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Homology of DNA sequences encompassing the malignant hyperthermia mutation site in the human, porcine, and zebrine *ryr1* gene*

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Abstract. The *RYR1* gene encoding the Ca^{2+} channel of sarcoplasmic reticulum of human skeletal muscle has been cloned and its nucleotide sequence has been determined earlier. We have used the polymerase chain reaction single strand conformation polymorphism (PCR-SSCP), and sequencing analysis for human, porcine (*Sus scrofa*), and zebrine (*Equus grevyi*) ryanodine receptor (*ryr1*) gene. The fragment of exon 17 of the *ryr1* gene was characterized by a high homology between all the analysed species (substitution of a nucleotide is underlined):
porcine *ryr1* ^{1834}GTG GCC GTG CGC TCC AAC CAA GAT CT^{1859}
human *RYR1* ^{1831}GTG GCC GTG CGC TCC AAC CAA GAT CT^{1856}
zebrine *ryr1* GTG GCC GTG CGC TCC AAC CAA GAC CT.

Key words: homology, *ryr1* gene.

The *RYR1* gene has been mapped to chromosome 19q13.1 band (MACKANZIE et al. 1990), and is composed of 106 exons (PHILLIPS et al. 1996). Size and complexity of the *RYR1* gene place it in a group of the largest known genes. The length of the *RYR1* gene is approximately 158 kb. A mutation identified in the porcine skeletal muscle ryanodine receptor gene, $Arg^{615} \rightarrow Cys$ (FUJII et al. 1991), is linked to malignant hyperthermia (MH), with a lod score of 102 for Θ max. = 0.0 (OTSU et al. 1991). The corresponding human *RYR1*

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mutation, $Arg^{614} \rightarrow Cys$, was found to be expressed in about 2% of MH families (DEUFEL et al. 1995). Another *RYR1* mutation, $Gly^{2433} \rightarrow Arg$, has been linked to 4% of MH families in Europe and Canada (KEATING et al. 1994). Anaesthetic-induced malignant hyperthermia has been also described in dogs, cats, horses, birds and in wild animals during capture in early seventies (SHORT, PADDLEFORD 1973, DE JONG et al. 1974, HENSCHEL, LOUW 1978). MH is a clinical syndrome in which individuals respond to the administration of potent inhalational anaesthetics and depolarising skeletal muscle relaxants with skeletal muscle rigidity, unstable blood pressure, tachycardia, arrhythmia, hypoxia, lactic and respiratory acidosis, hyperventilation, and high fever (BRITT 1987). In addition neurological, liver and kidney damage may occur.

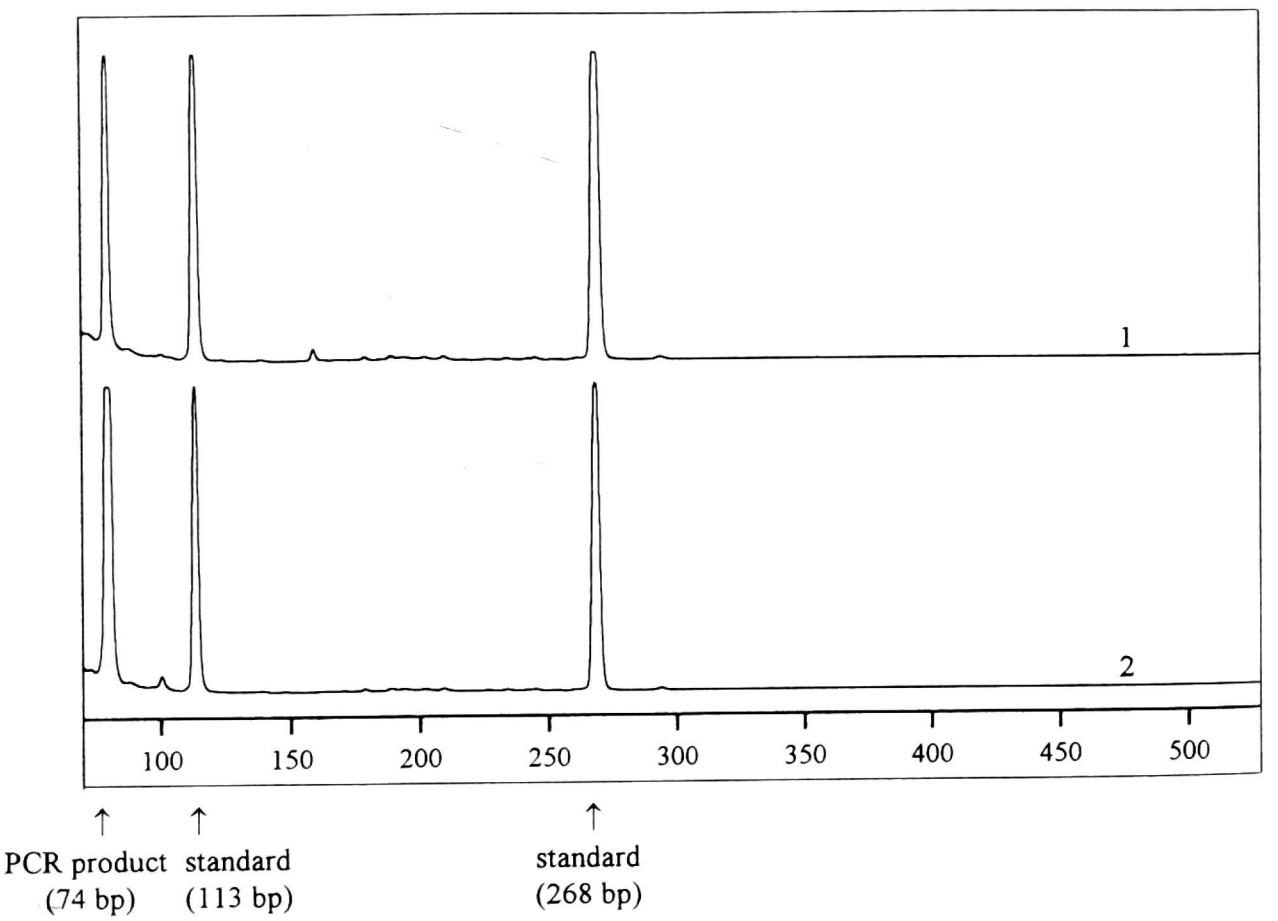

Figure 1. The analysed fragment of the zebrine (line 1), and porcine (line 2) exon 17 of the *ryr1* gene

The research was performed on zebrine (*Equus grevyi*), porcine, and human genomic DNA. Fragment of exon 17 of the *ryr1* gene was analysed, with substitution 1843C→T responsible for MH in pigs and 1840C→T in humans. The PCR reaction was performed in volume of 25 μL, and the mix contained 100 ng genomic DNA, 2.5 μL 10x Taq polymerase buffer, 25 pmols of each

primer, 10 nM dNTP, and 0.5 unit of Taq polymerase. The following primers labelled at the 5end with Cy5 were used:

RYR1 – F 5-GTTCCCTGTGTGTGTGCAATGGTG, and

RYR1 – R 5-GCCAGGGAGCAAGTTCTCAGTAAT.

The PCR conditions were as follows: 94°C for 5 min, followed by 32 cycles of 92°C for 45 sec, 55°C for 45 sec, 70°C for 1 min, with final extension at 70°C for 7 min. The amplification products were analysed in 6% polyacrylamide gel using the ALF*express*^{TM} DNA Sequencer (Pharmacia Biotech). 1.5 µL

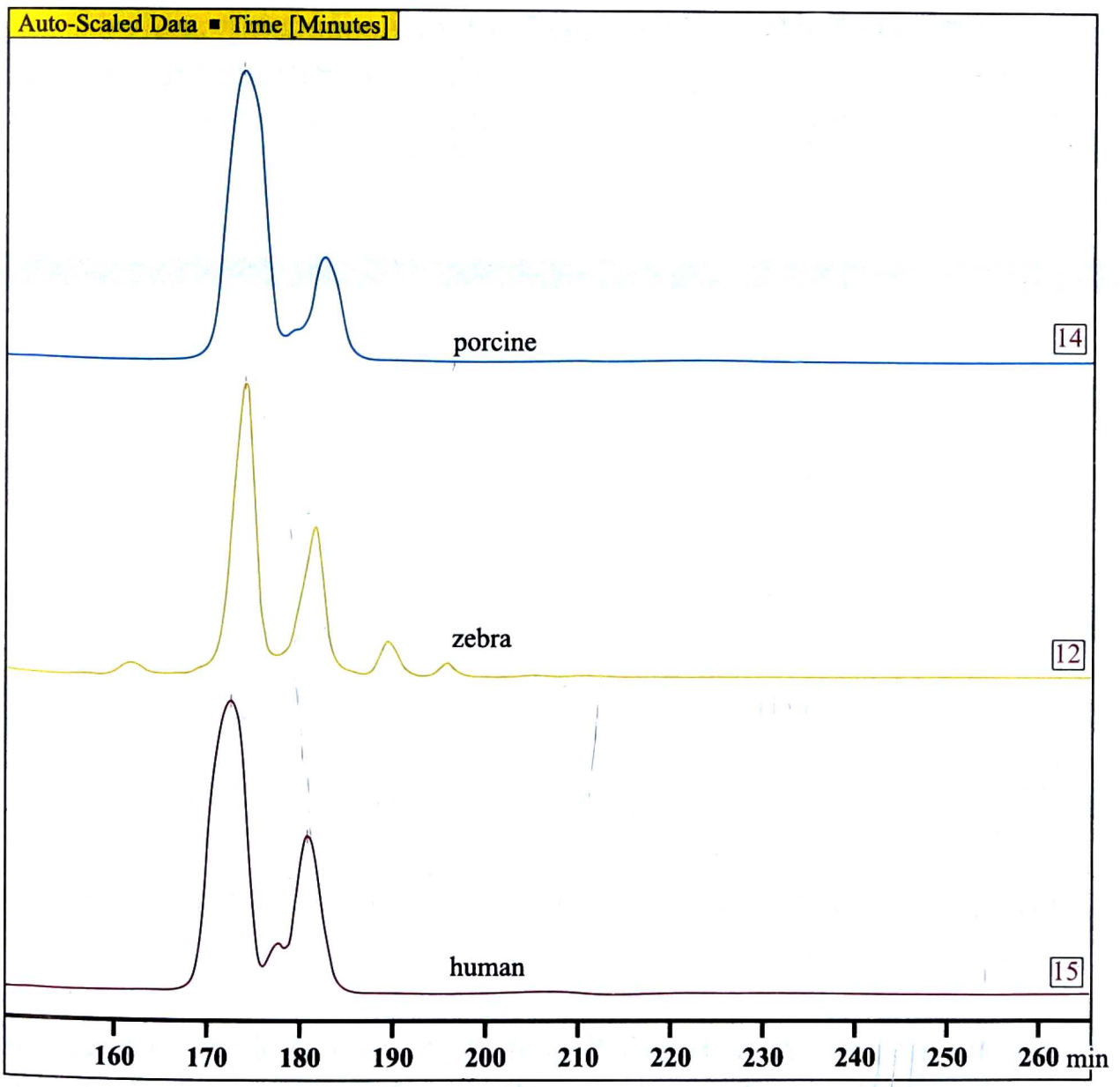

Figure 2. The single strand conformation polymorphism of the porcine, zebrine, and human exon 17 of the *ryr1* gene

of PCR products and 0.3 µL of each internal standard (113 and 268 bp) were loaded per lane after denaturation in formamide buffer at 95°C for 5 min. The analysed fragment of the porcine and zebrine *ryr1* gene has the same size of 74 bp as the human analogue (Figure 1). Screening for nucleotide changes

in the *ryr1* gene was carried out using the PCR-SSCP method (ORITA et. al. 1989). The PCR products were denatured in a formamide stop solution (temp. 80°C, 5 min.) and separated by automatic PCR-SSCP analysis on the ALF*express*^{TM} DNA Sequencer in 6% polyacrylamide gel (49 : 1 acrylamide : bis), 5% glycerol. The single strand conformation polymorphism between species was observed in porcine, zebrine, and human DNA (Figure 2). Usually, DNA conformers can be visualised after long-time polyacrylamide gel electrophoresis and autoradiography or silver staining. We have found that application of the ALF*express*^{TM} Sequencer simplifies analysis and makes it far more reliable than conventional methods. Polymorphism between species detected using the PCR-SSCP method was verified by a sequencing analysis.

The PCR products were purified using the Dynabeads method and then subjected to cycle sequencing and analysed on the ALF*express*^{TM} Sequencer.

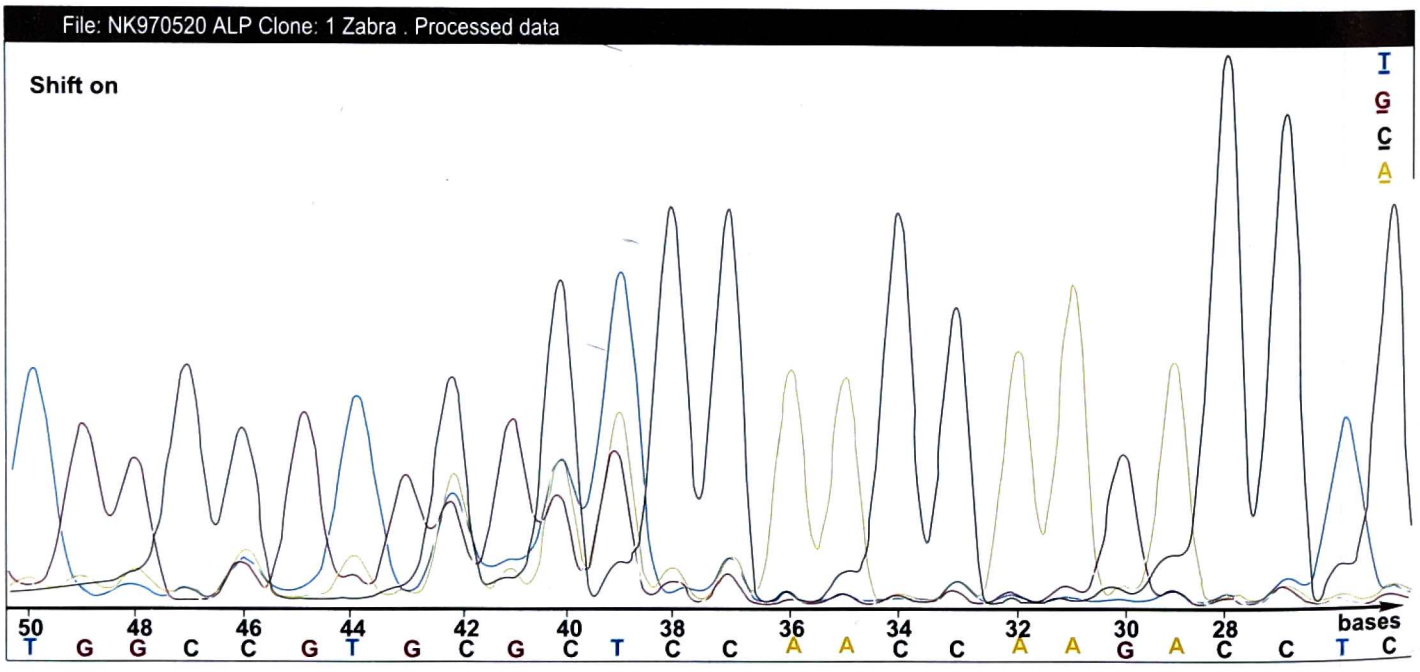

Figure 3. Sequence of the analysed fragment of the zebrine *ryr1* gene

The sequencing primers were labelled at the 5end with Cy5. The sequence of the zebrine fragment of the *ryr1* gene (Figure 3) was characterized by a high homology to the porcine and human *RYR1*.

porcine *ryr1* ^{1834}GTG GCC GTG CGC TCC AAC CAA GAT CT^{1859}
human *RYR1* ^{1831}GTG GCC GTG CGC TCC AAC CAA GAT CT^{1856}
zebrine *ryr1* GTG GCC GTG CGC TCC AAC CAA GA<u>C</u> CT
(substitution of a nucleotide is underlined).

This observation could have been expected, but the high homology of DNA of taxonomically distant individuals should be emphasized. The general knowledge about DNA allowed for a prior assumption that homology should also

concern the *ryr1* gene. It is well known that DNA is characterized by conservatism in nucleotide sequence as well as in organization of genomes in chromosomes.

According to STRANZINGER (1990) the genetic conservatism among species may reflect the existence of syntenic groups as well as linked genes and the structure of analogous genes or anonymous nucleotide sequences.

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