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EFFICIENCY OF BOVINE IGF-I GENE IN THE IMPROVEMENT OF MILK PRODUCTIVITY USING MARKER-ASSISTED SELECTION (MAS)

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Abstract: Because of insulin-like growth factor-I (IGF-I) gene plays an important regulatory function in milk secretion in cattle, IGF1 gene is potential quantitative trait locus and genetic marker (i.e, SNP) associated with milk production trait in cattle. Consequently, marker-assisted selection (MAS) will be useful to increase and accelerate the rate of genetic improvement on milk productivity. In this study, 48 female Holstein cattle reared under Egyptian conditions were selected based on their milk productivity and DNA from blood was extracted to amplify 249-bp of the gene encoding IGF-I. According to the breeding value, PCR products of IGF-I gene (249-bp) were sequenced only in the 15 highest and lowest milk productivity animals (GenBank accession numbers from gb|HQ183710| to gb|HQ183724|, sequentially). The result indicated that two single nucleotide polymorphisms (SNP's) at two different positions were observed in one of the highest milk productivity animals. Where, all 15 animals have adenine (A) and cytosine (C) bases at the positions 33 and 63, respectively, except, one animal (GenBank Acc. No. gb|HQ183711|) has thymine (T) and guanine (G) bases at the same positions (33 and 63, respectively). Thus, this finding can be used as marker-assisted selection (MAS) for high milk productivity in Holstein cattle.

Keywords: Holstein cattle, IGF-I gene, milk production, DNA sequencing

Introduction

Most traits of economic importance in farm animals are quantitative, in another words, are influenced by many genes and by environmental factors *(Zhang et al., 1998)*. For example, milk production trait is quantitative in nature. The

observed phenotype of this trait is the combined results of the action of large numbers of polygenes or quantitative trait loci (QTL) and environmental factors.

Marker assisted selection (MAS) is used for indirect selection of a genetic determinant of a trait of interest (milk productivity). The development of molecular genetic markers (AFLP's, RFLP's, SSCP's and SNP's) for genes (i.e. GH, Prl or IGF1) associated with quantitative productive traits in cattle will be the objective of this study for improvement of quantitative milk production trait using marker-assisted selection (MAS).

The growth hormone (GH)/insulin-like growth factor (IGF) system plays a critical endocrine role controlling nutrient metabolism in dairy cattle. In liver, growth hormone receptor (GHR) and IGF-1 are dynamically regulated by lactation and energy balance *(Rhoads et al., 2008)*. Insulin-like growth factor-I (IGF-I) gene plays an important regulatory function in milk secretion in cattle. Hence, the IGF-I gene is potential quantitative trait locus and genetic marker (RFLP's and SNP's) associated with milk production trait in cattle. Consequently, marker-assisted selection (MAS) will be useful to increase and accelerate the rate of genetic improvement on milk production trait *(Mackinnon and Georges, 1998; Reinecke et al., 2005; Reißmann et al., 2006)*.

Materials and Methods

Animals. Forty-eight female Holstein cattle reared under Egyptian conditions were chosen according to milk productivity (from the highest to the lowest milk production). 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 extraction.

DNA extraction. From the 48 blood samples, DNA extraction was carried out according to *Sharma et al. (2000)* 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 of IGF-I gene. A segment (249-bp) of IGF-I gene in 48 female Holstein cattle was amplified with the use of primer sequence *(Ge et al., 2001)*: 5'-ATTACAAAGCTGCCTGCCCC-3' (forward) and 5'-ACCTTACCCGTATGAAAGGAATATACGT-3' (reverse). 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 62°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 2-3% 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).

Statistical analysis. In 48 female Holstein cattle, the actual milk yield was corrected or adjusted for 305-day lactation length, age at first calving (AFC) and milking frequency (2x) using the equations described by Schmidt and Vanvleck (1974). Breeding value (BV) was calculated to rank animals according to their excellence in milk production using the equations described by *Falconer and Mackay (1996)*. BV= X□ + h^2 (X - X□), where: BV= breeding value, X□= average milk yield of the herd, h^2 = heritability for milk production trait (0.25) and X= corrected milk for animal.

Sequencing and analysis of the IGF-I gene. DNA sequencing for a fragment (249-bp) of the IGF-I gene (5' noncoding region) was performed according to *Sanger et al. (1977)* using 3130xl Genetic Analyzer (Applied Biosystems-Hitachi, Japan) at Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Scientific Research and Technology Applications, Alexandria, Egypt. Where, sequencing was carried out for the noncoding region (294-bp) of the IGF-I gene in 15 female Holstein cattle (the highest and lowest milk productivity). Consequently, fifteen different sequence submissions were submitted to NCBI GenBank database for getting the accession numbers. Using ClustalW (1.8), sequence alignment was compared with IGF-I genes that are available in the GenBank database (http://www.ncbi.nlm.nih.gov).

Results and Discussion

Genomic DNA from 48 female Holstein cattle (highest and lowest milk productivity) was extracted to amplify IGF-I gene. PCR amplification of the gene encoding IGF-I gene yielded 249-bp in length in all 48 selected animals (Figure 1). PCR products of the gene encoding IGF-I (249-bp) in 15 female Holstein cattle (ordered from high to low milk productivity) were sequenced and read. Consequently, DNA nucleotide sequences were submitted to the GenBank and the recorded accession numbers were as shown in Table 1. To demonstrate the sequence alignment of IGF-I gene (249-bp) among the 15 female Holstein cattle under study (selected and ordered according to the highest and the lowest milk

productivity), sequence alignment was carried out using ClustalW program (Figure 2).

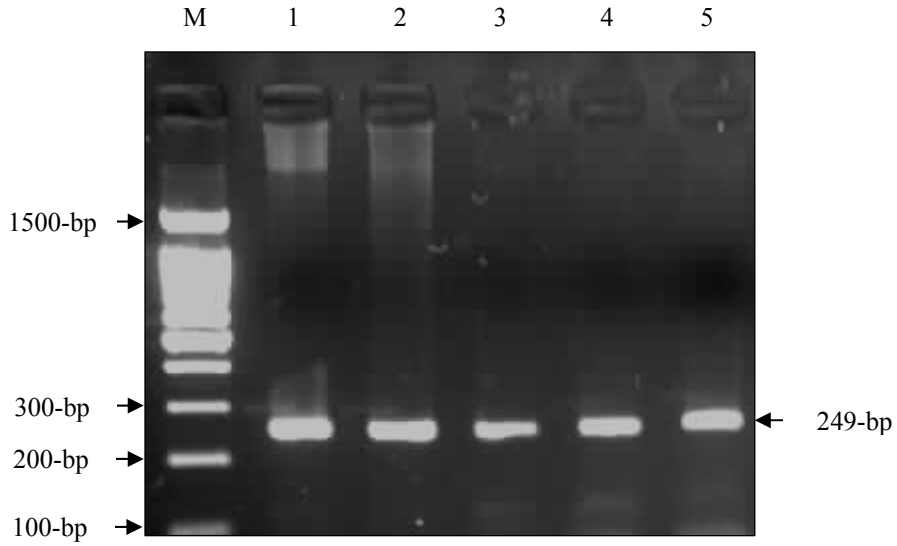

Figure 1. PCR products generated by the IGF-I gene primer. Where, lane M is DNA marker and lanes 1-5 are female Holstein cattle (as an example).

Cattle represent the most important part of animal husbandry in the most countries in the world and the genetic improvement of milk production in this farm animal is of economic importance, especially in development countries which have not arrived yet to the self-sufficiency. Components of the growth hormone (GH)/IGF system play an important role in the metabolic transition that favors high milk production after calving *(Lucy et al. 2001)*. For improvement milk production trait in cattle using marker-assisted selection (MAS), development of molecular genetic marker (SNP) for IGF-I gene was the objective of the present study.

Consequently, these two SNP's markers in bovine IGF-I gene may be useful in the genetic improvement of milk production trait in Holstein dairy cattle in general and in particular which reared under Egyptian conditions. Before leaving this part, it's important to note that animal number 1 is the only animal has two SNP's (T/G - 33/63), while all the other 14 animals have the same nucleotide sequence at the same positions (A/C - 33/63) including the highest milk productivity animal (animal number 2, BV = 11569). Because of there is no a big difference in milk productivity between animal number 1 (BV = 11553) and animal number 2 (BV = 11569), we are highly motivated for prediction to select the high

milk productivity animals using this experimental finding (marker-assisted selection).

Table 1. The animal numbers and their accession numbers.

Serial no.	*Animal no.*	*Accession no.*
1	*2*	*gb|HQ183710|*
2	*1*	*gb|HQ183711|*
3	*4*	*gb|HQ183712|*
4	*9*	*gb|HQ183713|*
5	*8*	*gb|HQ183714|*
6	*6*	*gb|HQ183715|*
7	*16*	*gb|HQ183716|*
8	*3*	*gb|HQ183717|*
9	*43*	*gb|HQ183718|*
10	*30*	*gb|HQ183719|*
11	*36*	*gb|HQ183720|*
12	*46*	*gb|HQ183721|*
13	*38*	*gb|HQ183722|*
14	*45*	*gb|HQ183723|*
15	*48*	*gb|HQ183724|*

In 48 female Holstein cattle reared under Egyptian conditions, 249-bp of IGF-I gene was amplified and only in 15 animals (selected according to the highest and the lowest milk productivity) was sequenced. Table 2 shows the nucleotide sequence variation among these 15 animals in 249-bp of IGF-I gene, which was only in 12 nucleotides. As indicated, two single nucleotide polymorphisms (SNP)

at two different positions were found in one of the two highest milk productivity animals (animal number 1, BV = 11553). Where, all the 15 animals have adenine base (A) at the position 33 except animal number 1 has thymine base (T) at the same position (33). Also all the 15 animals have cytosine base (C) at the position 63 except animal number 1 has guanine base (G) at the same position (63), see Figure 2. In contrast, we could not identify any SNP in the other 14 highest and lowest milk productivity animals in these 12 nucleotide sequence variations.

Thus, the present experiment showed that animals with T (SNP1) and G (SNP2) nucleotide sequence (33 and 63 positions) for the IGF-I gene can be used as marker-assisted selection (MAS) to select for high milk production trait.

In previous related two studies to link between IGF-I gene polymorphisms and milk production trait, *Siadkowsk et al. (2006)* indicated effect of polymorphism in IGF1 gene on milk production trait in Polish Holstein-Friesian cattle. Where, restriction analysis of PCR-RFLP-SnaBI of the IGF1 gene (249-bp) showed three genotypes AA (223- and 26-bp), AB (249-, 26- and 223-bp) and BB (undigested band, 249-bp). Cows carrying AB genotype yielded more FCM, VCM milk, more milk fat and more milk protein than those AA and BB genotypes. Also, *Mehmannavaz et al. (2010)* studied IGF1 gene (249-bp) polymorphism and milk production trait in Iranian bulls. Digestion of the 249-bp PCR product with SnaB1 restriction enzyme (C-T substitution creates a SnaB1 restriction site) yielded three genotypes TT (223- and 26-bp), TC (223-, 26-, 249-bp) and CC (249-bp). Results revealed that bulls with genotype TC had higher estimated breeding values of milk and fat yield compared to CC and TT genotypes.

Table 2. Nucleotide sequence variation among 15 animals ordered from high to low milk productivity.

Animal no.	*Breeding value (BV)*	*Nucleotide sequence variation*											
2	*11569*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*T*	*A*	*C*	*C*
1	*11553*	*C*	*T*	*-*	*C*	*G*	*T*	*G*	*T*	*A*	*T*	*A*	*C*
4	*11396*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
9	*11392*	*C*	*T*	*A*	*C*	*G*	*A*	*C*	*T*	*A*	*T*	*A*	*C*
8	*11390*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*T*	*T*	*A*	*C*	*C*
6	*11366*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*T*	*T*	*A*	*C*	*C*
16	*11321*	*C*	*T*	*A*	*C*	*G*	*A*	*C*	*T*	*A*	*T*	*A*	*C*
3	*11312*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*T*	*A*	*T*	*A*	*C*
43	*10348*	*-*	*T*	*C*	*G*	*T*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
30	*10345*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*A*	*C*	*C*
36	*10337*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*A*	*C*	*-*
46	*10316*	*C*	*T*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
38	*10279*	*C*	*-*	*A*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
45	*10249*	*C*	*A*	*-*	*C*	*G*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
48	*10036*	*-*	*T*	*C*	*G*	*T*	*A*	*C*	*C*	*A*	*T*	*A*	*C*
Nucleotide number		*18*	*22*	*23*	*24*	*25*	*33*	*63*	*175*	*191*	*192*	*193*	*194*

A is for adenine base, C is for cytosine base, G is for guanine base, T is for thymine base and - is absent base.

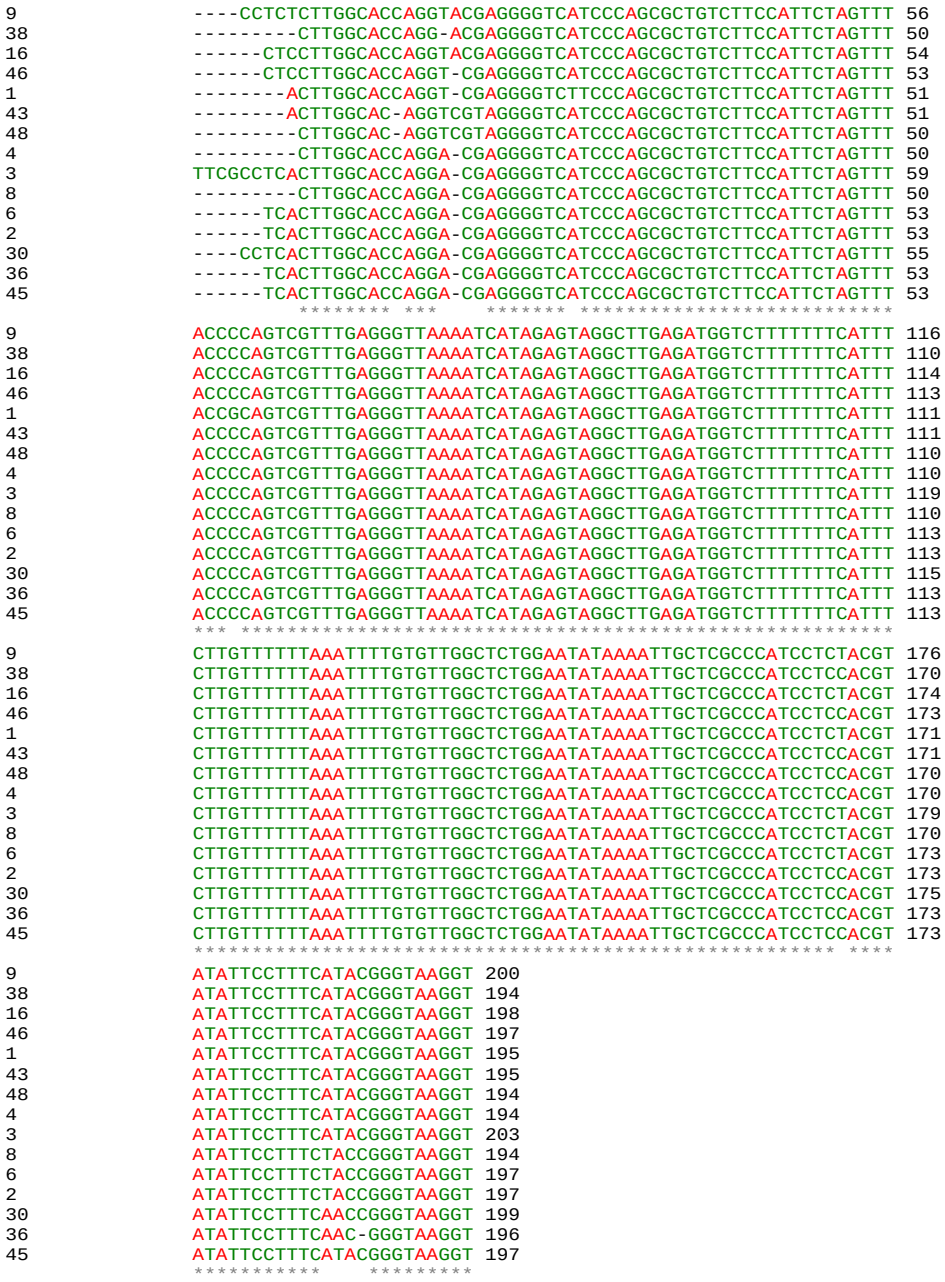

Figure 2. DNA sequence alignment of IGF-I gene (249-bp) among the 15 female Holstein cattle (9, 38, 16, 46, 1, 43, 48, 4, 3, 8, 6, 2, 30, 36 and 45). The asterisks represent the similarity.

Conclusion

In current study, PCR products of IGF-I gene (249-bp) were sequenced in 15 highest and lowest milk productivity animals (GenBank accession numbers from gb|HQ183710| to gb|HQ183724|, sequentially). Two single nucleotide polymorphisms (SNP's) at two different positions were found in one of the highest milk productivity animals. However, 14 animals have adenine (A) and cytosine (C) bases at the positions 33 and 63, respectively. While, animal number 1 (GenBank Acc. No. gb|HQ183711|) has thymine (T) and guanine (G) bases at the same positions (33 and 63, respectively). This finding can be used as a genetic marker associated with milk production trait. Hence, it could be used as marker-assisted selection (MAS) for high milk productivity selection in Holstein cattle.

Acknowledgment

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Efikasnost IGF-I gena goveda u poboljšanju produktivnosti proizvodnje mleka korišćenjem marker asistirane selekcije (MAS)

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Rezime

Zbog faktora porasta –I sličnog insulinu (IGF-I) gen ima važnu regulatornu funkciju u izlučivanju mleka kod goveda, IGF1 gen je potencijalni lokus kvantitativne osobine i genetski marker (npr. SNP) koji ima veze sa proizvodnjom mleka goveda. Zbog toga, marker-asistirana selekcija (MAS) će biti korisna za povećanje i ubrzanje genetskog napretka u proizvodnji mleka i produktivnosti. U ovom istraživanju, 48 ženskih grla rase holštajn odgajanih u uslovima u Egiptu su odabrana na bazi mlečnosti i DNK iz krvi je ekstrahovan da bi se amplificirao 249-bp gena koji šifrira IGF-I. Prema priplodnoj vrednosti, PCR proizvodi IGF-I gena (249-bp) su sekvencirani kod 15 grla sa najnižom i najvišom mlečnošću (GenBank pridruženi brojevi od gb|HQ183710| do gb|HQ183724|, redom). Rezultat ukazuje da dva pojedinačna nukleotidna polimorfizma (SNP's) na dve različite pozicije su

registrovani kod jedne od životinja sa najvećom proizvodnjom mleka. Takođe, svih 15 grla je imalo adenin (A) i citozin (C) baze na pozicijama 33 i 63, respektivno, osim jednog grla (GenBank Acc. No. gb|HQ183711|) koje je imalo timin (T) i guanin (G) baze na istim pozicijama (33 i 63, respektivno). S toga, ovi rezultati se mogu iskoristiti kao marker asistirana selekcija (MAS) za visoku proizvodnju mleka kod holštajn grla.

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