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A note on comparison of exon 2 of the IGF-I gene in four species of the family *Canidae*[*]

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ABSTRACT

A 131 bp long sequence comprising a fragment of the 5`untranslated region and a part of exon 2 of the insulin-like growth factor *IGF 1* gene, originating from the dog (*Canis familiaris*), Chinese raccoon dog *(Nyctereutes procyonoides procyonoides*), red fox (*Vulpes vulpes*) and arctic fox (*Alopex lagopus*) were studied with the use of SSCP, RFLP and DNA sequencing techniques. The SSCP mobility shifts revealed an interspecies, but not intraspecies variability of this sequence. Detailed searching for point mutations was carried out by direct DNA sequencing of a 131 bp fragment of the IGF-I gene. Three silent point mutations at positions: 21 (A>G) in the arctic fox, 57 (T>C) in the red fox, arctic fox and Chinese raccoon dog, and 93 (C>T) in the red fox and arctic fox were identified, when compared with the dog sequence, respectively. Since the above mentioned mutations are detectable by the RFLP technique, DNA samples originating from the studied species can be easily recognized due to the presence of the species specific intragenic haplotypes.

KEY WORDS: *IGF 1*, dog, red fox, arctic fox, raccoon dog

INTRODUCTION

The family *Canidae* comprise 36 species, including the dog (*Canis familairis)* and three species kept in captivity for fur production: the red fox (*Vulpes vulpes)*,

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the arctic fox (*Alopex lagopus*) and the raccoon dog (*Nyctereutes procyonoides procyonoides)*. A comparative analysis of mammalian genomes provides important insight into the structure, function and evolution of karyotypes and genes. Chromosome homologies among various canid species have been recently investigated by comparative chromosome painting (Graphodatsky et al., 2001), single-locus fluorescent *in situ* hybridization (Rogalska-Niznik et al., 2003) and linkage analysis of marker loci (Klukowska et al., 2002).

The insulin-like growth factor 1 is a small polypeptide (70-aminoacid), composed of three helical segments: helix I (Ala8-Cys 18), helix II (Gly 42-Phe 49) and helix III (Leu 54-Cys 61) (Wolf et al., 1996), which is structurally related to insulin. This protein plays a fundamental role in the regulation of the postnatal weight, metabolism and foetal development in mammals. Thus, the *IGF 1* gene is considered as a potential QTL, which could be used in the selection of fur animals for body weight. This characteristic is very important in fur animal breeding, since the value of the pelt is related to its size.

The aim of this study was to compare a 131bp fragment, representing a fragment of the 5`untranslated region (58bp) and a part of exon 2 (73 bp) of the *IGF 1* gene, in four species of the family *Canidae*: the dog, red fox, arctic fox and Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*) and to search for polymorphism in these species. It is known that a part of the amplified sequence (the last 73 bp) encodes helix I which corresponds to the B-chain helix in insulin involved in its receptor binding (Cascieri et al., 1988).

MATERIAL AND METHODS

DNA was obtained from 15 dogs, representing 5 breeds (Caucasian Shepherd Dog, Dachshund, Rottweiler, German Shepherd Dog, Cane Corso), 10 farm Chinese raccoon dogs, 15 farm silver foxes (a coat colour variant of the red fox), and 15 farm arctic fox, using phenol-chloroform procedures. PCR primers (5'Cy5-TCACATCTCTTCTACCTGG-3'; 5'AAGTAGAACCCCCTGTCTCC-3') were designed on the basis of the published sequence of the dog cDNA insuline-like growth factor 1a (L08254) and used with dog, Chinese raccoon dog, red fox and arctic fox genomic DNA to produce a 131 bp amplicon.

Approximately 200 ng of DNA were used for PCR amplification using the Biometra thermocycler. PCR (30 µl final volume) was performed using 3 µl 10 x PCR buffer (500 mM KCl, 100 mM TRIS-HCl pH 8.3, 15 mM $MgCl_2$, and 0.1% gelatin), 200 µM of each dNTP, 25 pM of each primer, 1 units of DyNAzyme DNA polymerase (Finnzymes, Finland) and sterile water. The temperature cycling was as follows: one cycle at 95ºC (10 min); 95ºC (30 s); 56ºC (30 s); 72ºC (1 min) for 35 cycles and 72ºC 10 min for one cycle.

For the SSCP analysis two microliters of PCR product diluted with 6 µl of loading dye (0.05% dextran blue in formamide) was denatured at 95ºC for 5 min and immediately plunged into ice for 10 min. The dilution was loaded onto the SSCP- gel and analysed in an ALFExpress II Automated Sequencer (Amersham Pharmacia, Uppsala, Sweden) using Cy5 fluorescently labeled primers with the Fragment Analyser 1.03 software for fragment analysis. The gel was run in 1x TBE at a constant voltage of 1200 mV at 20ºC for 700 min.

PCR products derived from one animal of each species was ligated into pUC18 and the denominated plasmids were used to transform *Escherichia coli* DH5α. Plasmid DNA was purified using WIZARD columns (Promega, Medison, USA) and double-stranded templates were sequenced from both sides by the dideoxy chain termination method according to the Cy5 AutoRed Sequencing Kit (Pharmacia Biotech, Uppsala, Sweden) protocol using the ALFExpress II Sequencer (Pharmacia Biotech).

A DNA sequence has been deposited with the EMBL DNA Sequence Database under accession numbers: Red fox - AY098635, Arctic Fox - AY098633 and Chinese raccoon dog - AY098634, respectively.

RESULTS AND DISCUSSION

The analysis of the SSCP patterns revealed differences between the studied species, but no intraspecies variability was found (Figure 1). The 131 bp fragment of the *IGF 1* gene, amplified from the genomes of the four species, was sequenced and a high level of the conservation was found. The estimated similarity of this fragment between the dog and the Chinese raccoon dog, red fox and arctic fox was 99.2, 98.5 and 97.7%, respectively. Single nucleotide substitutions, in comparison with the dog sequence, were detected at nucleotide position 21 (arctic fox) and 57 (raccoon dog). Moreover, the red fox and the arctic fox sequences differed from each other at position 93. The observed differences of the nucleotide sequence could be also detected by the RFLP approach (Table 1). Nucleotide substitutions were recognized by the following restriction enzymes: *Hae*III (position 21), *Msp*1 (position 57) and *Fok*I (position 93). The analysis of all the three substitution sites showed that the studied species have unique intragenic haplotypes. The identified substitutions did not alter the amino acid sequences of the *IGF 1* polypeptide.

The *IGF 1* gene has been characterized in the human (Rotwein et al., 1986), pig (Muller and Brem, 1990), horse (Otte et al., 1996), bovine (Fotsis et al., 1990), sheep (Wong at al., 1989), mouse (Bell et al., 1986) and dog (Delafontaine et al., 1993) genomes. A comparison of the amino acid sequences, described in the above mentioned species, shows extensive regions of homology among them. Within the amino-acid peptide region, coded by exon 2, all canids differ from the human, pig,

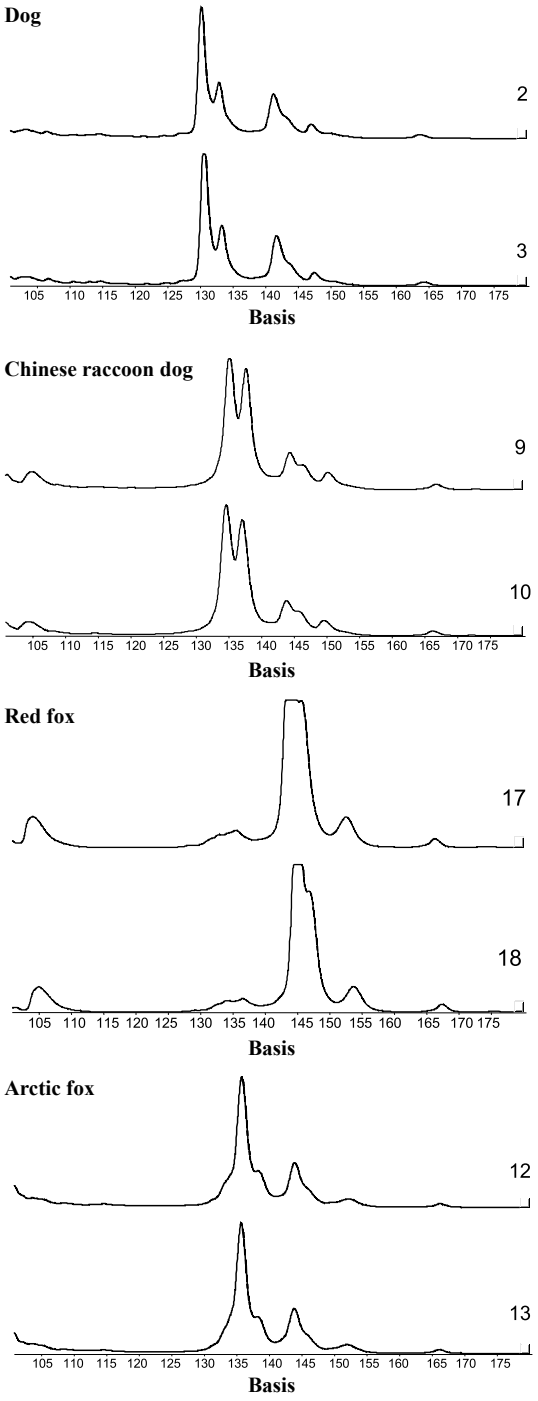

Figure 1. SSCP analysis of amplified 131 bp fragment of the *IGF 1* gene from four species of the family *Canidae* (for each species two samples are presented)

TABLE 1

Nucleotide substitutions, RFLP patterns and intragenic haplotypes of the part of the 5`untranslated regions and the part of exon 2 of the *IGF 1* gene in four species of the family *Canidae*

Species	Nucleotide substitutions and RFLP patterns (bp)						Intragenic haplotype
	Position 21 (*Hae*III)		Position 57 (*Msp*I)		Position 93 (*Fok*I)		
	nucleotide						
	nucleotide bp		fragments bp		fragments bp		
Dog	A	131	T	131	C	100 + 31	A-T-C
Chinese raccoon dog	A	131	C	75 + 56	C	100 + 31	A-C-C
Red fox	A	131	C	75 + 56	T	131	A-C-T
Arctic fox	G	110 + 21	C	75 + 56	T	131	G-C-T

horse, bovine, sheep and mouse sequences at a single amino acid residue. Threonine at position 14 in all species except sheep, and serine at position 16 have been replaced by prolines in the canine *IGF 1*. Bovine/ovine and pig sequences showed differences at position 12 - alanine and serine, respectively. Only mouse sequences showed one difference at position 39. From these comparisons a conclusion may be drawn that this gene fragment is evolutionary highly conservative.

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STRESZCZENIE

Ewolucja eksonu 2 genu IGF-1 u czterech gatunków z rodziny *Canidae*

Przeprowadzono porównanie odcinka 131 pz genu insulinopodobnego czynnika wzrostu -1 (IGF-1), obejmującego fragment sekwencji 5` oraz fragment eksonu 2 psa (*Canis familiaris*), jenota chińskiego (*Nyctereutes procyonoides*), lisa pospolitego (*Vulpes vulpes*) and lisa polarnego (*Alopex lagopus*). W badaniach wykorzystano techniki SSCP, RFLP oraz sekwencjonowania DNA. Wykonane analizy techniką SSCP wykazały wyraźne różnice międzygatunkowe, przy jednoczesnym braku zróżnicowania wewnątrzgatunkowego. Dokładną charakterystykę mutacji punktowych przeprowadzono przy pomocy sekwencjonowania badanego fragmentu DNA. Zidentyfikowano trzy ciche mutacje punktowe względem sekwencji nukleotydów psa: u lisa polarnego w nukleotydzie 21 (A>G), u lisa pospolitego, lisa polarnego i jenota chińskiego w nukleotydzie 57 (T>C) oraz u lisa pospolitego i lisa polarnego w nukleotydzie 93 (C>T). Występowanie tych mutacji potwierdzono techniką RFLP. Uzyskane wyniki pokazały, że badane gatunki mają specyficzne haplotypy w obrębie genu IGF-1.