ISSN: 2320-1983 Print ISSN: 2320-1991 Online Volume 2 Issue 4

Applied Cell Biology

Regular Paper

ACB, 2(4), 2013 [149-152]

Correlation between growth hormone gene polymorphisms and milk production trait in Holstein cattle

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Received: 13th October, 2013 ; Accepted: 16th November, 2013

ABSTRACT

PCR–RFLP technique was developed for association between growth hormone (*GH*) gene polymorphisms and milk production trait in Holstein cattle. Forty-eight female Holstein cattle reared under Egyptian conditions were precisely selected according to their milk productivity and DNA from blood samples of these animals was extracted to amplify 329-bp of the gene encoding *GH*. Based on the breeding value, the 48 animals were ordered from the highest to the lowest milk productivity. Restriction analysis of PCR-RFLP- *Hapa*II of the *GH* gene (329-bp) showed three various genotypes MM, MN and NN with frequencies 0.04, 0.25 and 0.71, respectively. The frequencies of the *M* and *N* alleles were 0.17 and 0.83, respectively. The results indicated that the MN cows yielded more milk than MM and NN cows. Sequencing (GenBank JF826521) revealed that six mutations (115C! T, 249C! T, 251C! A, 261T! C, 264T! C and 269T! C) occurred in genotype NN of Holstein cattle. These findings can be used as marker-assisted selection (MAS) for high milk production trait in Holstein cattle.

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KEYWORDS

Holstein cattle;
Milk production;
GH gene polymorphisms;
DNA sequencing.

INTRODUCTION

Restriction fragment length polymorphisms (RFLP's) and single nucleotide polymorphism (SNP's) can be used as molecular markers on the genetic chromosome. The development of molecular genetic markers (RFLP's and SNP's) for growth hormone (GH) gene will be the objective of this study for improvement of quantitative milk production trait in Holstein cattle using marker-assisted selection (MAS). Most quantitative traits such as meat and milk production in farm animals are influenced by many genes and by environmental factors. In other words, the observed phenotype of these traits is the combined results of the action of large numbers of polygenes and environmental factors[1,14].

The bovine growth hormone (bGH) gene has been intensively studied in farm animals owing to its defined key role in growth, body composition, metabolism regulation, mammary gland development and lactation[3].

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Components of the growth hormone (GH)/ insulin-like growth factor (IGF) system play an important role in the metabolic transition that favours high milk production after calving[4]. In liver, growth hormone receptor (GHR) and IGF-1 are dynamically regulated by lactation and energy balance[9]. Thus, growth hormone (GH) gene plays an important regulatory function in milk secretion in cattle. Consequently, the GH gene is potential quantitative trait locus associated with milk production trait in cattle and RFLP's and SNP's polymorphism can be used as marker-assisted selection (MAS) which will be useful for increasing and accelerating the rate of genetic improvement on milk production trait[5,7,8].

MATERIALS AND METHODS

Animals

Forty-eight female Holstein cattle live under Egyptian conditions were chosen based on milk productivity. Blood samples from these animals were collected by Jugular vein puncture into tubes containing an anticoagulant disodium EDTA. The samples were stored at -20 until needed for DNA isolation.

DNA isolation

DNA isolation was achieved according to Sharma *et al*. (2000)[12] as follows: 700 µl of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 µg of proteinase K (20 mg/ml) were added to 100 µl thawed blood. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted by equal volumes of phenol-chloroform-isoamylalcohol (25:24:1) and chloroform-isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume of double distillated water (ddH_2O).

PCR amplification and genotyping of GH gene

A 329-bp fragment of intron 3 of *GH* gene in 48 female Holstein cattle was amplified by PCR using forward (5'-CCCACGGGCAAGAATGAGGC-3') and reverse (5'-TGAGGAACTGCAGGGGCCCA-3' primers[6,15]. PCR was performed in a reaction volume of 25 µl using 25 ng of genomic DNA of each sample, 25 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/ µl Taq DNA polymerase (Bioron, Germany). Thermal cycling (Autorisierter Thermocycler and Mastercycler Gradient) was carried out by initial denaturation at 94°C for 4 min, followed by 34 cycles each at 94°C for 1 min, annealing temperature at 65°C for 1 min, polymerization temperature at 72°C for 1 min and final extension at 72°C for 10 min., then the samples were held at 4°C. The amplified DNA fragments were separated on 3% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada). For genotyping, digestion of 10 µl of amplified DNA was accomplished with 10 units *Hapa*II restriction enzyme for three hours at 37°C. Digested DNA was separated on 3% agarose gels in IX TBE buffer, stained with ethidium bromide, visualized under UV light and photographed.

Statistical analysis

After adjustment or correction of non genetic factors (lactation length (305-day), age at first calving (AFC) and milking frequency (2x)) according to Schmidt and Vanvleck, 1974[11], breeding value (BV) was calculated to rank animals according to their excellence in milk production for each animal using the following equation[2]: BV= X bar + h^2 (X – X bar), where: BV= Breeding value, X bar= Average milk yield of the herd, h^2= Heritability for milk production trait (0.25) and X= Corrected milk for animal. *M* and *N* alleles (*p* and *q*) and MM, MN and NN genotypes (p^2, 2pq and q^2) frequencies of the *GH* locus were estimated using the famous equations described also by Falconer and Mackay (1996)[2].

Sequencing and analysis of the GH gene

A 329-bp fragment of intron 3 of *GH* gene was subjected for DNA sequencing using *3130xl* Genetic Analyzer (Applied Biosystems-Hitachi, Japan) at Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technology Applications, Alexandria, Egypt. DNA sequencing was carried out only in the highest (seven animals) and lowest (six animals) milk productivity animals. Using ClustalW (1.8) program, DNA sequence alignment was compared among the sequenced thirteen female Holstein cattle.

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RESULTS AND DISCUSSION

PCR amplification of the gene encoding *GH* yielded 329-bp in length in all 48 female Holstein cattle (Figure 1). Restriction analysis of PCR-RFLP- *Hapa*II of the GH gene (329-bp) showed three different genotypes: MM genotype (329-bp undigested fragment), MN genotype (329-bp, 224-bp and 105-bp) and NN genotype (224-bp and 105-bp fragments), see Figure 2. The calculated frequencies of MM, MN and NN genotypes were 0.04, 0.25 and 0.71, respectively, and the frequencies of the *M* and *N* alleles were 0.17 and 0.83, respectively (TABLE 1). The results indicated that the MN genotype yielded more milk than MM and NN genotypes in Holstein cattle. For milk yield, our results agree with those obtained by Yao *et al*. (1996)[13], Sabour *et al*. (1997)[10] and Zhou *et al*. (2005)[15]. On the other hand, PCR products of *GH* gene (329-bp) were sequenced (GenBank JF826521) and the results

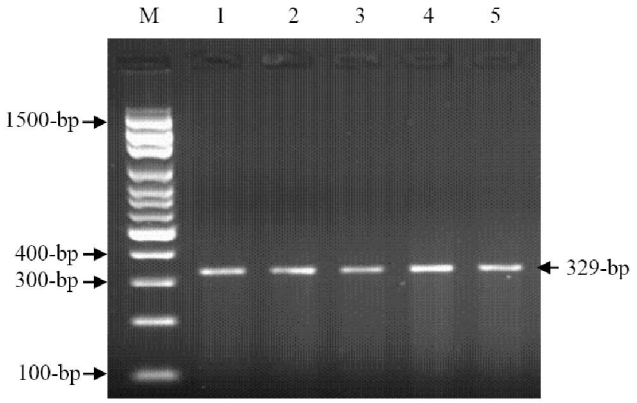

Figure 1 : PCR products (329-bp) generated by the *GH* gene primers. Where, lane M is DNA marker and lanes 1-5 are female Holstein cattle (as an example).

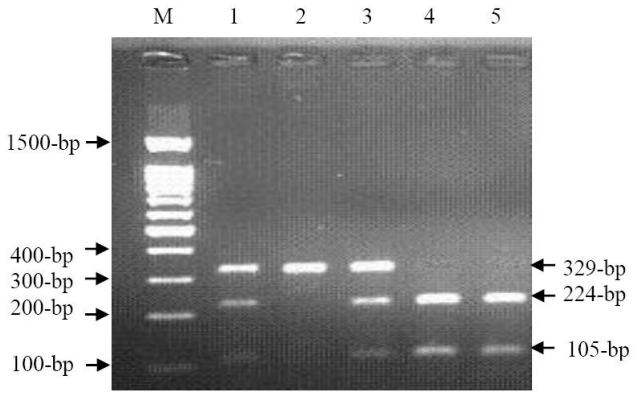

Figure 2 : Representative *Hapa*II restriction fragment pattern of *GH* gene (329-bp). Lanes 1 and 3 = MN genotype (329-bp, 224-bp and 105-bp), lane 2 = MM genotype (329-bp), lanes 4 and 5 = genotype NN (224-bp and 105-bp) and lane M = Molecular marker (100-bp).

TABLE 1 : Frequency of genotypes (MM, MN and NN) and alleles (*M* and *N*) in *GH* locus.

Breed	Total no. of cows	Genotypic frequency			Allelic frequency	
		MM(p^2)	MN(2pq)	NN(q^2)	M(p)	N(q)
Holstein cattle	48	0.04	0.25	0.71	0.17	0.83

of alignment among the sequenced animals showed six mutations (115C→T, 249C→T, 251C→A, 261T→C, 264T→C and 269T→C) occurred in genotype NN of Holstein cattle. However, these results preliminarily showed that *GH* gene is a genetic marker and closely linkage to the milk production trait and consequently, can be used as a marker-assisted selection (MAS) for high milk productivity animals.

CONCLUSIONS

For association between growth hormone (*GH*) gene polymorphisms and milk production trait, PCR products of *GH* gene (329-bp) were genotyped in 48 Holstein cattle and sequenced (GenBank JF826521). Restriction analysis of PCR-RFLP- *Hapa*II of the *GH* gene (329-bp) showed three genotypes: MM, MN and NN with frequencies: 0.04, 0.25 and 0.71, respectively. The frequencies of the *M* and *N* alleles were 0.17 and 0.83, respectively. Statistically, the results indicated that the MN cows yielded more milk than the MM and NN cows. Sequencing revealed that six mutations (115C→T, 249C→T, 251C→A, 261T→C, 264T→C and 269T→C) occurred in genotype NN of Holstein cattle. These findings can be used as genetic markers for selection the high milk productivity animals.

ACKNOWLEDGEMENTS

This work was achieved at Department of Nucleic Acid Research (NAR), Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (CSAT), Alexandria, Egypt.

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