# Genetic effects of melatonin receptor genes on chicken reproductive traits

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**ABSTRACT**: The melatonin receptors are G protein-coupled receptors (GPCR) that bind melatonin. Three types of melatonin receptors have been cloned. The *MTNR1A* (or *Mel1A* or *MT1*) and *MTNR1B* (or *Mel1B* or *MT2*) receptor subtypes are present in humans and other mammals, while an additional melatonin receptor subtype *MTNR1C* (or *Mel1C* or *MT3*) has been identified in amphibians and birds. Previous research has shown that the three common melatonin receptors regulate physiological processes, including seasonal reproduction and ovarian physiology. However, whether or not any polymorphisms of the different melatonin receptor subtypes are associated with reproductive traits in chickens is not known. In this study, we performed candidate gene analysis to identify single-nucleotide polymorphisms (SNPs) in the *MTNR1A*, *MTNR1B*, and *MTNR1C* genes in the Erlang Mountain Chicken population. SNP discovery was achieved by sequencing pooled DNA samples. Direct PCR-sequencing, PCR-SSCP/PCR-sequencing, and PCR-RFLP method were used to genotype the *MTNR1A*, *MTNR1B*, and *MTNR1C* genes, respectively. The GLM Procedure was used to estimate the statistical significance of association between genotypes at each locus and reproductive traits of chickens. In a sample of 460 chickens, four novel polymorphisms (JQ249890:g.384T>C, JQ249891:g.387T>C, JQ249894:g.63C>T, and JQ249896:g.294G>A) were detected in the melatonin receptor genes *MTNR1A*, *MTNR1B*, and *MTNR1C*, respectively. A statistically significant association ($P < 0.01$) was found between two SNPs (*MTNR1A* SNP, *MTNR1C* SNP) and reproductive traits: egg number at 300 days of age (EN) and age at first egg (AFE).

**Keywords**: *MTNR1A; MTNR1B*; *MTNR1C;* polymorphism; egg production traits

Based on phenotypic differences, more than 81 distinctive breeds of native chickens raised under extensive and/or intensive breeding systems have been recognized in China (Huifang et al., 2005). Meat from native chickens is more expensive in China, because it is more flavourful and contains less fat. Native chicken eggs are also more expensive than the commercial ones. The poultry industry regards growth and reproduction as the two most economically valued characteristics. The endocrine factor (Krishnan et al., 1993), environment factors such as lighting programs (wavelength, intensity, and duration) (Olanrewaju et al., 2006), and different feeding allowances could influence growth and reproduction (Liu et al., 2004). Nevertheless, the genetic factor is the prerequisite. Egg production is a polygenic inheritance trait with low to moderate heritability, which depends on the period involved (Acharya et al., 1969; Luo et al., 2007). Moreover, production and fitness traits are negatively correlated (Pinard-van der Laan et al., 1998). Multitraits selection to improve fitness and simultaneously increase egg yield is therefore difficult to accomplish by traditional, direct phenotypic selection. Thus selecting individuals with additional information on their genotype

Supported by the National Modern Technology System on Layer Chicken Industry (Project No. CARS-41).

for markers associated with QTLs for fitness and reproduction (marker-assisted selection, MAS) is preferred (Deeb and Lamont, 2002). Molecular markers were used to map QTLs related to chicken growth and reproduction such as body weight (BW), egg number at 300 days of age (EN), and age at first egg (AFE) in the past decade (Xu et al., 2011a, b).

Melatonin (*N*-acetyl-5-methoxytryptamine), an indole hormone, is synthesized from serotonin in the pineal gland and other extra-pineal tissues and regulates various biological functions through three different receptor subtypes – *MTNR1A*, *MTNR1B*, and *MTNR1C* (Sundaresan et al., 2009; Li et al., 2011a). In mammals, melatonin influences reproduction by activating receptor sites within the hypothalamic-pituitary-gonadal axis (Malpaux et al., 2001). In birds, melatonin also regulates circadian rhythm, hibernation, feeding pattern, thermoregulation, and neuroendocrine functions (Courtillot et al., 2010). Melatonin is found in ovarian follicular fluid (Rönnberg et al., 1990), suggesting a direct effect of this hormone on ovarian function. The effects of melatonin on ovarian function vary with tissue structure, cell type, and with the fact whether the species is a seasonal or non-seasonal breeder (Soares et al., 2003). Two high-affinity melatonin receptor types, *MTNR1A* and *MTNR1B*, have been cloned in humans, sheep, Siberian hamsters, mice, and rats (Nishiyama et al., 2009) and found to exhibit different molecular structures and chromosomal locations among these species. An additional receptor subtype, *MTNR1C* (*Mel1C*), has been identified in amphibians and birds but not in mammals (Ebisawa et al., 1994).

Melatonin binding sites were identified in the ovaries of birds, suggesting a possible role of melatonin in various ovarian functions (Poon and Pang, 1994). All three subtypes of melatonin receptors exhibit nearly identical pharmacological profiles and have been identified in neural tissues of chickens (Natesan and Cassone, 2002). The ovarian *MTNR1A*, *MTNR1B*, and *MTNR1C* transcripts are equivalent to the brain receptors recently characterized in chickens and their expression suggests a direct influence of melatonin on female reproductive processes of domestic chickens (Sundaresan et al., 2009). While the *MTNR1A*, *MTNR1B*, and *MTNR1C* genes are potential candidate genes for QTLs, no studies of their association with reproduction in chickens have been reported. In this paper, the association of the three melatonin receptor genes with reproductive traits in chickens was explored.

## MATERIAL AND METHODS

### Population and phenotypic traits

The Erlang Mountain Chicken is a cultivated breed, that was successfully developed from local chicken breeds in Sichuan province, P.R. China (Xiao et al., 2011; Li et al., 2011b). According to the feather colour, we defined two different strains of this breed. Four hundred sixty Erlang Mountain Chickens from one hatch were raised in cages under the same conditions and diet, and six reproductive traits were measured: body weight at first egg (BWAFE), weight of first egg (WFE), age at first egg (AFE), number of eggs at 300 days of age (EN), body weight at 300 days of age (BWTA), and egg weight at 300 days of age (EWTA). These experiments were conducted in accordance with the Law of the People's Republic of China on Animal Protection.

### SNP discovery and genotyping

Genomic DNA was phenol-extracted from blood samples following standard procedures (Sambrook and Russell, 2001). A DNA pool containing 100 ng DNA from each of the least closely related 60 chickens (30 birds from two different strains) was constructed. Polymerase chain reactions containing the first four pairs of primers listed in Table 1 were performed that included 2 µl of pooled DNA, 12.5µl 2×*Taq* PCR MasterMix (TianGen Biochemical Technology Co. Ltd., Shanghai, P.R. China), 1.25 µl (10 pmol/µl) of each primer, and $ddH_2O$ to 25 µl. PCR conditions were as follows: 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing from 49 to 63°C for 45 s, 72°C for 50 s, and a final extension at 72°C for 5 min. SNPs were identified by looking for multiple peaks at the same base pair by direct sequencing. We may have missed the low frequency SNPs, some of which could have significant effects on reproductive traits.

Three pairs of primers (synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd., Shanghai, P.R. China) that flank each SNP discovered were designed using Primer Premier 5.0 software based on the complete DNA

Table 1. Primers and sequencing information

| Gene | Primer | Primer sequences (5'→3') | Annealing temperature (°C) | Length (bp) | Method |
|---|---|---|---|---|---|
| *MTNR1A* exon 1 | MT1P1S<br>MT1P1AS | ACACTTCGGGGGAAACTT<br>CAACCCAATCAGAACAGCA | 54 | 680 | Pooled DNA sequencing |
| *MTNR1A* exon 2 | MT1P2S<br>MT1P2AS | GAAGTAATGGAAGAGAGCAAATGAG<br>TGAAACAGATTCTTTATTTGGATGC | 59.5 | 984 | Pooled DNA sequencing |
| *MTNR1B* exon 2 | MT2P2S<br>MT2P2AS | ATTGTTTCCCCTACAGACCCTATTG<br>AAGGTCCCCCAGAGTAAGCAAGAAT | 63 | 1121 | Pooled DNA sequencing |
| *MTNR1C* exon 2 | MT3P2S<br>MT3P2AS | CAAATGGTAGGTGGAGTGAAGG<br>TAGGCACATGGTGAAAGATGGTAT | 59 | 1229 | Pooled DNA sequencing |
| *MTNR1A* SNP | MT1snpS<br>MT1snpAS | GGGCAACCTCCTGGTCATC<br>GCAACCCAATCAGAACAGCA | 59.5 | 386 | DNA sequencing |
| *MTNR1B* SNP | MT2snpS<br>MT2snpAS | TTGCCATCACCAATACCTTA<br>CATTTCACCCAAAGTCCATC | 54.5 | 227 | PCR-SSCP genotyping |
| *MTNR1C* SNP | MT3snpS<br>MT3snpAS | GGTGTATCCGTATCCTCTAA<br>GACAGTGGGACAATGAAGT | 49 | 372 | PCR-RFLP genotyping |

sequence of *Gallus gallus* melatonin receptor genes (http://uswest.ensembl.org/index.html). These primers, the last three pairs listed in Table 1, were used to amplify fragments containing each SNP in each of the 460 chickens. Directed DNA sequencing of the *MTNR1A* SNP was performed by using ABI 3730 automated sequencer (Applied Biosystems, Carlsbad, USA). PCR-SSCP analysis of the *MTNR1B* SNP was performed according to Orita et al. (1989). An *Mbo*I recognition site was created by the *MTNR1C* SNP. The PCR products of this locus were digested with the restriction enzyme *Mbo*I (BBI Enzymes (UK) Ltd., Pontypool, UK) at 37°C for 16 h, ran on a 2% agarose gel, and stained with ethidium bromide (Ota et al., 2007) to detect the SNP by cleavage of the *MTNR1C* amplicon. All PCR products were purified with an EZ Spin Column DNA Gel Extraction Kit (Shanghai Sangon Biological Engineering Technology & Services Co. Ltd., Shanghai, P.R. China), sequenced in both directions on an ABI 3730 automated sequencer (Applied Biosystems, Carlsbad, USA), and the sequences were deposited in GenBank (accession numbers JQ249890–JQ249896). The primers used to amplify the fragments were also used for sequencing.

### Traits and statistical analysis

Associations between genotype and reproductive traits were assessed using the least-squares method (GLM Procedure, Statistical Analysis System, Version 8.02, 2001). The model used to analyze the data was assumed to follow:

$$Y_{ij} = \mu + G_i + S_j + (G \times S)_{ij} + e_{ij}$$

where:

$Y_{ij}$ = trait measured in the chickens
$\mu$ = population means of the trait
$G_i$ = fixed effect associated with the genotype
$S_j$ = fixed effect of strain
$G \times S$ = interaction effects of genotype and strain
$e_{ij}$ = residual error

Significance of the least squares means was tested with the Duncan's Multiple Range test. Pearson's chi-square test was used to check for Hardy-Weinberg equilibrium of the four SNPs discovered in the sample population. The linkage disequilibria $D'$ value and $r^2$ of the SNPs were estimated by Haploview (Barrett et al., 2005).

## RESULTS

### Identified SNPs, allele and genotype frequencies

Four SNPs were identified in the sequences of the pooled DNA sample and included as independent variables ($G$) in the regression analysis. Three genotypes were found in all three melatonin receptor genes (Figure 1) by the direct sequencing, PCR-SSCP

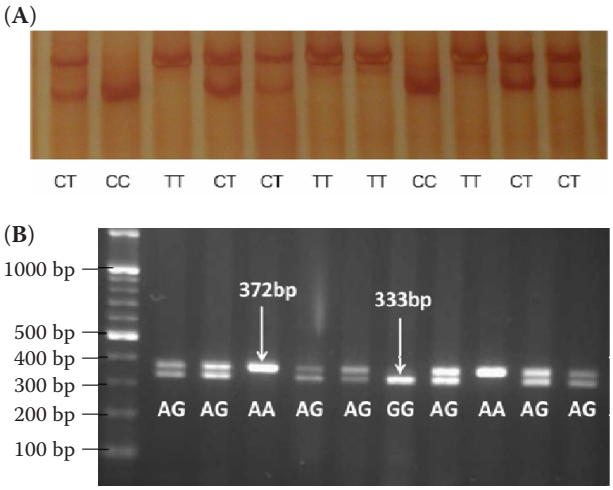


Figure 1. (**A**) PCR-SSCP patterns of *MTNR1B* SNP with three detected genotypes (*CC*, *CT*, and *TT*); (**B**) PCR-RFLP patterns of *MTNR1C* SNP, three genotypes (*AA*, *AG*, and *GG*) were observed

or PCR-RFLP genotyping methods. While two SNPs (JQ249890:g.384T>C, JQ249891:g.387T>C) were identified in the *MTNR1A* gene, they were completely linked ($D'$ = 1.0 and $r^2$ = 1.0), providing only three possible haplotypes (*EE*, *EF*, and *FF*). If the two loci are far apart on the same chromosome or even on different chromosomes, LD should be quite low (Schrider and Hahn, 2010). Low levels of linkage disequilibrium ($D'$ = 0.028 and $r^2$ = 0.001) were observed between the *MTNR1A* and *MTNR1C* genes in this study. *MTNR1A* and *MTNR1C* were also assessed using individual SNPs based on genotypes. These SNPs, their corresponding allele and genotype frequencies, and *P*-values for the Hardy-Weinberg equilibrium test are presented in Table 2. The *MTNR1A* and *MTNR1C* SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$), but the *MTNR1B* genotypes exhibited a statistically significant excess of heterozygous genotypes. PCR-SSCP patterns of *MTNR1B* SNP and PCR-RFLP patterns of *MTNR1C* SNP are shown in Figure 1.

## Association of variations in the two candidate genes with reproductive traits

Effects of the SNPs on reproductive traits are shown in Table 3. Data are presented as LSM ± SEM. The JQ249894:g.63C>T SNP of the *MTNR1B* gene was statistically significantly associated with WFE, and both the *MTNR1A* and *MTNR1C* SNPs were statistically significantly associated with AFE and EN. Birds with the *TT* genotype for the JQ249894:g.63C>T SNP of *MTNR1B* exhibited greater WFE than those of the *CC* and *CT* genotypes ($P < 0.01$). Birds with the *FF* genotype for the *MTNR1A* SNP and the *AG* genotype for the *MTNR1C* SNP had shorter AFE than those of the *EE* and *EF* genotypes ($P < 0.01$). While birds with the *AA* genotype for the *MTNR1C* SNP, lacking the *MTNR1C Mbo*I restriction site, exhibited statistically significantly higher WFE ($P < 0.05$), they exhibited statistically significantly lower EN values ($P < 0.01$) than those with both the *GG* and *AG* genotypes that were homozygous and heterozygous for the restriction site, respectively. No statistically significant association was found between the JQ249894:g.63C>T allele and BWFE, AFE, EN, BWTA or EWTA ($P > 0.05$) and between the JQ249896:G.294G>A allele and BWFE or EWTA ($P > 0.05$) (Table 3).

Table 2. Frequency of alleles and genotypes and the Hardy-Weinberg (H-W) equilibrium test

| Gene | SNP | Genotype | No. of animals | Frequency | Allele | Frequency | H-W test, *P* |
|---|---|---|---|---|---|---|---|
| | chromosome 4 | *EE* | 100 | 0.23 | *E* | 0.45 | 0.32 |
| *MTNR1A* | JQ249890:g.384T>C | *EF* | 217 | 0.47 | *F* | 0.55 | |
| | JQ249891:g.387T>C | *FF* | 143 | 0.31 | | | |
| | chromosome 1 | *CC* | 47 | 0.10 | *C* | 0.33 | 0.64 |
| *MTNR1B* | JQ249894:g.63C>T | *CT* | 207 | 0.45 | *T* | 0.67 | |
| | | *TT* | 206 | 0.45 | | | |
| | chromosome 4 | *AA* | 79 | 0.17 | *A* | 0.49 | < 0.01 |
| *MTNR1C* | JQ249896:g.294G>A | *AG* | 294 | 0.64 | *G* | 0.51 | |
| | | *GG* | 87 | 0.19 | | | |

Table 3. Least squares means and standard errors for the effects of the melatonin receptor genotypes on reproductive traits

| Gene | Genotype | BWFE (g) | AFE (days) | WFE (g) | EN (count) | BWTA (g) | EWTA (g) |
|---|---|---|---|---|---|---|---|
| | *EE* | 2457.60 ± 30.75$^{Ba}$ | 163.740 ± 1.18$^{Aa}$ | 42.78 ± 0.79$^{a}$ | 85.06 ± 2.06$^{C}$ | 2807.80 ± 33.75 | 58.54 ± 0.44 |
| *MTNR1*A | *EF* | 2508.71 ± 20.90$^{Bb}$ | 160.730 ± 0.80$^{Ab}$ | 40.32 ± 0.54$^{b}$ | 94.27 ± 1.40$^{B}$ | 2811.54 ± 22.94 | 59.09 ± 0.30 |
| | *FF* | 2614.17 ± 26.23$^{A}$ | 154.670 ± 1.00$^{B}$ | 40.66 ± 0.68$^{b}$ | 101.65 ± 1.75$^{A}$ | 2738.03 ± 28.78 | 58.70 ± 0.38 |
| | *CC* | 2518.89 ± 44.86 | 161.210 ± 1.72 | 38.55 ± 1.14$^{Ba}$ | 95.020 ± 3.03 | 2831.49 ± 48.66 | 59.03 ± 0.64 |
| *MTNR1B* | *CT* | 2555.92 ± 21.57 | 159.082 ± 0.83 | 39.77 ± 0.55$^{Bb}$ | 94.145 ± 1.46 | 2804.83 ± 23.39 | 58.87 ± 0.31 |
| | *TT* | 2518.89 ± 22.09 | 159.558 ± 0.85 | 42.70 ± 0.56$^{A}$ | 94.835 ± 1.49 | 2761.24 ± 23.96 | 58.79 ± 0.31 |
| | *AA* | 2494.76 ± 34.62 | 162.456 ± 1.32$^{a}$ | 43.162 ± 0.88$^{a}$ | 80.630 ± 2.21$^{B}$ | 2865.44 ± 37.06$^{a}$ | 58.84 ± 0.49 |
| *MTNR1C* | *AG* | 2528.42 ± 17.93 | 158.255 ± 0.68$^{b}$ | 40.38 ± 0.46$^{b}$ | 98.67 ± 1.15$^{Aa}$ | 2746.89 ± 19.20$^{b}$ | 58.59 ± 0.25 |
| | *GG* | 2568.15 ± 34.52 | 161.092 ± 1.32$^{ab}$ | 40.92 ± 0.88$^{b}$ | 93.22 ± 2.21$^{Ab}$ | 2856.78 ± 36.95$^{a}$ | 59.74 ± 0.49 |

BWFE = body weight at first egg, AFE = age at first egg, WFE = weight at first egg, EN = total number of eggs at 300 days of age, BWTA = body weight at 300 days of age, EWTA = egg weight at 300 days of age

Least squares means in a column with different superscripts are significantly ($^{a,b}P < 0.05$) or highly significantly ($^{A,B}P < 0.01$) different

## DISCUSSION

Egg production is an important economic trait in the poultry industry (Xu et al., 2011b). Our findings confirm the influence of melatonin receptors on female reproductive traits (WFE, AFE, and EN) of a Chinese domestic chicken breed. In poultry breeding programs, egg number at 300 days of age (EN) is used as the most valuable indicator of total egg production potential. Recently, many researchers have sought correlations between markers of candidate genes and reproductive traits in chickens (Xu et al., 2011a). The *MTNR1A* and *MTNR1C* genes studied in this paper are located on chromosome 4 to which highly significant QTL effects on production traits have been mapped in previous studies (Tuiskula-Haavisto et al., 2002). Chickens with the *AG* genotype at *MTNR1C* and *FF* at *MTNR1A* produced their first eggs earlier (but, perhaps consequentially, eggs of lower weight) and produced more eggs at 300 days of age than chickens with other genotypes. Interestingly, the expression of the *MTNR1A* (*Mel-1a*) receptor has not been found in the granulosa layer of the chickens' ovarian follicles. However, the *MTNR1B* (*Mel-1b*) and *MTNR1C* (*mel-1c*) receptors are known to be expressed at significantly higher levels in the granulosa layer (Sundaresan et al., 2009).

The search for molecular markers that influence reproductive traits of chickens has been well reported (Zhou et al., 2010). Number of eggs at 300 days of age (EN) and age at first egg (AFE) are the most economically important reproductive traits and much effort has been put into improving them (Kang et al., 2011; Xu et al., 2011a, b). Dunn et al. (2004) identified polymorphisms in the *GnRHR* (gonadotropin-releasing hormone receptor) and *NPY* (neuropeptide Y) genes but discovered no association of the two polymorphisms with total egg production (Dunn et al., 2004). Cui et al. (2006) reported a 24-bp indel at the site of −358 of the *PRL* gene that was associated with egg production (Cui et al., 2006). In the present study, a statistically significant association of the JQ249896:g.294G>A, JQ249890:g.384T>C, and JQ249891:g.387T>C SNPs with EN was found, demonstrating that animals with the *FF* and *AG* genotypes produce their first egg at a lower age (AFE) and produce more eggs at 300 days of age (EN) than those with the *EE*, *EF*, *AA*, and *GG* genotypes ($P < 0.01$). This apparent production advantage to the *AG* genotype at *MTNR1C* having a lower AFE and higher EN is consistent with the excess of heterozygous genotypes at this locus compared to equilibrium expectations, illustrated in Table 2, suggesting strong balancing selection (overdominance). In contrast, the effect of allele *T* on EN is additive and may reflect the influence of positive selection. Another significant production trait, age at first egg (AFE), an important indicator for sexual maturation in female chickens, is controlled by polygenes (Xu et al., 2011a). Previous studies have found that the *GnRH-I* gene (Xu et al., 2011a), signal transducers and activators of transcription 5B *(STAT5B)* gene (Ou et al., 2009), vasoactive intestinal polypeptide receptor-1 (*VIPR-1*)

gene (Zhou et al., 2008a, b), follicle-stimulating hormone receptor *(FSHR)* gene (Kang et al., 2011), and the exon 9 of mitochondrial phosphoenolpyruate carboxykinase gene (Torkamanzehi and Kuhnlein, 2007) significantly affects AFE or broodiness. In the current study we have shown that JQ249890:g.384T>C, JQ249891:g.387T>C in the *MTNR1A* gene, and JQ249896:g.294G>A in the *MTNR1C* gene are associated with AFE in the Erlang Mountain Chickens. It was shown in this study that the lower the body size at the onset of lay and the earlier the age at first egg, the greater the effect on the total number of eggs at 300 days of age. Therefore, it appears that the *MTNR1A* and *MTNR1C* genes are significantly associated with both AFE and EN in chickens. These results provide some insight into the genetics of chicken production and possible markers for selective breeding.

## Acknowledgement

Our thanks are due to Ted Doherty for revising the manuscript.

## REFERENCES

Acharya R., Dhillon J., Tiwana M. (1969): Age at first egg and egg production – their inheritance and expected response to different methods of selection. British Poultry Science, 10, 175–181.

Barrett J., Fry B., Maller J., Daly M. (2005): Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics, 21, 263.

Courtillot C., Chakhtoura Z., Bogorad R., Genestie C., Bernichtein S., Badachi Y., Janaud G., Akakpo J.P., Bachelot A., Kuttenn F., Goffin V., Touraine P. (2010): Characterization of two constitutively active prolactin receptor variants in a cohort of 95 women with multiple breast fibroadenomas. The Journal of Clinical Endocrinology and Metabolism, 95, 271–279.

Cui J., Du H., Liang Y., Deng X., Li N., Zhang X. (2006): Association of polymorphisms in the promoter region of chicken prolactin with egg production. Poultry Science, 85, 26–31.

Deeb N., Lamont S. (2002): Genetic architecture of growth and body composition in unique chicken populations. Journal of Heredity, 93, 107.

Dunn I., Miao Y., Morris A., Romanov M., Wilson P., Waddington D. (2004): A study of association between genetic markers in candidate genes and reproductive traits in one generation of a commercial broiler breeder hen population. Heredity, 92, 128–134.

Ebisawa T., Karne S., Lerner M.R., Reppert S.M. (1994): Expression cloning of a high-affinity melatonin receptor from Xenopus dermal melanophores. Proceedings of the National Academy of Sciences of the United States of America, 91, 6133.

Huifang L., Shuangjie Z., Kuanwei C., Qingping T., Yushi G., Guohong C. (2005): Analysis of genetic diversity among domestic concernful chicken breeds in China. Journal of China Aricultural University, 10, 21–24.

Kang L., Zhang N., Zhang Y., Yan H., Tang H., Yang C., Wang H., Jiang Y. (2011): Molecular characterization and identification of a novel polymorphism of 200 bp indel associated with age at first egg of the promoter region in chicken follicle-stimulating hormone receptor (*FSHR*) gene. Molecular Biology Reports, 39, 2967–2973, doi: 10.1007/s11033-011-1058-x.

Krishnan K., Proudman J., Bolt D., Bahr J. (1993): Development of a homologous radioimmunoassay for chicken follicle-stimulating hormone and measurement of plasma FSH during the ovulatory cycle. Comparative Biochemistry and Physiology, Part A: Physiology, 105, 729–734.

Li D.Y., Li Q.Q., Zhao X.L., Xu H.L., Zhao B.Y., Zhu Q. (2011a): Research progress on melatonin receptor in poultry. Energy Procedia, 11, 2252–2257.

Li D.Y., Zhao B.Y., Zhao X.L., Xu H.L., Li Q.Q., Wu Y.Q., Zhu Q. (2011b): Bioinformatic analysis and characteristics of *Mel1a* gene product from chicken. Energy Procedia, 11, 2246–2251.

Liu H., Lilburn M., Koyyeri B., Anderson J., Bacon W. (2004): Preovulatory surge patterns of luteinizing hormone, progesterone, and estradiol-17beta in broiler breeder hens fed *ad libitum* or restricted fed. Poultry Science, 83, 823–829.

Luo P., Yang R., Yang N. (2007): Estimation of genetic parameters for cumulative egg numbers in a broiler dam line by using a random regression model. Poultry Science, 86, 30–36.

Malpaux B., Migaud M., Tricoire H., Chemineau P. (2001): Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. Journal of Biological Rhythms, 16, 336.

Natesan A.K., Cassone V.M. (2002): Melatonin receptor mRNA localization and rhythmicity in the retina of the domestic chick, *Gallus domesticus*. Visual Neuroscience, 19, 265–274.

Nishiyama K., Shintani Y., Hirai K., Yoshikubo S. (2009): Molecular cloning and pharmacological characterization of monkey *MT1* and *MT2* melatonin receptors showing high affinity for the agonist ramelteon. Journal of Pharmacology and Experimental Therapeutics, 330, 855–863.

Olanrewaju H., Thaxton J., Dozier W., Purswe J., Roush W., Branton S. (2006): A review of lighting programs for broiler production. International Journal of Poultry Science, 5, 301–308.

Orita M., Suzuki Y., Sekiya T., Hayashi K. (1989): Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics, 5, 874–879.

Ota M., Fukushima H., Kulski J.K., Inoko H. (2007): Single nucleotide polymorphism detection by polymerase chain reaction-restriction fragment length polymorphism. Nature Protocols, 2, 2857–2864.

Ou J.T., Tang S.Q., Sun D.X., Zhang Y. (2009): Polymorphisms of three neuroendocrine-correlated genes associated with growth and reproductive traits in the chicken. Poultry Science, 88, 722–727.

Pinard-van der Laan M.H., Siegel P.B., Lamont S.J. (1998): Lessons from selection experiments on immune response in the chicken. Poultry and Avian Biology Reviews, 9, 125–141.

Poon A.M., Pang S.F. (1994): Differential effects of guanosine 5'-O-(3-thiotriphosphate) (GTP gamma S) on the 2-[125I]iodomelatonin binding sites in the chicken bursa of Fabricius and spleen. Neuroscience Letters, 173, 167–171.

Rönnberg L., Kauppila A., Leppäluoto J., Martikainen H., Vakkuri O. (1990): Circadian and seasonal variation in human preovulatory follicular fluid melatonin concentration. The Journal of Clinical Endocrinology and Metabolism, 71, 492.

Sambrook J., Russell D.W. (2001): Molecular cloning: a laboratory manual. 3rd Ed. Cold Spring Harbor Laboratory Press, New York, USA.

Schrider D.R., Hahn M.W. (2010): Lower linkage disequilibrium at CNVs is due to both recurrent mutation and transposing duplications. Molecular Biology and Evolution, 27, 103–111.

Soares J.M., Masana M.I., Erşahin Ç., Dubocovich M.L. (2003): Functional melatonin receptors in rat ovaries at various stages of the estrous cycle. Journal of Pharmacology and Experimental Therapeutics, 306, 694.

Sundaresan N.R., Marcus Leo M.D., Subramani J., Anish D., Sudhagar M., Ahmed K.A., Saxena M., Tyagi J.S., Sastry K.V., Saxena V.K. (2009): Expression analysis of melatonin receptor subtypes in the ovary of domestic chicken. Veterinary Research Communications, 33, 49–56.

Torkamanzehi A., Kuhnlein U. (2007): Restriction fragment length and single strand conformational polymorphisms in chicken mitochondrial phosphoenol-pyruate carboxykinase gene and its association with egg production. Pakistan Journal of Biological Sciences, 10, 4075–4080.

Tuiskula-Haavisto M., Honkatukia M., Vilkki J., De Koning D., Schulman N., Maki-Tanila A. (2002): Mapping of quantitative trait loci affecting quality and production traits in egg layers. Poultry Science, 81, 919–927.

Xiao L.H., Chen S.Y., Zhao X.L., Zhu Q., Liu Y.P. (2011): Association of cellular retinol-binding protein 2 (*CRBP2*) gene polymorphism with egg production in Erlang mountainous chicken. The Journal of Poultry Science, 48, 162–167.

Xu H., Zeng H., Luo C., Zhang D., Wang Q., Sun L., Yang L., Zhou M., Nie Q., Zhang X. (2011a): Genetic effects of polymorphisms in candidate genes and the QTL region on chicken age at first egg. BMC Genetics, 12, 33.

Xu H.P., Zeng H., Zhang D.X., Jia X.L., Luo C.L., Fang M.X., Nie Q.H., Zhang X.Q. (2011b): Polymorphisms associated with egg number at 300 days of age in chickens. Genetics and Molecular Research, 10, 2279–2289.

Zhou M., Lei M., Rao Y., Nie Q., Zeng H., Xia M., Liang F., Zhang D., Zhang X. (2008a): Polymorphisms of vasoactive intestinal peptide receptor-1 gene and their genetic effects on broodiness in chickens. Poultry Science, 87, 893–903.

Zhou M., Liang F., Rao Y., Zeng H., Zhang D., Zhang X. (2008b): Association of twelve polymorphisms of the *VIPR-1* gene with chicken early egg production traits. Chinese Journal of Animal and Veterinary Sciences, 39, 1147–1152.

Zhou M., Du Y., Nie Q., Liang Y., Luo C., Zeng H., Zhang D.X. (2010): Associations between polymorphisms in the chicken *VIP* gene, egg production and broody traits. British Poultry Science, 51, 195–203.

Received: 2012–02–28
Accepted after corrections: 2012–08–27

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