Nucleotide sequence polymorphism of the growth hormone gene in American mink (*Neovison vison*)

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Abstract

The nucleotide sequence variation of the American mink (*Neovison vison*) growth hormone gene was analysed. For this purpose 1,113 bp region of the mink genomic DNA, encompassing four exons and three introns of somatotmropin gene, was amplified by nested-PCR and sequenced. The study included 116 individuals of wild mink (Nova Scotia, Canada), ranch mink (Scanblack, Wild, Sapphire, Pearl, Black Cross and Sapphire Cross; West Pomerania, Poland) and feral mink (Poland and Iceland). Sequencing allowed to determine the nucleotide composition of previously unrecognized non-coding regions, as well as exons and to identify 12 new SNP substitutions, one single nucleotide deletion and one ins/del polymorphism. The average incidence of SNPs in coding sequences was equal to 1/156.6 bp and in non-coding – 1/86.9 bp. Analysis of the observed number and location of substitutions revealed its non-random distribution and significant preponderance of transitions over transversions. The results also revealed a significant differences between originally wild mink and domesticated animals (both ranch and feral) in identified SNPs type and distribution. The results of the present study, supported by further analysis, can help in effective genetic monitoring of feral mink, as some differences were also found between ranch and feral mink.

Keywords: feral mink, genetic polymorphism, growth hormone gene, ranch mink, wild mink

Introduction

Growth hormone (GH, also called somatotrophin, STH) is a peptide hormone secreted by eosinophilic cells of anterior lobe of the pituitary gland (Machnik and Lechniak, 2000). GH is a very comprehensive and systemic hormone, affects target cells in direct and indirect manner (Kmieć *et al.*, 2007). Its most important function is to stimulate growth and development of cells, tissues and the whole body (Kmieć *et al.*, 2008). Functionally, growth hormone belongs to the somatotrophic axis, together with its receptor (GHR) and somatoliberin (GHRH), as well as prolactin (PRL), prolactin receptor (PRLR), insulin-like growth factor-I (IGF-I), transcription factor Pit-I and the transcription factor STAT5 (Zwierzchowski *et al.*, 2004).

Nucleotide sequence of mink growth hormone gene's cDNA was first reported by Shoji *et al.* in 1990. The open reading frame includes 648 bp and product of its translation – 216 amino acids with app. 24.5 dalton. Signal peptide consist of 26 amino acids and mature hormone – 190 (Shoji *et al.*, 1990).

The growth hormone gene shows high structural-functional similarity to genes encoding prolactin and placental lactogen (LH). All these genes belong to one gene family (Wallis, 1992; Ohta, 1993; Goffin *et al.*, 1995; Soares, 2004), evolved from one common gene, whose age is determined on the 400 million years (Miller and Eberhardt, 1983). It is worth noting that while the same nucleotide sequence of the GH gene has a relatively high degree of similarity to the PRL gene, their protein products show only 16% homology (Krzymowski and Przała, 2005).

The aim of this study was to investigate the nucleotide sequence of mink GH gene, as well as to analyse its polymorphism.

Material and methods

The study included 116 individuals – 22 of wild mink (Nova Scotia, Canada), 58 of ranch mink (six colour types – Scanblack, Wild, Sapphire, Pearl, Black Cross and Sapphire Cross from West Pomerania, Poland) and 36 of feral mink (from Poland and Iceland). Genomic DNA was isolated from muscle tissue, using the High Pure PCR Template Preparation Kit from Roche (Germany). Target fragment of mink growth hormone gene include 1,113 bp region of the mink genomic DNA, encompassing whole 3rd and 4th exon and whole 2nd, 3rd and 4th intron, as well as parts of 2nd and 5th exon.

In order to amplify the target fragment of the growth hormone gene, nested-PCR with outer primers, flanking 1,220 bp region, and inner primers, flanking 1,113 region was carried out. In both reactions DNA amplification was performed in a mixture with a volume of 15.0 µl, containing ready-to-use PCR mix (A&A Biotechnology), each outer primer, water and DNA template (genomic DNA for first reaction and first reaction mix, which was used as DNA template for the second reaction). The PCR reaction was performed in a T3-type oil-free thermocycler of the Biometra (Germany) company with time-temperature programme, especially optimized for these reactions. The specificity and efficiency of amplification was assessed by checking the PCR products by electrophoresis in a 1.5% agarose gel with ethidium bromide.

The nucleotide sequence of the amplicons, as well as its sequence variation was analysed by sequencing, performed using BigDye® Terminator v3.1 kit (Applied Biosystems, Life Technologies). Sequencing reaction products were separated on a capillary sequencer 3730xl DNA Analyzer and 3130xl Genetic Analyzer (Applied Biosystems, Life Technologies).

Results

Sequencing allowed determining nucleotide composition of previously unrecognized non-coding regions, as well as exons. These include three introns, at positions: intron 2nd, 171 bp; intron 3rd, 175 or 176 bp; and intron 4th, 272 or 290 bp. Also 14 new polymorphisms were identified: 12 SNP substitutions (one in 2nd intron: G→C, five in 3rd exon: G→A, T→C, three in 3rd intron: G→C, A→G, C→T, and three in 4th intron: A→G, C→G, T→C), one single nucleotide deletion (in 3rd intron: C/-) and one ins/del polymorphism (18 bp; in 4th intron). The average incidence of SNPs in coding sequences was equal to 1/156.6 bp and in non-coding – 1/86.9 bp. Analysis of the observed number and location of substitutions revealed its non-random distribution (more

than 56% substitution is located in a central part of the amplicon) and significant preponderance of transitions over transversions, representing only app. 21% of all substitutions.

Comparing the genetic polymorphism of wild, ranch and feral mink the most important are differences were found in heterozygosity rate and presence of ins/del polymorphism in the 4th intron. Namely, average heterozygosity rate in wild mink was equal to 18.75%, in ranch mink – 15.87% and in feral mink – 23.38%. Ins/del polymorphism (deletion of 18 bp fragments) in the 4th intron was detected only in wild mink, and its prevalence was equal to 66.67%.

Discussion

The results revealed some significant differences between originally wild mink and domesticated animals (both ranch and feral) in identified SNPs type and distribution. Very interesting is investigation of the heterozygosity rates of mink from different environments. Firstly, smaller number of heterozygotes in farm animals, in comparison with wild ones, is an evidence of breeding work and selection pressure, under whose influence is the first of these groups. In turn, a significant increase in the heterozygosity rate in feral mink seems to indicate the influence of environmental factors in shaping genetic variability of animals under high natural selection pressure in new environments.

Further analysis are needed to determine the possibility of use the results of the present study in effective genetic monitoring of feral mink, as some differences were also found between ranch and feral mink. This could be used to develop an alternative to microsatellite genetic profiling and differentiation of wild, ranch and feral mink's populations.

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