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Novel polymorphisms of the growth hormone gene and their effect on growth traits in Chinese goats

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ABSTRACT

The polymorphisms of the growth hormone (GH) gene were analyzed in 686 individuals from four goat populations, Three haplotypes (A, B and C) and three observed genotypes (AA, AB and AC) were detected at the P2 locus, and three haplotypes (E, F and G) and three observed genotypes (EE, EF and EG) were also detected at the P4 locus. In addition, five single nucleotide polymorphisms (SNPs)—A112G, C142T (Gly>Ser), C214T (P2 locus), C266A (Pro>His) and C214T (P4 locus, Arg>Trp), were identified by *GH* gene sequencing and PCR-SSCP analysis. The SNPs loci were in Hardy–Weinberg disequilibrium in three goat populations ($P<0.05$). Association of polymorphisms with growth traits was done in BG, F1 and F1 populations, which were shown to be associated with growth traits in three goat populations. The SNPs in the goat *GH* gene had significant effects on growth traits ($P<0.05$). suggesting that the *GH* gene is a strong candidate gene that affects growth traits in goat.

1. Introduction

Growth traits are physiological functions under the control of several genes. Genotyping animals for all the genes encoding a polygenic trait seems impractical and so it is more realistic to focus on only a few genes having effects that account for a significant part of the genetic variation in growth traits (Li et al., 2010; Dekkers, 2004; Zhang et al., 2009). With the development of molecular biology and biotechnology, it is now possible to achieve more accurate and efficient selection goals by marker-assisted selection (MAS). For the goat, a special polytocous species with limited litter size, MAS plays a more important role than in other polytocous species in selection programs due to the fact that growth is greatly associated with litter size (Hua et al., 2009; Fan et al., 2010). In general, elucidating and validating the genetic markers of growth traits are the initial and crucial steps to establish a MAS system (Allan et al., 2007).

Since the 1920s, it is well documented that growth hormone (GH) influences animal processes such as growth (Breier, 1999), lactation (Baldi, 1999), reproduction (Scaramuzzi, Murray, Downing & Campbell, 1999) and metabolism (Bauman, 1999). Growth hormone (GH) is released by the exocytosis of somatotrophs in the adenohypophysis through a series of physiological stimuli which involve, among others, the actions of GH releasing hormone (GHRH) and somatostatin and fluctuations in the blood concentrations of glucagon, insulin, IGF-I and -II, estrogen hormones and thyrotrophin releasing hormone (Enright et al., 1993). Circulating GH binds to the extracellular domain of specific transmembrane receptors (GHR), which are expressed in various tissues, but especially in the liver (Sorensen, Chaudhuri, Louveau, Coleman & Etherton, 1992). This process is the signal for a cascade of intracellular metabolic events and culminates in the production of IGF-I by the target tissues, notably the liver (Renaville, Hammadi & Portetelle, 2002). Directly or indirectly, through the action of IGF-I, GH is the main regulator of postnatal somatic growth, stimulating anabolic processes such as cell division, skeletal growth and protein synthesis (Connor, Ashwell & Dahl, 2002; Curi, de Oliveira, Silveira & Lopes, 2005; Katoh, Kouno, Okazaki, Suzuki & Obara, 2008). In addition, GH is involved in the regulation of fat oxidation (lipolytic activity), in the inhibition of glucose transport to peripheral tissues (diabetogenic activity), and in the regulation of the activity of ribosomes involved in the translation process, which in turn influences protein synthesis (Goodman, 1993). These processes are directly implicated in the metabolism of nutrient distribution to different tissues and, consequently, in carcass composition and quality (Schlee et al., 1994). Based on the above considerations, the objectives of the present study were to estimate the allele and genotype frequencies of goat *GH* gene polymorphisms and to determine associations between these polymorphisms and the performance of these animals when submitted to an intensive production model.

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2. Materials and methods

2.1. DNA samples

All procedures involving animals were approved by Boer goat breeding centers and Green Century Biology Development Company in Shaanxi Province, China. A total of 686 female goats were examined, including 190 Xinong Saanen goats (SG), 168 Boer goats (BG), 136 F1 generation of Boer × Guanzhong dairy goats (F1) and 192 F2 generation of Boer × Guanzhong dairy goats (F2). The traits evaluated included body weight, withers height, body length and chest girth at 3 months of age. Approximate 5 ml blood per goat was collected aseptically from the jugular vein and kept in a tube containing anticoagulant ACD (10:27:38 Citric acid:Sodium citrate:$C_6H_{12}O_6$). All samples were delivered to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using a standard phenol-chloroform extraction protocol (Joseph & David, 2002). The DNA samples were dissolved in TE buffer which was made from 10 mM Tris–Cl (pH7.5) and 1 mM EDTA (pH 8.0) and were stored at −20 °C until used.

2.2. PCR conditions

According to the goat *GH* gene (GenBank accession no. D00476), four pairs of primers (Table 1) were designed to amplify exons 1, 3, 4 and 5 of the *GH* gene. The 25 μl volume contained 50 ng genomic DNA, 12.5 μL 2× reaction mix (including 500 μM dNTP each; 20 mM Tris–HCl, pH 9; 100 mM KCl; 3 mM MgCl2), 0.5 μM of each primer, and 0.5 units of Taq DNA polymerase. The cycling protocol was 5 min at 95 °C, 35 cycles of denaturing at 94 °C for 30 s, annealing at X °C (Table 1) for 30 s, extending at 72 °C for 40 s, with a final extension at 72 °C for 10 min.

2.3. Single strand conformation polymorphism (SSCP) and DNA sequencing

PCR products (5 μl) were mixed with 5 μl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene–cyanole and 0.025% bromophenol blue), heated for 10 min at 98 °C and chilled on ice. Denatured DNA samples were subjected to PAGE (80 × 73 × 0.75 mm) in 1× TBE buffer and constant voltage (200 V) for 4.5 h. The gel (29:1 acrylamide:bis) was stained with 0.1% silver nitrate (Ji, & Cao, 2007; An et al., 2009). After the polymorphisms were detected, each of the DNA bands on the SSCP gel was extracted and the PCR products of the different electrophoresis patterns were sent for sequencing in both directions (repeated three times) in an ABI 377 DNA analyzer (Applied Biosystems) and the sequences were determined with DNAstar software (version 7.1) and blast in NCBI (National Center for Biotechnology Information).

2.4. Statistical analysis

The genotypic frequencies, heterozygosity (He) and polymorphism content (PIC) were calculated using Cluster-analysis software (version 1.2), and Hardy–Weinberg equilibrium for each population was analyzed using χ^2-test, which was performed by SPSS software (version 16.0), and the parameters of Linkage Disequilibrium between *GH* gene exons 3 and 5 were calculated using PowerMarker software (version 3.25). The software SPSS (version 16.0) was also used to determine the relationship between genotypes and growth traits in goat. Taking into account the variety of breeds, the adjusted linear model with fixed effects was established and included effects of sire, dam within sire, breed and genotype. Adjusted linear model: $Y_{ijklm} = \mu + S_i + D_{ij} + B_k + G_l + E_{ijklm}$, where Y_{ijklm} is the trait measured on each of the *ijklm*th animal, μ is the overall population mean, S_i is the fixed effect associated with the *i*th sire, D_{ij} is the fixed effect associated with *j*th dam with sire *i*, B_k is the fixed effect due to the *k*th breed, G_l is the fixed effect associated with *l*th genotype, and E_{jiklm} is the random error. Effects associated with farm and season of birth (spring vs fall) were not matched in the linear model, as the preliminary statistical analyses indicated that these effects did not have a significant influence on the variability of traits in the analyzed populations.

Table 1
Primer sequences and information of the *GH* gene.

Names	Sequences(bp)	Tm (°C)	Product size (bp)	Amplicons
P1	F: 5-ATAAAAAGGGCCCAGCAGAGACC-3 R: 5-GGACACATCTCTGGGGAGCTTACA-3	66.5	531	Exon 1
P2	F: 5-TAGAAATGGGGGTGTGTGGGGT-3 R: 5-CATCCTCCACTGCCATCCAACA-3	66	320	Exon 3
P3	F: 5-AGGGTGTTGGATGGCAGTGGA-3 R: 5-GACCCAACAACGCCATCCTCA-3	66	307	Exon 4
P4	F: 5-TAGGGGAGGGTGGAAAATGGA-3 R: 5-TCTAGGAAGGCACGGGGAGG-3	65	302	Exon 5

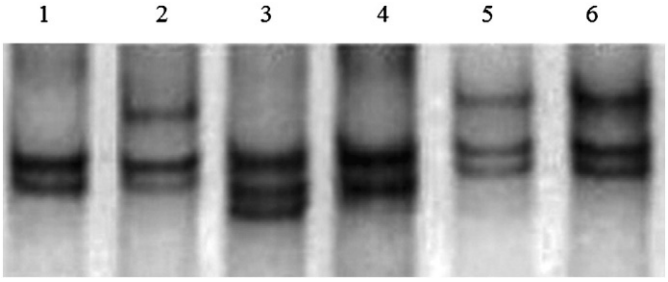

Fig. 1. SSCP analysis of PCR products using P2 in the goat populations 1 and 4: AA genotype; 2, 5, and 6: AB genotype; 3: AC genotype.

2.5. Analysis of DNA and protein sequences

The sequencing results of the PCR products of different SSCP patterns were compared with those from the Genbank, respectively. In addition, the amino acid sequences of different genotypes were compared with DNAstar software (version 7.1).

3. Results

3.1. Polymorphisms of GH gene in four goat populations

The *GH* gene exons 1, 3 4 and 5 including 1460 bp were amplified by the P1–P4 primers. In the PCR products of four pairs of primers, only that of P2 and P4 had polymorphisms. According to international practice and Gupta, Pandey, Malik & Gupta (2009) and Shi et al. (2009) about the naming of SSCP patterns, in P2 primer locus, different SSCP patterns

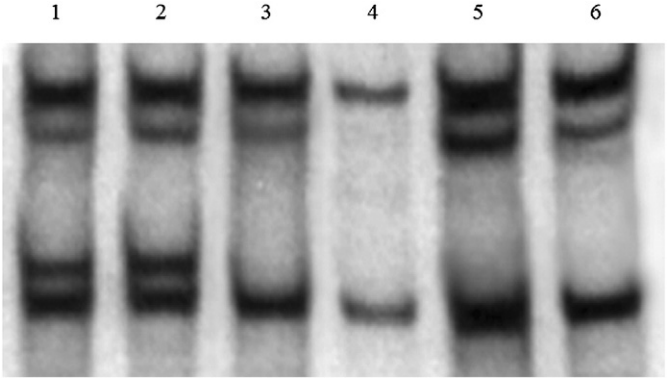

Fig. 2. SSCP analysis of PCR products using P4 in the goat populations 1 and 2: EG genotype; 3, 5 and 6: EF genotype; 4: EE genotype.