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A TG-repeat polymorphism in the 5′-noncoding region of the goat growth hormone receptor gene and search for its association with milk production traits

Andrzej Maj *, Małgorzata Korczak, Emilia Bagnicka, Lech Zwierzchowski, Mariusz Pierzchała

Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, 05-552 Wólka Kosowska, Poland

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Abstract

A variable TG-repeat polymorphism was found in the goat growth hormone receptor (GHR) gene 5′-noncoding region. In total 235 goats belonging to two dairy breeds were genotyped, 10 alleles of the GHR gene were detected. The length of the repeat region variants (alleles) was from 305 to 346 bp. Among the 10 alleles identified, eight occurred in homozygous systems. The frequency of the homozygous genotypes was 0.25 for the Polish White Improved breed and 0.28 for the Polish Fawn Improved breed. The mean heterozygosity (H) and polymorphic content coefficients (PIC) at this locus were similar for both breeds. The association of the TG-repeat variants was studied with milk production traits in 157 goats using REML method with repeatability, multi-traits Animal Test-Day Model. No associations were found with dairy traits—milk yield and content of the major milk components (fat, protein, and lactose). Also, no effect was shown of the GHR genotype on the somatic cell count (SCC).

Keywords: Growth hormone receptor; Gene polymorphism; Microsatellites; Milk traits; Goat

1. Introduction

The biological effects of growth hormone (GH) involve a variety of tissues and the metabolism of all nutrient classes: carbohydrates, lipids, proteins, and minerals. In farm ruminants, these coordinated changes in tissue metabolism alter nutrient partitioning and thus play a key role in increasing growth performance and milk yield (Etherton and Bauman, 1998). Therefore, there is a great interest in using growth hormone to improve production in farm animals. Moreover, the gene encoding GH and other genes related to the so called "somatotropic axis" are considered promising candidate markers for selection purposes (Parmentier et al., 1999).

Growth hormone actions on target cells depend on GH receptor (GHR) (Burton et al., 1994). GH binding to GHR causes its dimerization, activation of the GHR-associated JAK2 tyrosine kinase, and tyrosyl phosphorylation of both JAK2 and GHR (Zhu et al., 2001). These events activate a variety of signalling molecules, including MAP kinases, insulin receptor substrates, phosphatidylinositol 3′-phosphate kinase, diacylglycerol, protein kinase C, intracellular calcium, and STAT transcription factors.

The GH receptor is a member of the cytokine/hematopoietin superfamily of receptors. The gene coding for

* Corresponding author. Tel.: +48 22 756 17 11; fax: +48 22 756 16 99.
E-mail address: A.May@ighz.pl (A. Maj).

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GHR of most mammalian species consists of nine exons (from 2 to 10) in the translated part and of a long 5′-noncoding region that includes several alternative untranslated exons, of which only exons 1A, 1B, and 1C have been studied in detail in bovine GHR gene (Jiang and Lucy, 2001). Distinct promoters regulate transcription from the alternative exons. The P1 promoter which regulates growth hormone receptor expression in liver, is associated with exon 1A in cattle and sheep (Jiang et al., 1999).

From recent publications it is known that some productive traits of cattle, e.g. milk yield and composition are associated with polymorphism of the GHR gene (Aggrey et al., 1999; Falaki et al., 1996; Blott et al., 2003). Lucy et al. (1998) found a length polymorphism in a TG-repeat (microsatellite) in the P1 promoter located 86 bp upstream from the start site of exon 1A in the bovine GHR gene. They found that an 11-TG-repeat allele commonly occurred in *Bos indicus* cattle while alleles with 16–20 consecutive TGs are most common in taurine breeds. However, the shorter 11-TG-repeat allele can be found at low frequency among European cattle, e.g. in Aberdeen Angus. The goat GHR gene has not been investigated yet. The objective of this study was to search for polymorphic microsatellite repeats within the 5′-region of the goat GHR gene and to verify possible association between this putative polymorphism and productive traits.

2. Materials and methods

2.1. Animals

Studies of the GHR gene TG-repeat polymorphism were conducted on 115 dairy Polish White Improved (PWI) and 120 Polish Fawn Improved (PFI) goats (does, bucks and kids). For studying associations between the GHR polymorphism and milk production traits data for 157 dairy goats being from 1st to 6th lactation were used. Goats of the PWI breed were mated with PWI and Saanen bucks, and goats of the PFI breed with PFI and Alpine bucks. The goats were maintained in three herds. The goats were kept in a loose barn with outside run (except winter). During the test period the animals were fed according to the INRA-system (Jarrige, 1988); water was available ad libitum.

An authorized veterinarian collected blood samples from jugular vein to tubes containing K_3EDTA. DNA for GHR gene sequencing and genotyping was isolated from blood by the method of Kanai et al. (1994) and amplified by PCR.

2.2. Sequencing of the caprine GHR gene 5′-region

Basing on the available sequences of the bovine (GenBank U15731) and ovine (O'Mahoney et al., 1994) GHR genes, and using the Primer3 software (www.genome.wi.mit.edu), primers were designed aimed at PCR amplification of overlapping fragments of the caprine GHR gene 5′-region (Table 1). Polymerase chain reactions were performed using a PCR-mix with: primers at 5.0 pmol/μl, 1 U *Taq* polymerase (Polgen, Łódź, Poland), 1 μl *Taq* polymerase buffer, four dNTPs, each at a final concentration of 0.2 mM, 100 ng of genomic DNA, and H_2O up to 10 μl. The number of cycles and temperature of annealing used for each pair of primers are given in Table 1. The yield and specificity of PCR products were evaluated after electrophoresis in 2% agarose gel (Gibco) stained with ethidium bromide. Then the PCR products were purified with GenElute PCR DNA Purification Kit (Sigma–Aldrich Corporation, St. Louis, MO, USA) and sequenced in an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were analysed using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA) software.

2.3. Analysis of GHR genotypes

Primers GHR3 (labelled at 5′ end with fluorescein dye C5) and GHR4 (Table 1) were used for the analysis of the TG-repeat (microsatellite) polymorphism. The PCR products were analysed after 5 min denaturing in a 50% formamide solution containing blue dextran. The fluorescent PCR products were separated in 6% denaturing polyacrylamide gels, using an ALFexpress DNA Sequencer (Amersham Biosciences Corporation, Piscataway, NJ, USA). In each lane 1 μl of PCR products were resolved together with a size marker. After automated allele calling using the Allele Links 1.01 software (Amersham Biosciences), individual genotypes were checked by manual inspection before exporting the genotypes to Excel.

2.4. Analysis of milk composition

The goats were milked twice a day. Milk samples were taken from each goat once a month during the whole lactation period. The milk yield and content of the major milk components – fat, protein, and lactose and also somatic cell count (SCC) – were collected. The daily milk yield was determined and the fat, protein and lactose content in milk samples were estimated in fresh milk using Milko Scan 104A/B (FOSS A/S, Hillerød,