of them was proved to be the disease-causing mutation.

To date, only expensive, sophisticated, laborious or unpractical methods (sequencing, fragmentation analysis, polyacrylamide gel electrophoresis) have been used for genotyping of the delAAGA mutation of the canine *RPE65* gene in Briards (Aguirre et al., 1998; Veske et al., 1999; Switonski et al., 2002). The main goal of this study was to develop an effortless, reliable and less expensive method suitable for routine genotyping of the causative mutation and its application in genetic screening of the Czech population of Briards.

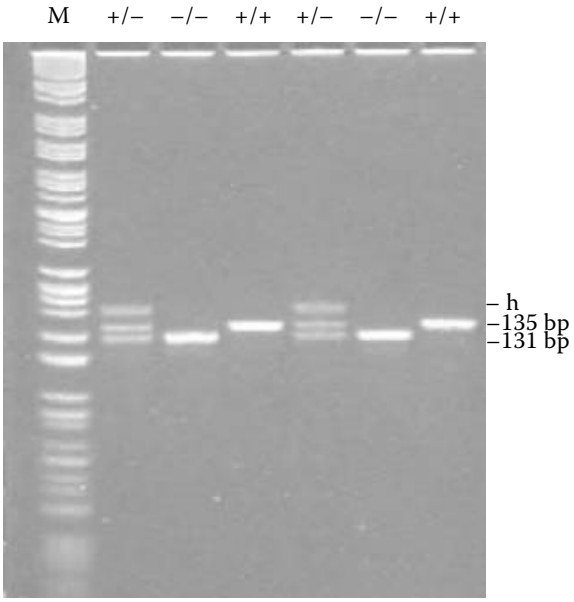

Figure 1. The Spreadex EL600 gel electrophoresis of the *RPE65* gene polymorphism showing the mutant allele delAAGA. Genotypes are indicated at the top of each line – +/+ normal homozygote; +/– heterozygote and –/– mutant homozygote. M–M3 ladder (Elchrom Scientific, Switzerland); h – heteroduplex

MATERIAL AND METHODS

The investigated sample population comprised 173 both related and unrelated individuals of the Briard breed from the Czech Republic and a few animals from foreign countries (Poland, Hungary and Slovakia) besides.

The amplification of exon 5 of the *RPE65* gene harbouring deletion was carried out with primers designed by Veske et al. (1999):
RPE71: 5'-TTTCTTACTTCCGAGGAGTG-3'
RPE186: 5'-GCTTAATTGTCTCCAGGGTC-3'

The PCR was performed in 25 µl reactions containing 100 ng genomic DNA, 200µM dNTP, 10 pmol of each primer, 2% DMSO, 1× LA PCR Buffer, 2.0mM $MgCl_2$ and 1 U LA DNA Polymerases Mix (Top-Bio, Prague, Czech Republic). The PCR-profile included 2 min at 95°C, 35 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 68°C, followed by a final extension of 7 min at 68°C.

Obtained PCR products were analyzed on the gel Spreadex EL600 using Elchrom's SEA2000 equipment (Elchrom Scientific, Switzerland). The mixtures consisting of 2 µl PCR product, 1 µl loading buffer and 2 µl 30mM TAE buffer were loaded on the gel and separated for approximately 2.5 h at a voltage 100 V and room temperature. The gel was stained with ethidium bromide (0.4 µg per ml) for 20 min and destained in distilled water for 5 min. The bands were visualized under UV light. The wild (+; normal) and deleted (–; mutant) alleles result in amplicons of the lengths 135 bp and 131 bp, respectively (Figure 1).

The contingency table chi-square test was used for a comparison of genotype frequencies between particular populations.

RESULTS AND DISCUSSION

Methods previously described for the detection of the delAAGA polymorphism in the *RPE65* gene (Aguirre et al., 1998; Veske et al., 1999; Switonski et al., 2002) exhibited several drawbacks, e.g. high laboriousness and environmental toxicity (PAGE), inability to detect heterozygotes (sequencing), and requirements for technical equipment (such as genetic analyzer). The application of Spreadex gels and Elchrom's SEA2000 apparatus was proved to be a worthwhile option for routine genotyping of the causative mutation. Compared to the former methods, this approach is highly advantageous for routine testing in diagnostic laboratories for its robustness, serviceability and low expense.

Table 1. Observed genotype and allele frequencies in delAAGA mutation of the *RPE65* gene in the Czech population of Briard dogs and their comparison to foreign populations

Population	*n*	Genotype frequencies			χ^2	Allele frequencies	
		+/+	+/–	–/–		+	–
Czech Republic	173	0.884	0.110	0.006		0.939 ± 0.013	0.061 ± 0.013
Poland[1]	24	0.917	0.083	0	0.298/NS	0.958 ± 0.029	0.042 ± 0.029
USA[1]	108	0.898	0.102	0	0.678/NS	0.949 ± 0.015	0.051 ± 0.015

[1]Switonski et al. (2002)

NS = non-significant differences (critical values: $\chi^2_{(2;\,0.05)} = 5.99$; $\chi^2_{(2;\,0.01)} = 9.21$)

In Figure 1, all three genotypes, +/+ (normal), +/– (heterozygote) and –/– (mutant) are shown. A slowly migrating zone of heteroduplex is present in heterozygotes.

The benefits of this method were verified in a molecular genetic analysis of the Czech population of Briards. The frequencies of the genotypes and alleles observed in the studied population of Briards from the Czech Republic are presented in Table 1. The incidence of the mutant allele in the population (0.061) was approaching that noticed in Poland (0.042) and in the USA (0.051) (Switonski et al., 2002). Similarly, no significant differences were identified in genotype frequencies between the Czech population and the Poland or the American populations (Table 1). It is known that the Polish population was established on animals imported from the Czech Republic and France. However, the American Briards are broadly unrelated to them. In spite of this, the variability of the polymorphism was surprisingly comparable in all populations.

Moreover, we analysed several Briards from other Central European countries – 8 dogs from Poland, 7 from Hungary and 5 from Slovakia. One, two and three heterozygotes were identified in them, respectively. However, it must be kept in mind that these population samples consisted of related individuals. Therefore, the incidence of the mutant allele was significantly affected by genetic drift.

Despite of the seemingly low frequency of the mutant allele, more than 10% of heterozygous animals were observed in the Czech population. Therefore, the genotyping of sires and dams is an essential precondition for the breeding of healthy offspring.

The method of electrophoresis in the gel Spraedex EL600 can be widely used for genotyping of the *RPE65* gene as a basis of proper genetic counselling and an improvement of genetic health in the Briard populations.

REFERENCES

Aguirre G.D., Baldwin V., Pearce-Kelling S., Narfström K., Ray K., Acland G.M. (1998): Congenital stationary night blindness in the dog: common mutation in the *RPE65* gene indicates founder effect. Mol. Vis., 4, 23.

Dekomien G., Epplen J.T. (2003): Evaluation of the canine *RPE65* gene in affected dogs with generalized progressive retinal atrophy. Mol. Vis., 9, 601–605.

Gu S., Thompson D.A., Srikumari C.R.S., Lorenz B., Finckh U., Nicoletti A., Murthy K.R., Rathmann M., Kumaramanickavel G., Denton M.J., Gal A. (1997): Mutations in *RPE65* cause autosomal recessive childhood-onset severe retinal dystrophy. Nat. Genet., 17, 194–197.

Kondo H., Qin M., Mizota A., Kondo M., Hayashi H., Hayashi K., Oshima K., Tahira T., Hayashi K. (2004): A homozygosity-based search for mutations in patients with autosomal recessive retinitis pigmentosa using microsatellite markers. Invest. Ophthalmol. Vis. Sci., 45, 4433–4439.

Marlhens F., Bareil C., Griffoin J.M., Zrenner E., Amalric P., Eliaou C., Liu S.Y., Harris E., Redmond T.M., Arnaud B., Claustres M., Hamel C.P. (1997): Mutations in *RPE65* cause Leber's congenital amaurosis. Nat. Genet., 17, 139–141.

Morimura H., Fishman G.A., Grover S.A., Fulton A.B., Berson E.L., Dryja T.P. (1998): Mutations in the *RPE65* gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis. Proc. Natl. Acad. Sci. USA, 95, 3088–3093.

Moiseyev G., Chen Y., Takahashi Y., Wu B.X., Ma J. (2005): *RPE65* is the isomerohydrolase in the retinoid visual cycle. Proc. Natl. Acad. Sci. USA, 102, 12413–12418.

Narfström K., Wrigstad A., Nilsson S.E. (1989): The Briard dog: A new animal model of congenital stationary night blindness. Brit. J. Ophthalmol., 73, 750–756.

Nicoletti A., Wong D.J., Kawase K., Gibson L.H., Yang-Feng T.L., Richards J.E., Thompson D.A. (1995): Molecular characterization of the human gene encoding an abundant 61 kDa protein specific to the retinal pigment epithelium. Hum. Mol. Genet., 4, 641–649.

Switonski M., Konieczny P., Klukowska J., Janyga B., Aguirre G. (2002): Microdeletion in the *RPE65* gene causing hereditary retinal dystrophy (HRD) disease segregates in the Polish population of Briards. Med. Weter., 58, 946–949.

Veske A., Nilsson S., Narfström K., Gal A. (1999): Retinal dystrophy of Swedish Briard/Briard-Beagle dogs is due to a 4-bp deletion in *RPE65*. Genomics, 57, 57–61.

Received: 2007–09–20
Accepted after corrections: 2007–11–26

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