Genetic Polymorphism of Prolactin, Bone Morphogenetic Protein Receptor 1B and Insulin- like Growth Factor 1 Genes in Two Selected Lines of Japanese Quail

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Abstract: Quail is the smallest avian species raised for meat and egg production. It has several advantages as a laboratory bird for biological and biomedical investigations. In this study, we performed single nucleotide polymorphism (SNP) detection in prolactin (*PRL*), Bone Morphogenetic Protein Receptor IB (*BMPR-1B*) and Insulin- like Growth Factor 1 (*IGF-1*) genes in egg and meat selected lines of Japanese quail. Meat line recorded significant superior body weight measurements at 2nd, 3rd, 4th, 5th and 6th week of age (45.15, 85.64, 118.90, 164.22 and 176.67 g; respectively), compared those of egg line (36.72, 63.00, 84.36, 122.11 and 143.55 g; respectively). DNA was extracted from blood samples using commercial kits and amplified using polymerase chain reaction (PCR). Nucleotide polymorphisms between two selected lines were detected by DNA sequencing. Five nucleotide changes in *PRL* and *BMPR-1B* genes were identified and there was no nucleotide difference in *IGF-1* gene between egg and meat selected lines. The further study was required to find mutation in other site of *IGF-1* and the SNPs discovered in this study provided suitable markers for association studies of candidate genes with important economic traits in Japanese quail.

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1. Introduction

Quail are an economically important avian species and provide an alternative to the more commonly used chicken. They require less space and low initial investment. Quail are in the genus *Coturnix*, family *Phasianidae* and order *Galliformes* (Van Tuinen & Dyke, 2004; Kan *et al.*, 2010). Several aspects account for the utility of this bird as an experimental bird in both research and education. First, it has attained economic importance as an agricultural species producing eggs and meat that are enjoyed for their unique flavor. Second, the low maintenance cost associated with its small body size (80–300 g) coupled with its short generation interval, resistance to diseases and high egg production; render it an excellent laboratory animal. Third, Japanese quail is phylogenetically closely related to the chicken (Stock & Bunch, 1982).

Although the Japanese quail has several advantages as a laboratory bird, its genome sequence is not currently available. The Japanese quail genome sequence will provide key genomic resources to facilitate various studies as well as to establish distinct Japanese quail lines.

The development of molecular biology and especially DNA based markers during the past three decades created new means for studying livestock genetics and animal breeding. Selection according to genotype has the power to increase productivity of farm animals, as well as to enhance environmental adaptation and maintain genetic diversity (Hayes *et al.*, 2009). Molecular genetic markers represent one of the most powerful tools for the analysis of genomes and enable the association of heritable traits with underlying genomic variation. Additionally, studies of candidate genes and their effect on the phenotypic traits are the basis for market-associated selection (MAS) (Kulibaba & PodStreshnyi, 2012).

Prolactin (*PRL*) is one of the pituitary hormones, regulates important physiological functions, ranging from well-known effects in mammalian reproduction to osmoregulation in fish and its roles are not yet understood extensively in birds, but its major function is believed to be manifested during incubation and feeding of nestlings (Hui-Fang *et al.*, 2009). The Japanese quail prolactin gene is 10 KB in size and is composed of 5 exons and 4 introns, encoding 229 amino acids. The Japanese quail *PRL* has an overall similarity with a comparable region of chicken (96.5%), turkey (93%), duck (93.4%), goose (93.4%), and ostrich (91.3%) *PRL* (Kansaku *et al.*, 2008).

The bone morphogenetic proteins (*BMPs*) belong to the transforming growth factor-β (TGF-β) superfamily and play a key role in ovarian physiology of domestic animals (Shimasaki *et al.*, 1999). In chickens, reproduction is characterized by egg production which is fully associated with ovulation rate. *BMPR-1B* is expressed in the granulosa and theca of chicken ovary, with the granulosa having higher mRNA levels in all follicles than the theca (Onagbesan *et al.*, 2003), implying an important role of chicken *BMPR-1B* in follicle maturation. However, the genetic effect of *BMPR-1B* on chicken ovulation and egg

production traits remains largely unknown.

Insulin- like growth factor 1 (*IGF-1*) is one of the most important candidate genes for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens (Zhou *et al.,* 2005). Insulin-like growth factors belong to the family of polypeptide hormones; they are structurally associated with insulin and also have a similar function (Keating, 2008).

The aim of the present study was to determine Prolactin (*PRL*), Bone Morphogenetic Protein Receptor 1B *(BMPR-1B)* and Insulin- like Growth Factor 1 *(IGF-1)* polymorphism in meat and egg selected lines of Japanese quail.

2. Material and Methods

This study was carried out at the experimental building belonging to Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University.

Experimental flock

One hundred and fifty day old birds of Japanese quail, egg and meat selected lines were used. Egg line was selected for six generations (high egg production for 120 days), while meat line was selected for four generations (high body weight at 4th week of age). Birds were obtained from the experimental unit belonging Faculty of Agriculture, Cairo University. Quails were housed at colony cages (65 x 50 x 29 cm). During the experiment, quails were fed with a diet consisting of 2900 k cal/ kg metabolic energy and 24% crude protein as *adlibitum*. Unlimited water was supplied during the experiment. Birds received standard requirements of lighting, ventilation, as well as vaccination program. A total of 50 quails weighed from each line periodically at hatch, 1st, 2nd, 3rd, 4th, 5th and 6th week of age.

Genomic DNA extraction

Blood samples were collected at 8th week of age from both selected lines birds in sterilized vacutainer tubes containing EDTA as anticoagulant and then stored at −20°C until extraction of DNA. Genomic DNA was extracted from whole blood using Gene JET whole blood genomic DNA purification mini kit (Fermentas) following the manufacturer protocol. The quality of the extracted DNA was evaluated by 0.7 % agarose gel electrophoresis and the quantity was measured by UV spectrophotometer taking optical density (OD) at 260 and 280 nm. The 260/280 nm absorbance ratio ranged from 1.7 to 1.9 indicating high quality DNA.

PCR amplification

The primers used for the amplification of the *PRL*, *BMPR-1B* were those described by Cui et al., (2006), Zhang et al., (2008), respectively. The primers designed by myself were used to amplify the target region of *IGF-1* genes. The corresponding sequences were 5′-TTT GCC AGA AGA GGG AGA GA-′3 (forward) and 5′-GCA GAA GCA GAC AAC ACA CA-′3 (reverse). PCR was carried out using T-professional thermal cycler (Biometra, Germany) and DreamTaq Green PCR Master Mix (ThermoScientific, fermentas). Each reaction mixture consisted of 12.5 µl of the master mix, 2 µl of the DNA template, 1 µl of each primer (10 pmol/µl) and some deionized water making up a final volume of 25 µl. Cycles applied were: 95°C for 5 min; followed by 40 cycles of 20 Sec at 95°C, 20 Sec at 56°C for *PRL* and *BMPR-1B*, 53°C for *IGF-1*, 30 Sec at 72°C; and a final extension of 5 min at 72°C. The PCR products were checked by agarose gel electrophoresis using 1.5% agarose gel in 1×TAE buffer and were visualized in gel documentation system under UV light by means of transilluminator.

DNA sequencing

PCR products were run in 1% agarose gel and the band of interest was purified with Gene JET PCR purification kit (Fermentas) according to the manufacturer's instructions. The purified DNA fragments were directly sequenced using both the forward and reverse primers of PCR amplification. The sequencing process was performed by European Custom Sequencing Centre (GATC Biotech AG, Germany). The obtained sequences were edited manually using Chromas Lite Ver. 2.01,(http://www.technelysium.com.au/chromas.html) and aligned with Clustal Omega software to identify nucleotide substitutions.

Statistical Analysis

Data were analyzed using SPSS/PCT system package (**Foster,** 2001). Least Squares Means (LSM) ± standard errors were calculated and tested for significance using "T" test (Steel & Torrie, 1960).

3. Results and Discussion

The number of confirmed causative genes associated with traits of biomedical, economic and evolutionary importance is still small and the list of identified candidate genes is limited. However, the candidate gene approach is useful for quickly determining the association of a specific genetic variant with traits of economic importance (Zhu & Zhao, 2007).

Table 1 summarized body weight measurements for egg and meat lines at different ages. Non significant differences were recorded at hatch and 1st week of age. However, from the 2nd week of age, meat line recorded significant superior measurements at 2nd, 3rd, 4th, 5th and 6th

week of age (45.15, 85.64, 118.90, 164.22 and 176.67 g; respectively), compared those of egg line (36.72, 63.00, 84.36, 122.11 and 143.55 g; respectively). Our findings confirmed those obtained previously (Giuseppe *et al.*, 2009). They recorded that, average weekly gain of meat type quails at 2nd, 3rd, 4th and 5th week of age (48.1, 51.9, 44.6 and 33.1g; respectively) were superior compared egg type (23.5, 27.8, 26.0 and 14.1 g; respectively).

Table 1 Least Square Means ± Standard Errors (LSM ± SE) of body weight measurements for egg and meat selected lines of Japanese quail at different ages.

Age	Egg line	Meat line	*P* value
Hatch	6.76 ± 0.35	7.87± 0.25	NS
1st week	22.24 ± 1.35	22.64 ± 1.96	NS
2nd week	36.72 ± 2.21	45.15 ± 2.83	*
3rd week	63.00 ± 4.61	85.64 ± 3.24	**
4th week	84.36 ± 8.61	118.90 ± 9.39	*
5th week	122.11 ± 4.06	164.22 ± 6.29	**
6th week	143.55 ± 7.06	176.67 ± 6.78	**

NS. Non Significant. * Significant at level ($p < 0.05$). ** Significant at level ($p < 0.01$).

Molecular characterizations of the *PRL* gene promoter in Japanese quail are rare, and most of reported data are limited to native or commercial chickens (Cui *et al.*, 2006; Emamgholi-Begli *et al.*, 2010; Alipanah *et al.*, 2011 and Rashidi *et al.*, 2012).

A DNA fragment of 439 bp was amplified in egg and meat selected line birds using the primer specifically designed for amplification of the chicken *PRL* gene (Cui *et al.*, 2006). A clear and distinct band of size 439 bp was obtained (Figure 1A). The amplified products were confirmed by nucleotide sequencing using the same set of primers. Sequence comparison of the *PRL* promoter region between egg and meat selected line birds specified the existence of one SNP at 407 (T/C) (Figure 1B). Cui *et al.*, (2005) identified seven polymorphisms (C-2402T, C-2161G, T-2101G, C-2062G, T-2054A, and G-2040A) from direct sequencing of the *PRL* promoter region in chicken.

The *PRL* gene has been cloned in a variety of avian species; including chicken, turkey, quail, duck, and pigeon (Liu *et al.*, 2008). The avian *PRL* gene consists of five exons and four introns (Li *et al.*, 2009; Yousefi *et al.*, 2012). Since the avian *PRL* gene was cloned and sequenced, most of the research has concentrated on identifying new polymorphic sites in this gene.

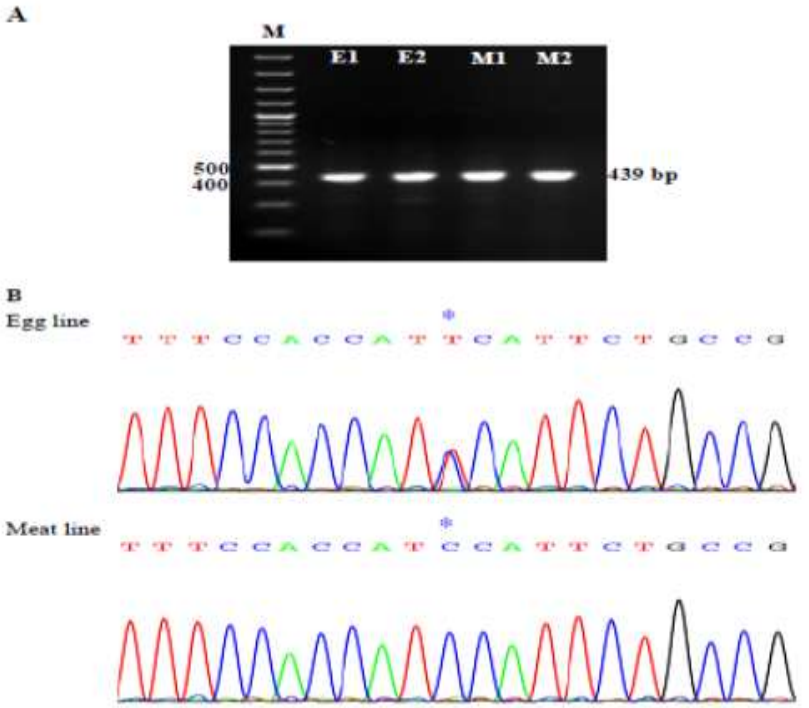

Figure 1 (A) PCR product of Prolactin promoter region in egg (E) and meat (M) selected line of Japanese quail. M: 100 bp plus ladder. (B) Sequencing analysis of the Prolactin promoter region in egg and meat selected line of Japanese quail. Asterisks (*) refer to site of base change.

The results of experiments on chickens showed a significant association between single

nucleotide polymorphisms (SNPs) in exon 2 and body weight at hatch, age at sexual maturity, and between SNPs in exon 5 and egg number (Rashidi *et al.,* 2012). It was found that the presence of a 24-bp insertion in the promoter region of the avian prolactin gene is positively correlated with the intensity of egg-laying activity in birds and broody behavior (Jing et al., 2009; Kulibaba & Podstreshnyi, 2012). The results of Jiang *et al.,* (2005) have shown that *PRL* could be a genetic marker in breeding against broodiness in chickens.

Most *PRL* gene polymorphisms were found in the 5'-flanking region, 3'-flanking region, and coding region of the signal peptide (Li *et al.* 2009). Furthermore, the 5'-flanking region of the avian prolactin gene has been considered as an excellent experimental model for studying both tissue specific and hormonally regulated activation of gene transcription.

Differences were found in the length of 5' promoter between mammals and birds. Compared to the extensive research on the promoter region of the mammalian *PRL* gene, information on the 5´ promoter of chicken *PRL* gene is rather limited (Liang *et al.,* 2006). Sequence variation in the 5'-flanking region of the prolactin gene may result in changes in transcription factor binding sites and contribute to the release of the hormone prolactin (Rashidi *et al.,* 2012). Experiments with birds and mammals provide evidence that PIT-1, CCAAT-enhancer binding protein, estrogen receptors and other proteins are crucial to the regulation of *PRL* gene expression (Rashidi *et al.,* 2012). However, the molecular mechanisms of the PIT-1 protein on *PRL* gene activation are not completely understood (Ohkubo *et al.,* 2000).

The length of the amplified product of *BMPR-1B* was 575 bp in the two selected line of Japanese quails (Figure 2). In contrast, a 581 bp of amplified product was found in both Zang and Jining Bairi chicken using the same set of primers (Zhang *et al.,* 2008). There were some deletions of nucleotides in the intronic region of quail *BMPR-1B* gene causing a decrease in the length of PCR product to 575 bp as compared to 581 bp of chicken. These deletions could be utilized as a maker for species differentiation/ identification.

The 575 bp of amplified fragment of quails *BMPR-1B* gene comprised of part of exon 6, complete intron 6, and partial of exon 7. Alignment of the nucleotide sequences in egg and meat selected lines revealed four nucleotide changes: A/G at 165, C/T at 211, A/T at 290, and A/G at 430 (Figure 3). Zhang *et al.,* (2008) identified five nucleotide differences (T/C at 35, C/T at 166, G/C at 224, A/G at 287, and G/A at 303) between Jining Bari and Zang chickens. Two of them, C35T and A287G, were confirmed and analyzed for their associations with egg production. The association results revealed no association of SNP C35T with egg production, while the SNP A287G was found to be significantly associated with egg production from 47 to 56 wks in a synthetic broiler line.

The *BMPR-1B* fragment obtained by us had a 93% (575/581) homology with those of chicken (Genbank accession No. EF530593.1). The *BMPR-1B* exon 6 and exon 7 sequences of chicken and japanase quail were identical except for the T/C site. At the base position of 35, it is replaced by A in the rat and by T in the mouse. The C/T substitution does not alter the amino acid that it encodes, and the difference among species may indicate codon preference.

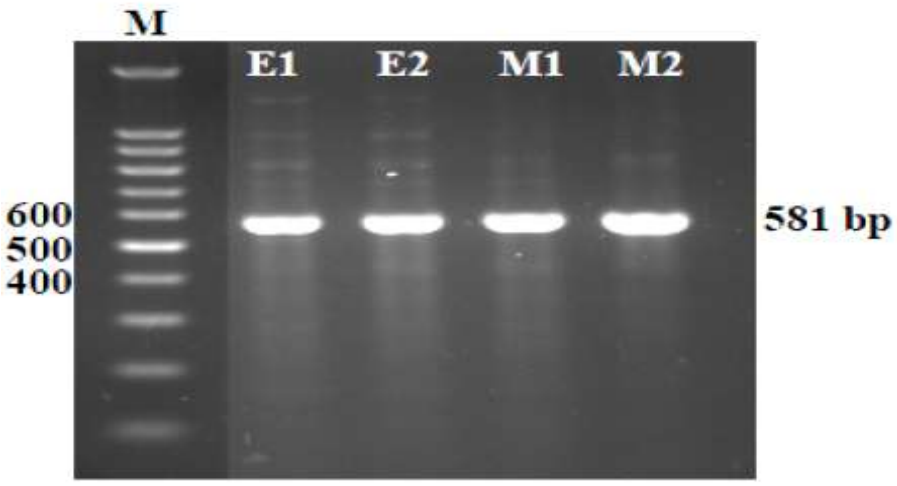

Figure 2 Amplification of *BMPR-1B* gene exon 6 to exon 7 in egg (E) and meat (M) selected lines of Japanese quail. M: 100 bp ladder.

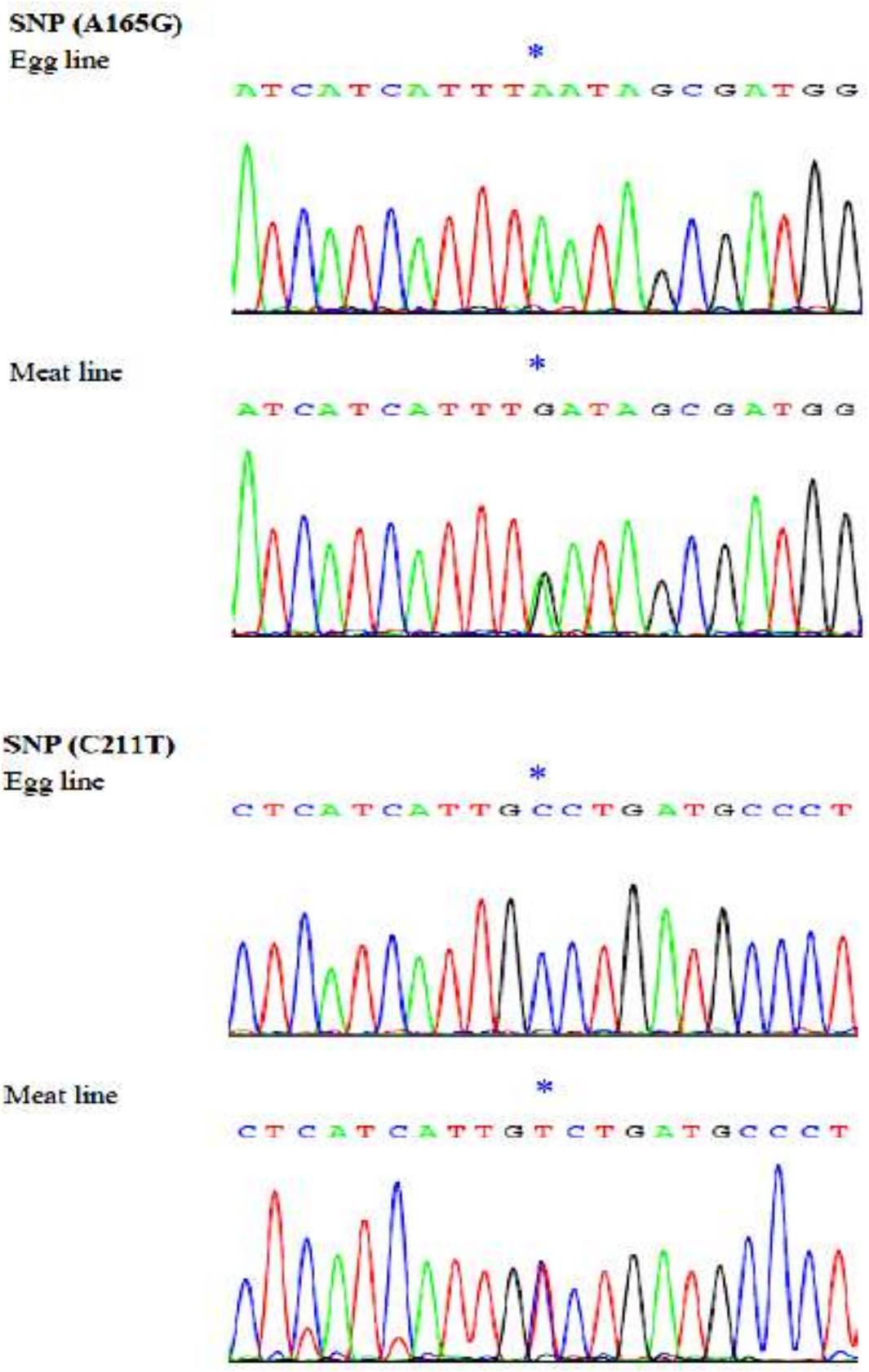

Figure 3 Sequencing analysis of the *BMPR-1B* gene in egg and meat selected line of Japanese quail. Asterisks (*) refer to site of base change.

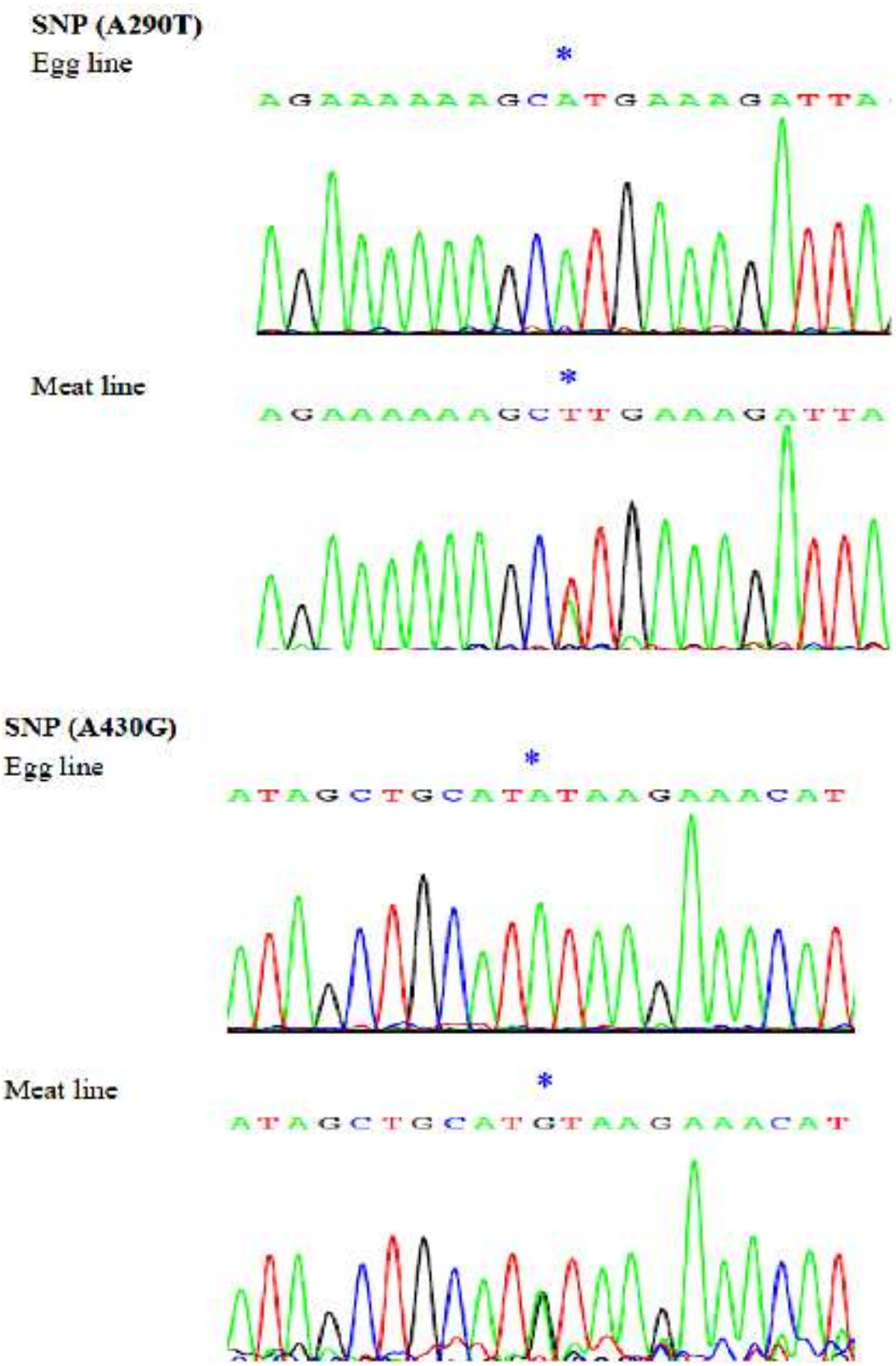

Figure 3 (*Continued*)

The BMP signaling, including *BMPR-1B*, is involved in chick diencephalic development, and the expression level of *BMPR-1B* decreases in the theca of chicken ovary from F1 to F3 follicles (Lim *et al.*, 2005). It also involved in follicular differentiation and maintenance of the follicular hierarchy. Therefore, the expression level or the activity of *BMPR-1B* in the granulosa and/or theca of chicken ovary may be associated with oocyte maturation (Onagbesan *et al.*, 2003).

The amplification product of the *IGF-1* exon1 was 418 bp in length (Figure 4A). PCR product of multiple individuals was sequenced separately. The obtained sequences were analyzed by chromas software (lit 2.01). Sequence comparison of the *IGF-1* coding region between egg and meat selected lines birds showed that there was no nucleotide difference in this region (Figure 4B). These results are in agreement with the study of Nikzad *et al.* (2012).

Several studies is done in the chicken *IGF-1* genes and mutations in different regions was detect, for example, Zhou *et al.* (2005) & Sato *et al.* (2012) in the promoter region, Hui-fang *et al.* (2009) in introns 2 & 5, and Bian et al., (2008) in 5'-flanking, exon 3 and 3'-flanking regions of *IGF*. *IGF-1* plays a key role in the development of muscle tissue and positively affects the growth of muscle (Duclos, 2005). *IGF* also regulates glucose, fat and muscle protein metabolism (Yun *et al.*, 2005).

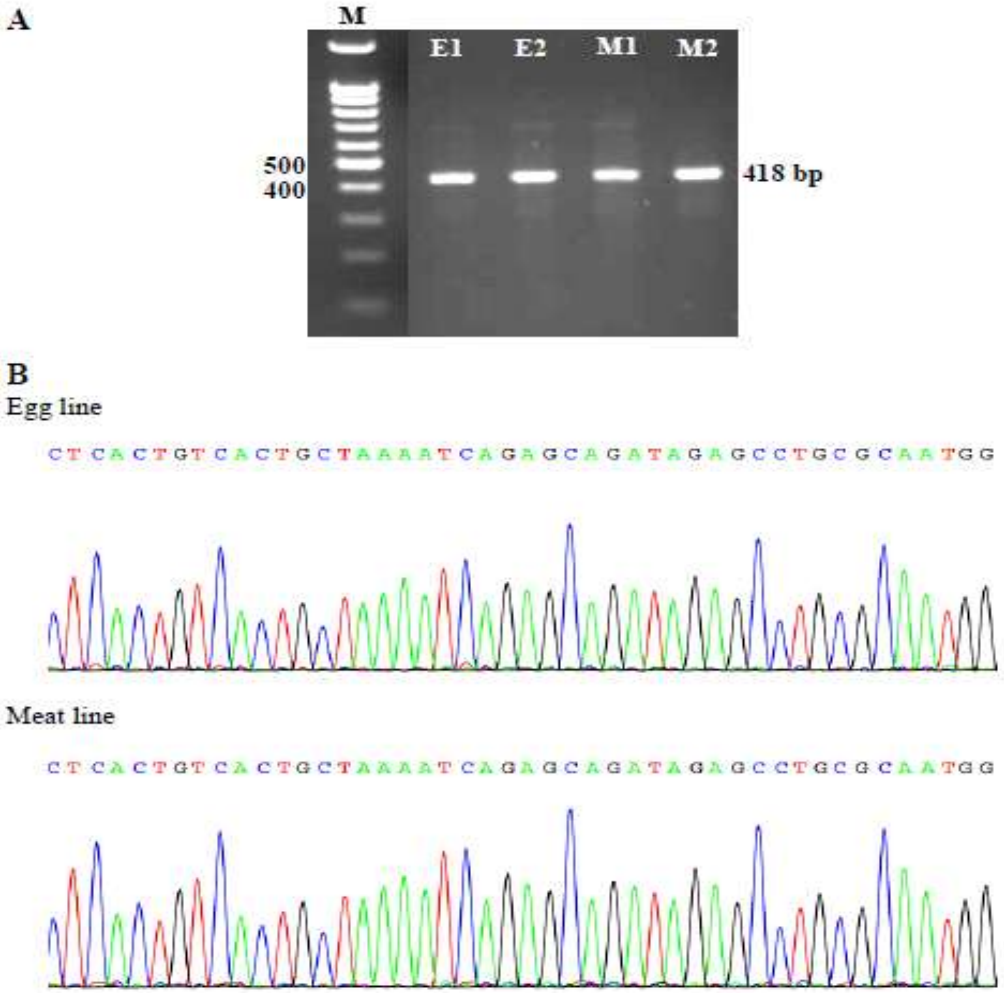

Figure 4 (A) Electrophoretic profile of *IGF-1* gene in egg (E) and meat (M) selected line of Japanese quail. M: 100 bp ladder. (B) Relative sequenced peaks in IGF-1 gene in egg and meat selected line of Japanese quail.

In birds, *IGF1R* is known to mediate two IGFs with different affinities. Therefore, the functional difference associated with the *IGF1R* SNP, together with the biological actions of other chromosomal SNPs with line specificity, may influence growth traits only in these selected lines. Thus, divergent lines should be valuable resource populations for further studies on the identification of candidate genes for these growth and metabolism traits (Moe *et al.*, 2007).

Conclusion

Meat selected line of Japanese quail recorded significant superior body weight measurements compared those of egg selected line. Single nucleotide polymorphisms were detected in *PRL* and *BMPR-1B*, but no difference in *IGF-1*. Consequently, further study was required to sequence other regions of *IGF-1*. The SNPs discovered in this study provided suitable markers for association studies of candidate genes with important economic traits in Japanese quail.

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