A note on ryanodine receptor gene (\textit{ryr \text{1}})
occurrence in the anserine genome

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ABSTRACT

The PCR products of the \textit{ryr\text{1}} gene amplified from anserine genomic DNA were analysed. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to investigate the anserine ryanodine receptor (\textit{ryr\text{1}}) gene. The anserine fragment of the \textit{ryr\text{1}} gene was the same size as exon 17 of the porcine \textit{ryr\text{1}} gene.

KEY WORDS: ryanodine receptor gene, goose, PCR-RFLP

INTRODUCTION

The typical avian karyotype consists of a few distinguishable macrochromosomes and a high number of very small microchromosomes (Ladjali et al., 1993; Rodionov, 1996). The presence of numerous cytogenetically indistinguishable mi-
crochromosomes complicates the mapping of genes and other DNA fragments and integrating the genetic maps of the karyotype.

Henschel and Louw (1978) reported malignant hyperthermia (MH) in birds. MH is linked to mutations in the ryanodine receptor which is a calcium release channel protein on the intracellular Ca$^{2+}$-store in skeletal muscle. The human and rabbit ryanodine receptor genes (RYR1) have been mapped (MacKanzie et al., 1990) and sequenced (Zorzato et al., 1990; Phillips et al., 1996), and the porcine ryr1 gene has been localised (Davies et al., 1988); cDNAs have been cloned (Fujii et al., 1991). The RYR1 gene is composed of 106 exons, of which two are alternatively spliced (Phillips et al., 1996). In studies on the genetic basis of malignant hyperthermia (MH), the a1843C→T mutation in the porcine ryr1 gene corresponding to an Arg$^{615}$ to Cys alteration has been identified (Fujii et al., 1991) and linked to malignant hyperthermia (MH) with a lod score of 102 for $\theta$ max. = 0.0 (Otsu et al., 1991). The corresponding human RYR1 mutation, Arg$^{614}$ to Cys, was found to be expressed in about 2% of MH families (Gillard et al., 1992).

The primary aim of this study was to determine whether the ryr1 gene occurs in the anserine genome.

MATERIAL AND METHODS

Animals

The research was performed on genomic anserine DNA: e.g. White Italian goose (Anser anser) (n=20), White Italian goose, strain WD3 (Anser anser) (n=20), Garbonosa goose (Anser anser x Anser cygnoides) (n=20) originating from Poland, and Polish Large White swine (n=20). The anserine blood samples were taken from the vena cutanea ulnaris.

Methods

In this study, consensus primers were designed based on the porcine ryanodine receptor (ryr1) cDNA sequences where natural polymorphism occurs, and used to amplify a fragment of exon 17 of the anserine ryanodine receptor gene. The PCR reactions were performed in a 25 ml mix containing: 0.1 mg genomic DNA, 10 pmol primer RYR1F 5'-Cy5-GTTCCCTGTGTGTGCAATGGTG-3', which corresponds to porcine ryr1 cDNA nucleotides 1811 to 1834, and 10 pmol RYR1R 5'- (GCCAGGGAGCAAGTTCTCAGTAAT-3') in which the last 24 nucleotides are complementary to ryr1 cDNA nucleotides 1861 to 1884, dNTPs fc 300 μM, 2.5 μl 10 x PCR buffer and 0.5U Taq DNA Polymerase. Thermocycling was performed by initial denaturation for 5 min at 94°C, 30 cycles of 45 s denaturation at 92°C, 45
Annealing at 55°C, 60 s hybridisation at 70°C and 7 min final extension at 70°C. For automatic analysis of PCR products on ALFexpress™ Sequencer (Pharmacia LKB) primers were labelled at the 5' end with Cy5. Electrophoresis was performed on 10% polyacrylamide gel at 30°C using the following conditions: 25 W, 60 mA, 200 V. Allele size was quantified using Fragment Manager V 1.2® (Pharmacia Biotech) software. Because the 1843C→T mutation changes, a HinP1 site to a HgiAl site the PCR-RFLP method was used. The PCR-RFLP for detection of mutant alleles was performed using HinP1 enzyme (10,000 U/ml, Biolabs) as follows: 10 ml of the amplified products were digested with HinP1 (10 units) for 3 h at 37°C in the appropriate buffer containing BSA.

RESULTS AND DISCUSSION

The goose PCR product was of the same size as the porcine one (74 bp) (Figure 1). In the analyzed geese material, no individuals with substitution of T for C at nucleotide 1843 of the ryr1 gene were found. The PCR products were digested with HinP1, and the resulting fragments were separated by electrophoresis on a 10% polyacrylamide gel. The gel was stained with ethidium bromide and visualized under UV light. The expected fragment sizes were 74 bp and 50 bp for the wild-type and mutant alleles, respectively. The gel image showed a single band at 74 bp for all the samples, indicating the absence of the 1843C→T mutation in the goose population.

Figure 1. The analysed fragment of the ryr1 gene amplified with oligonucleotides based on porcine cDNA sequence. Line 34 White Italian goose, WD3 strain (Anser anser); line 35 White Italian goose (Anser anser); line 36 Garbanosa goose (Anser anser x Anser cygnoides); line 40 porcine
otide 1843 were detected. Nonetheless, since the study involved a relatively small population, it is difficult to draw any statistical conclusions. However, only C/C genotypes were observed.

CONCLUSIONS

This analysis shows that PCR based on primers for a known porcine cDNA sequence may be suitable for the homologous gene sequence of other genomes. Molecular techniques applied in this study permitted the analysis of a fragment of the \( ryr1 \) gene in the goose. It seems that the gradual mapping of the goose genome should make the identification of genes responsible for genetic defects feasible. Creation of marker maps of goose genomes may help in explaining their evolution.

This study may be a model for other genes, especially for those species which, despite being closely related to one another, display cytogenetic differences. It seems that localization and identification of animal disease genes are the most important aspects of animal gene mapping.

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STRESZCZENIE

Gen receptora ryanodyny występuje u gęsi

Badania molekularne przeprowadzono na DNA gęsi. W oparciu o startery skonstruowane na podstawie znanej sekwencji cDNA genu ryrl świni przeprowadzono reakcję amplifikacji oraz wykonano analizę PCR-RFLP. Otrzymane wyniki pozwalają sądzić, że gen receptora ryanodyny najprawdopodobniej występuje w genomie ptaków, czego dotychczas nie stwierdzono. Jednoznacznym potwierdzeniem tych pierwszych obserwacji będzie na dalszym etapie badań poznanie sekwencji i określenie stopnia homologii pomiędzy gatunkami. W badanej grupie zwierząt nie zaobserwowano mutacji 1843C→T.