Journal of Animal and Feed Sciences, 9, 2000, 721 – 726

Sequence polymorphism of exon 17 of the ryanodine receptor gene (*ryr1*) in the Canidae

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(Received 26 May 2000; accepted 4 October 2000)

ABSTRACT

Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), and sequencing analysis were used to study the ryanodine receptor gene (*ryr1*) of the blue fox (*Alopex lagopus*), wolf (*Canis lupus*), and domestic dog (*Canis familiaris*). PCR-amplified *ryr1*-gene-specific fragments were cloned in a pGem5 Zf(-) plasmid. Double-stranded templates were sequenced by the Sanger dideoxy chain termination method, using Cy-5' labelled primers. We detected G→C transversion (position 1836) in the wolf *ryr1* gene, and T→C transition (position 1857) in the blue fox (these mutations do not alter the respective amino acid sequences) and a C→T transition (position 1846) in the *ryr1* of the blue fox that changes the amino acid from serine to proline. We did not detect the 1843C→T mutation in the Canidae *ryr1* gene.

KEY WORDS: ryanodine receptor gene, Canidae genetics, polymorphism

INTRODUCTION

The development of molecular techniques enables genomic investigations to be conducted despite incomplete cytogenetic information. The DogMap project is more advanced than that of the blue fox (*Alopex lagopus*). However, the por-

cine ryanodine receptor gene (*ryr1*) and point mutation 1843C→T in the *ryr1* gene, which is responsible for malignant hyperthermia (MH), have not yet been found in any of the above-mentioned species. Ryanodine receptors (RYR) are a subunit of the calcium release channel proteins of intracellular Ca^2-stores in the brain, skeletal and cardiac muscles. The RYR1 gene has been mapped in man to chromosome 19 in the q13.1 band (MacKenzie et al., 1990), and assigned to chromosome 6 in the pig through linkage between GPI and HAL (Davies et al., 1988). Using *in situ* hybridisation, the calcium release channel (*CRC*) gene was mapped to chromosome 6 (Harbitz et al., 1990). It is composed of 106 exons of which 2 are alternatively spliced (Phillips et al., 1996). In studies of the genetic basis for malignant hyperthermia (MH), a mutation, Arg^{615} to cysteine (Fujii et al., 1991), was identified in the porcine skeletal muscle ryanodine receptor gene, which is linked to MH (Otsu et al., 1991). MH is a clinical syndrome in which genetically susceptible individuals respond to the administration of potent inhalant anaesthetics and depolarising skeletal muscle relaxants with skeletal muscle rigidity, tachycardia, hypoxia, arrhythmia, unstable blood pressure, lactic and respiratory acidosis, hyperventilation, and high fever (Ooms et al., 1982; Britt, 1987).

Anaesthetic-induced malignant hyperthermia (MH) has been observed in dogs, cats, horses, birds, and also in wild animals during detainment (Short and Paddleford, 1973; De Jong et al., 1974; Henschel and Louw, 1987). However, it has not yet been described in species such as the fox and wolf. There are reports of sudden death of dogs during administration of anaesthetics (Short and Paddleford, 1973).

The aim of this study was to investigate the region of exon 17 containing the 1843 nucleotide in the *ryr1* gene of the dog (*Canis familiaris*), wolf (*Canis lupus*), and blue fox (*Alopex lagopus*).

MATERIAL AND METHODS

Genomic DNA of *Alopex lagopus* (n=35), *Canis lupus* (n=1) and *Canis familiaris* (n = 35) was isolated from 10 ml of peripheral blood according to the salt-out procedure described by Miller et al. (1988). Approximately 0.1 µg of DNA was subjected to 30 cycles of PCR as described by Fujii et al. (1991). The PCR reactions were performed in 25 µl mixes containing 0.1 µg genomic DNA, 10 pmol primer RYR1-F 5'-GTTCCCTGTGTGTGTGCAATGGTG, which corresponds to porcine RYR1 cDNA nucleotides 1811 to 1834, 10 pmol RYR1-R 5'-GCCAGGGAGCAAGTTCTCAGTAAT which is complementary to *ryr1* cDNA nucleotides 1861 to 1884, dNTPs at a final concentration of 300 µM, 2.5 µl 10xPCR buffer and 0.5U Taq DNA polymerase. PCRs were performed

with reagents provided by Amersham. For automatic analysis of PCR products on ALFexpress™ Sequencer (Pharmacia Biotech., Uppsala, Sweden) primers were labelled at the 5'end with Cy5. The following cycling conditions were used: 94°C for 5 min followed by 32 cycles of 92°C for 45 sec, 55°C for 45 sec, and 70°C for 1 min with a final extension at 70°C for 7 min. The PCR products were separated in 10% polyacrylamide gel. For non-radioactive PCR-SSCP analysis, the PCR products were denatured to a single-strand state by mixing a 4 µl aliquot of the product with 6 µl of stop solution and separated in 10% polyacrylamide gels (20x20 cm, 49:1 acrylamide: bis, 5% glycerol, 20°C, 0.5xTBE, 70V, 12 h).

PCR-amplified exon 17 fragments were cloned in a pGem5 Zf(-) plasmid. Plasmid DNA was purified using Wizard™ columns (Promega, Madison, USA). Double-stranded templates were sequenced by the Sanger dideoxy chain termination method (Sanger et al., 1979), using the following Cy-5' labelled primers:
– universal primer - 5'-Cy5-d[CGACGTTGTAAAACGACGGCCAGT]
– reverse primer - 5'-Cy5-d[CAGGAAACAGCTATGAC]
according to the Cy5™ AutoRed™ Sequencing Kit protocol (Pharmacia Biotech., Uppsala, Sweden). Sequencing products were analysed with the ALFexpress™ sequencing system (Pharmacia Biotech., Uppsala, Sweden).

RESULTS AND DISCUSSION

PCR primers were designed based on the porcine ryanodine receptor cDNA sequence, and used to amplify a fragment of exon 17 of the *ryr1* gene in the Canidae (Figure 1). Previously, we successfully used these primers to amplify a zebrine (Gronek et al., 1998), ovine (Gronek et al., 1999a) and anserine (Gronek et al., 1999b) fragment homologous to the porcine exon 17 of the *ryr1* gene. Analysis of the product length showed that its size in all the analyzed species was exactly the same. The PCR-SSCP technique was applied to study the DNA structure. Conformation polymorphism of the DNA was observed between species, but no differences in the SSCP pattern within species were detected (Figure 2).

Comparison of the sequence of the *ryr1* gene in the region of nucleotide 1843, the site of a mutation responsible for MH in pigs and humans, allowed the differences between the species to be investigated (Figure 3). G→C transversion (position 1836) in the wolf *ryr1* gene and T→C transition (position 1857) in the vulpine *ryr1* gene do not alter the amino acid sequences. However, transition T→C (position 1846) in *ryr1* of the blue fox changes the amino acid from a serine to a proline.

In the analysed material of the Canidae, no individuals with the 1843C→T mutation were detected.

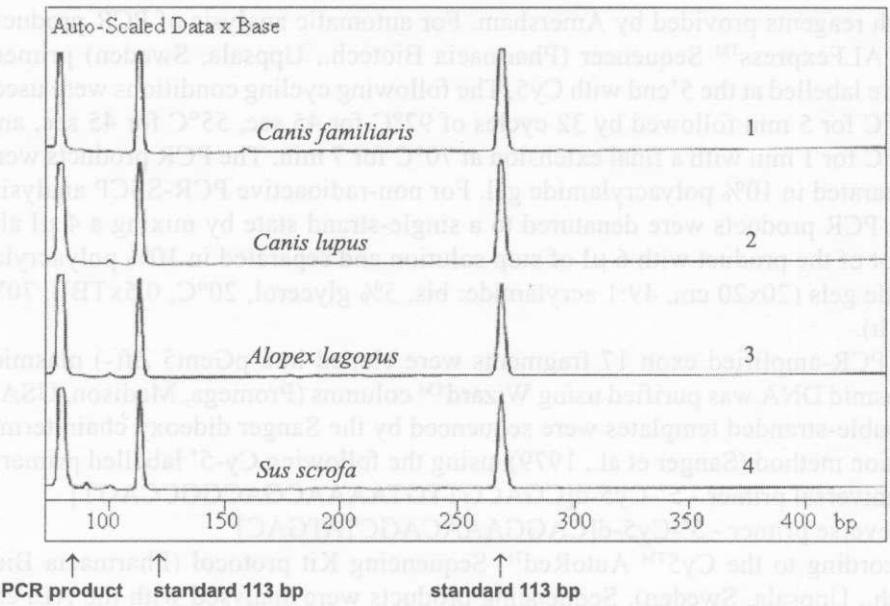

Figure 1. The analysis of *ryr1* gene fragment length amplified with oligonucleotides based on porcine cDNA sequence. Line 1 *Canis familiaris*; line 2 *Canis lupus*; line 3 *Alopex lagopus*; line 4 *Sus scrofa*

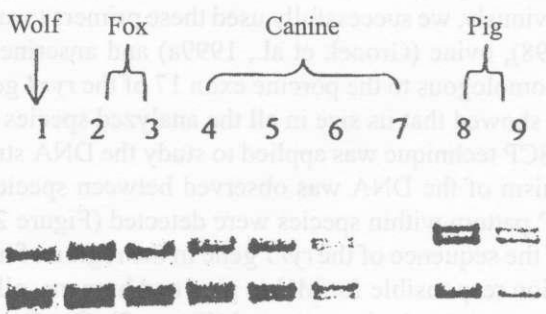

Figure 2. Homology of the DNA sequence encompassing malignant hyperthermia mutation site in the *ryr1* gene of the family Canidae. Shown here is SSCP analysis of the 74bp amplified fragment of exon 17, separated by electrophoresis through a 6% polyacrylamide gel (49:1 acrylamide: bis), 5% glycerol in 0.5 x TBE, and silver stained. Lane 1 is wolfish, lanes 2-3 vulpine, lanes 4-7 are canine, lanes 8-9 are porcine *ryr1* gene

Alopex lagopus 1843 1846 1857
GTG GCC GTG **CGC** CCC AAC CAA GAC CTC
V A V R P N Q D L

Canis lupus 1836
GTC GCC GTG **CGC** TCC AAC CAA GAT CTC
V A V R S N Q D L

Canis familiaris
GTG GCC GTG **CGC** TCC AAC CAA GAT CTC
V A V R S N Q D L

Figure 3. Nucleotide and amino acids deduced sequence alignment of *ryr1* gene in the region of nucleotide 1843 (in bold). The substitutions of the nucleotides or amino acids are underlined

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STRESZCZENIE

Polimorfizm sekwencji eksonu 17 genu receptora ryanodiny u psowatych

Badania molekularne prowadzono na DNA lisa polarnego (*Alopex lagopus*), wilka (*Canis lupus*) i psa domowego (*Canis familiaris*) stosując techniki PCR-SSCP i sekwencjonowanie. Analizowano ekson 17 genu receptora ryanodiny. Zamplifikowane fragmenty genu *ryr1* klonowano w plazmidzie pGem5 Zf(-). Fragmenty sekwencjonowano metodą Sangera używając starterów znakowanych Cy na końcu 5'. Stwierdzono następujące mutacje: transwersję G→C (pozycja 1836) w genie *ryr1* wilka, tranzycję T→C (pozycja 1857) u lisa polarnego, które nie zmieniają sekwencji aminokwasowej, oraz tranzycję C→T (pozycja 1846) u lisa polarnego, która zmienia sekwencje aminokwasową z seryny na prolinę. W analizowanym materiale nie stwierdzono mutacji 1843C→T.