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ORIGINAL ARTICLE

Association of Polymorphisms in the *Cryptochrome-1* gene (*turCry1*) with Growth and Reproductive Traits in Turkeys, *Meleagris gallopavo*

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Abstract

Circadian clock genes including Cryptochrome-1 are involved in the molecular mechanism of the biological clock which controls the behavioral, physiological and biochemical circadian rhythms of animals. The turkey Cryptochrome1 (turCry1) gene for DNA sequence variations was screened and evaluated the relationships among haplogroups with the performance traits. DNA sequences of turCry1 (15.0 kb) gene were screened using a total of 290 turkey birds by sequencing of the individual amplicons. In the turCry1 gene, seven SNPs including four and three in the introns 3 and 6 were identified, respectively. The D´ among SNPs ranged from 0.48 to 0.89. The pairwise F_{ST} ranged from 0.004 to 0.67 among turkey birds. Haplotypes observed from the 7 SNPs were grouped into seven haplogroups with the frequencies ranging from 0.02 to 0.96, were significantly associated with average daily gain (ADG) for the period of 35 - 68 days, feed conversion ratio (FCR) for the periods of 160 - 231 days and 34 - 231 days, egg production and ejaculate volume ($p \leq 0.05$). DNA sequence variations of the turCry1 gene may have some regulatory role in the molecular mechanism of the circadian clock which may affect the overall mechanism of the circadian clock. The turCry1 is a good candidate gene for association studies in turkey. In general, these data support our hypothesis that DNA sequence variations of clock genes at the nucleotide and haplotype levels are associated with the differences in performance traits. In conclusion, the reported genomic information in the present study would be valuable for future genotype-phenotype evaluation studies between turCry1gene and other traits in the turkey using candidate gene approach.

Keywords: *Cryptochrome-1 gene, Nucleotide polymorphism and Association, Growth and Reproductive traits*

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1. Introduction

The biological clock controls the wide variety of activities including behavioral, physiological and biochemical circadian rhythms. The molecular components of the circadian clock have been identified in diverse animal species (Bailey et al. 2002). The transcriptional and translational feedback loop characterizes the basic molecular mechanism of the circadian clock (Helfer et al. 2006). Several genes including *Clock, Bmal1, Period1* (*Per1*)*, Period2 (Per2*)*, Period3* (*Per3*)*, Cryptochrome1* (*Cry1*) and *Cryptochrome2* (*Cry2*) are involved in the molecular mechanism of the circadian clock (Froy 2007).

The *Cry1* gene was first identified in *Arabidopsis*. Then, it was discovered in other plants and various animal species including insects, fish, amphibians, birds and mammals (Liedvogel and Mouritsen 2010). In animals, *Cryptochrome*, one of the four groups of clock genes, that generate transcriptional and translation negative feedback loop, plays a major role as a component of the circadian clock. Under *Cry* family, basically there are two paralogues; *Cry1* and *Cry2*. In mice, it has been shown that mice lacking both *Cry* genes behave arrhythmically suggesting that proper functioning of *Cry* genes are crucial in generating of circadian rhythms (Bailey et al. 2002). In the transcriptional and translation model, *Crys* are activated via E-box enhancers by a positive element referred to as BMAL-CLOCK heterodimer and expression of *Crys* are suppressed by PERs and CRYs proteins (Yamamoto et al. 2001). The *Cry* genes have been identified in the chicken, house sparrow and quails and are reported to be rhythmically expressed. The expression of *Cry1* gene is high during the light phase while *Cry2* is shown to be expressed at high levels during late night (Yamamoto et al. 2001; Bailey et al. 2002; Helfer et al. 2006).

Even though many studies on polymorphisms of clock genes and its association have been carried out in human and wild birds, association studies of circadian clock genes are limited in poultry. It is believed that turkey genome sequence and genomic resources provide the tools that are required to improve the growth and reproductive traits in the turkey industry. In addition, the emerging turkey genome sequence provides a unique opportunity to understand the DNA sequence variations of the clock genes. Here, it was hypothesized that the differences in the DNA sequence variation of turkey clock genes may be associated with performance traits including growth and reproductive traits. The objectives of the current study were to screen the *turCry1* gene for DNA sequence variations and to evaluate the relationships among its haplotypes with growth and reproductive traits of turkeys.

2. Materials and Methods

2.1 Genotyping and screening the population

A total of 290 birds including hybrid turkeys (CC) and seven different heritage turkeys (Narragansett (NA), Royal Palm (RP), Blue Slate (BS), Spanish Black (SB), Midget White (MW), White Holland (WH) and Bourbon Red (BR)) were used. The management and measurement of growth and reproductive traits were described elsewhere (Adikari et al. 2016). Birds

were in the same age and physiological status. Genomic DNA from 290 turkey birds were isolated using a standard salting out procedure (Guan et al. 2007). The DNA sequences of the *turCry1* gene were used to design primers using Primer 3 software (Rozen and Skaletsky 2000). The information for the primers including the sequences, annealing temperature, and expected sizes of the Polymerase Chain Reaction (PCR) amplicon are presented in Table 1. Amplification was in a final volume of 25 uL consisting of standard reagents including Taq DNA polymerase (Takara Bio, Inc., Japan), 200 uM dNTPs, and 2 mM $MgCl_2$. The PCR reaction was performed for a total of 30 cycles in a GeneAmp, PCR System 9700 (Applied Bio-system, CA,). Following PCR, each amplicon was purified using Diffinity RapidTips (Diffinity Genomics, Inc., West Henrietta, NY), and sequenced (VBI, Blacksburg, VA) using the BigDye Terminator, Version 3.1, Sequencing kit (Applied Biosystems, Carlsbad, CA). The sequences were analyzed for SNPs using Phred, Phrap, Polyphred, and Consed as previously described by Guan et al. (2007).

2.2 Statistical analyses

Allele, genotype and haplotype frequencies were determined by standard counting. The computer program, Arlequin ver3.5 (Excoffier and Lischer 2010) was used to estimate the pairwise linkage disequilibrium (LD) among SNP loci, to test genotype frequencies for Hardy-Weinberg Equilibrium (HWE) and to estimate the fixation index (F_{ST}) among turkeys. Haplogroups were determined based on the output from Visual Haplotypes (VH1) software (http://gvs.gs.washington.edu/GVS/).

Data were analyzed with the PROC GLIMMIX of SAS 9.3 (SAS Inst. Inc., Cary, NC). The following statistical model was used for the analysis of associations between the genotype and phenotypic traits.

$$Y = \mu + L + S + G + (L \times G) + (G \times S) + e_i$$

where Y is the trait measured and estimated on turkeys, μ is the overall population mean, L is the fixed effect of the turkey variety, S is the fixed effect of sex, G is the fixed effect associated with the genotype, $(L \times G)$ is the interaction between the turkey variety and genotype, $(G \times S)$ is the interaction between the sex and genotype and it was excluded from the model if its effect was $P \geq 0.05$ and e is the residual error. A separate ANOVA was run for BW at each measurement day, ADG and FCR for each period, and each reproductive parameter. Multiple comparisons were analyzed using Tukey's test. The values were presented as least square means ± standard error. Results were considered significant at $P \leq 0.05$.

3. Results and Discussion

3.1 turCry1 gene variation

The amplicons produced by the three primer-pairs spanned a 15.0 kb region that included the *turCry1* gene (Table 1). A total of 7 SNPs were detected in the sequences scanned and validated. The complete list of the SNPs, the sequence contexts, alleles, and GenBank identification (*dbSNP*) are presented in Table 2. Of the 7 SNPs identified, four and three SNPs were detected in introns 3 and 6, respectively. The putative SNPs discovered in the current study have not been published earlier in the *dbSNP*, NCBI and therefore, these SNPs

Table 1: Primer sequences, the expected sizes of amplicons and annealing temperature used for the *turCry1* gene.

Primer ID	Primers[1]	Sequences	Tm[2] (^{0}C)	Amplicon length[3] (bp)
*Cry1*_1	For(54615366)	5′-TACTGTAAGCTAAGTGTAACTAGGATGTGGAC-3′	65.9	5000
	Rev(54610288)	5′-TATGCTTTTAGTGGTAGGCTACTTCTCTACTC-3′	65.9	
*Cry1*_2	For(54610609)	5′-ATAGTACAATATTTCTTCTGTGTGTCTAGATG-3′	62.1	4500
	Rev(54606117)	5′-TACTAATTTCTACAAAGCAGGAAACACAAG-3′	61.9	
*Cry1*_3	For(54606311)	5′-TAATACAAATAGTAATGACCACAGAAGATGGT-3′	62.1	5500
	Rev(54600808)	5′-TTAAGTGGTATCTCCTCTTCTAATAATGCTAC-3′	63.4	

[1]*For, forward primer; Rev, reverse primer.* Primer-binding sites in the turkey genome (GenBank accession No: LOC100008577) are presented in parentheses.

[2]The optimized annealing temperature at which a single amplicon of the expected size was obtained.

[3]Length in base pairs (bp) of the expected amplicon based on the binding sites of the forward and reverse primers.

Table 2: Characteristics of single nucleotide polymorphisms (SNPs) identified in the *turCry1* gene in eight divergent turkey varieties.

SNP	Location	Nucleotide position[1]	Sequence Context[2]	*dbSNP* Identification[3]	Genotype	Genotype Frequency %	MAF[4]
Cry1-1	Intron 6	54606291	GATGC(*A*/G)TGAAC	rs397507156	A/A	12.75	0.17
					A/G	7.93	
					G/G	79.32	
Cry1-2	Intron 6	54606392	CTTCC(*A*/G)AAGCT	rs397507157	A/A	42.07	0.43
					A/G	1.72	
					G/G	56.21	
Cry1-3	Intron 6	54606661	ATCAT(C/*T*)GTCAT	rs397507158	C/C	82.41	0.16
					C/T	4.13	
					T/T	13.46	
Cry1-4	Intron 3	54609933	GAAGA(*C*/G)ATTTT	rs397507159	C/C	13.10	0.13
					C/G	0.00	
					G/G	86.90	
Cry1-5	Intron 3	54610176	CCATT(*C*/T)AATCA	rs397507160	C/C	41.38	0.46
					C/T	9.31	
					T/T	49.31	
Cry1-6	Intron 3	54610182	AATCA(C/*A*)AACAA	rs397507161	C/C	73.45	0.21
					C/A	11.38	
					A/A	15.17	
Cry1-7	Intron 3	54610265	GCTTT(*C*/T)AGTGG	rs397507162	C/C	43.45	0.46
					C/T	4.82	
					T/T	51.73	

[1]Position of the SNP in Ensembl on the forward strand of chromosome 1 of the *Meleagris gallopavo* genome sequence.
[2]Within each sequence context, alleles at the SNP locus appear in parentheses. The minor allele is italicized in the parentheses.
[3]*rs* prefix indicates novel SNPs detected in the present study and available in *dbSNP*, NCBI
[4]Minor allele frequency (MAF) of 7 SNPs markers.

represent novel nucleotide variants of the *turCry1* gene.

Most of the SNPs detected in the present study were C-T/ A-G transitions. Within the 290 birds screened, the minor alleles ranged in frequency from 0.13 to 0.46 with the observed heterozygosity of 0.23 and 0.50, respectively. Across all SNPs, D´ ranged from 0.48 to 0.89. The correlation coefficient (r^2) for the SNPs ranged from 0.03 to 0.42 (Table 3). All the D´ and r^2 values were significant ($p \leq 0.05$). The pairwise F_{ST} estimated for the eight turkey varieties ranged from 0.004 to 0.67. The highest F_{ST} reported between BR and RP turkeys (0.67) while the lowest reported between the MW and NA turkeys (0.004). Most of the F_{ST} values were significantly different ($p \leq 0.05$) (Table 4). The relatively low F_{ST} estimate suggests low genetic differentiation between the two genetic lines and probably a reflection of their common ancestry.

The haplotypes observed from the 7 SNPs were grouped into seven haplogroups based on the VH1 output. The haplogroups ranged in frequency from 0.02 to 0.96 in the turkey varieties (Table 5). The most common haplogroup identified for the BR, NA and SB turkeys was Hap1, with the frequencies of 0.96, 0.82 and 0.46, respectively. The predominant haplogroup identified for the BS, CC, MW, RP and WH turkeys was Hap4, Hap3, Hap2, Hap6 and Hap5 with the frequencies of 0.67, 0.68, 0.81, 0.56 and 0.73, respectively.

The turkey *Cry1* (14.46 kb region) gene located in the chromosome 1 and contains 13 exons and 12 introns. Only one transcript of the *turCry1* gene has been identified so far with a length of 1761 bp (www.ensembl.org). However, nucleotide variants of the *turCry1* gene though detected in the introns, have not been published yet according to our knowledge since the turkey genome sequence has been published recently. The SNPs that detected in the present study would be novel variants of the *turCry1* gene. We compared the genetic structure of *turCry1* gene with chicken and zebra finch genome to verify the sequence identity. The genetic structure of the turkey *Cry1* gene had 90 and 91 % sequence similarity with chicken and zebra finch genome respectively according to BLAST result (http://blast.ncbi.nlm.nih.gov/) suggesting that most of the nucleotides of the *Cry1* gene are also conserved within these avian species. Low *Fst* value between the MW and NA and SB and NA turkeys indicated that closer relatedness between those turkeys, which is inconsistent with the previous reports by Smith et al. (2005) and Kamara et al. (2007).

3.2 Associations of turCry1 haplogroups with growth parameters

There was significant association of haplogroups with some of the growth and reproductive parameters of the turkeys. Haplogroups were not significantly associated with BW at 1, 34, 68, 159, 231 and 309 days (d) of ages ($p \geq 0.05$). However, Hap3 numerically appeared be the advantageous haplogroup for BW at 1, 34, and 68 d of ages while Hap4 reported the highest BW at 231 and 309 d of ages (Table 6). As shown in Table 7, haplogroups were significantly associated with ADG ($p \leq 0.05$) during the period

Table 3: Linkage disequilibrium as measured by *D´* and r^2 between the 7 segregating SNPs in the *turCry1* gene.

SNPs[1]	***Cry1-1***	***Cry1-2***	***Cry1-3***	***Cry1-4***	***Cry1-5***	***Cry1-6***	***Cry1-7***
Cry1-1		0.74	0.70	0.61	0.48	0.50	0.83
Cry1-2	0.19		0.66	0.52	0.73	0.56	0.83
Cry1-3	0.41	0.18		0.65	0.74	0.69	0.71
Cry1-4	0.31	0.08	0.29		0.56	0.67	0.82
Cry1-5	0.03	0.23	0.10	0.04		0.89	0.73
Cry1-6	0.17	0.16	0.39	0.25	0.17		0.72
Cry1-7	0.14	0.42	0.12	0.12	0.38	0.16	

[1]SNP identification (*Cry1-1 – Cry1-7*).

All *D'* and r^2 values are significant ($p \leq 0.05$).

D' values are listed in upper right section, and r^2 values are listed in lower left section.

Table 4: The pairwise fixation index (*Fst*) estimated for turkey varieties using the *turCry1* gene variants.

Turkey varieties[1]	BR	BS	NA	MW	SB	WH	RP	CC
BR	0.0							
BS	0.50	0.0						
NA	0.08	0.29	0.0					
MW	0.06	0.34	0.004*	0.0				
SB	0.17	0.20	0.02*	0.05	0.0			
WH	0.50	0.25	0.22	0.28	0.20	0.0		
RP	0.67	0.33	0.49	0.54	0.39	0.51	0.0	
CC	0.66	0.22	0.47	0.52	0.38	0.45	0.16	0.0

[1]Seven different varieties of heritage turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) and Commercial (CC) turkeys were used for the study.

* indicates non-significant *Fst* ($p \geq 0.05$).

Table 5. Haplogroup frequencies of *turCry1* gene in eight turkey populations.

Turkey Varieties		Haplogroups						
	N	**Hap1**	**Hap2**	**Hap3**	**Hap4**	**Hap5**	**Hap6**	**Hap7**
BR	23	0.96	0.00	0.00	0.00	0.00	0.00	0.04
BS	36	0.17	0.00	0.00	0.67	0.00	0.05	0.11
CC	50	0.02	0.00	0.68	0.02	0.08	0.12	0.08
MW	31	0.00	0.81	0.00	0.00	0.00	0.03	0.16
NA	33	0.82	0.00	0.00	0.00	0.00	0.15	0.03
RP	39	0.03	0.05	0.08	0.00	0.00	0.56	0.28
SB	37	0.46	0.00	0.00	0.00	0.00	0.11	0.43
WH	37	0.08	0.00	0.00	0.00	0.73	0.05	0.14

[1]Seven different varieties of heritage turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) and Commercial (CC) turkeys were used for the present study.

N = numbers of birds from each variety used for calculation of haplogroup frequencies.

Table 6. Associations between haplogroups of the *turCry1* gene and the body weight (BW) at different ages of turkeys.

Haplogroups	BW[1] (kg)					
	1 d	**34 d**	**68 d**	**159 d**	**231 d**	**309 d**
Hap1	0.046 ± 0.001[a]	0.69 ± 0.02[a]	2.01 ± 0.05[a]	6.64 ± 0.15[a]	8.73 ± 0.18[a]	9.03 ± 0.18[a]
Hap2	0.045 ± 0.001[a]	0.71 ± 0.05[a]	1.98 ± 0.13[a]	6.42 ± 0.35[a]	8.66 ± 0.41[a]	8.82 ± 0.40[a]
Hap3	**0.048 ± 0.001[a]**	**0.74 ± 0.03[a]**	**2.23 ± 0.09[a]**	6.56 ± 0.25[a]	8.55 ± 0.30[a]	9.14 ± 0.31[a]
Hap4	0.045 ± 0.001[a]	0.73 ± 0.04[a]	2.12 ± 0.11[a]	6.55 ± 0.30[a]	**8.91 ± 0.35[a]**	**9.21 ± 0.35[a]**
Hap5	0.044 ± 0.001[a]	0.66 ± 0.04[a]	1.94 ± 0.10[a]	6.41 ± 0.27[a]	8.47 ± 0.32[a]	8.69 ± 0.32[a]
Hap6	0.047 ± 0.001[a]	0.70 ± 0.02[a]	1.95 ± 0.06[a]	6.56 ± 0.17[a]	8.75 ± 0.21[a]	9.11 ± 0.20[a]
Hap7	0.047 ± 0.001[a]	0.71 ± 0.02[a]	2.07 ± 0.06[a]	**6.65 ± 0.15[a]**	8.86 ± 0.18[a]	8.90 ± 0.18[a]

[a]Means within columns with different superscripts are significantly different ($p \leq 0.05$).

[1]BW, body weight (kg) was measured at 1, 34, 68, 159, 231 and 309 days (d). Least square means **±** SE.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 7: Associations between haplogroups of *turCry1* gene and the average daily gain (ADG) by period of age for turkeys.

Haplogroups	ADG[1] (kg)					
	1-34 d	**35 – 68 d***	**69 – 159 d**	**160 – 231 d**	**232 – 309 d**	**1 – 309 d**
Hap1	0.019 ± 0.001[a]	0.039 ± 0.001[a,b]	0.050 ± 0.002[a]	0.029 ± 0.002[a]	0.004 ± 0.002[a]	0.029 ± 0.001[a]
Hap2	0.020 ± 0.001[a]	0.038 ± 0.003[a,b]	0.049 ± 0.004[a]	0.031 ± 0.005[a]	0.002 ± 0.005[a]	0.029 ± 0.001[a]
Hap3	**0.021 ± 0.001[a]**	**0.044 ± 0.002[a]**	0.048 ± 0.003[a]	0.029 ± 0.003[a]	**0.005 ± 0.004[a]**	**0.031 ± 0.001[a]**
Hap4	0.020 ± 0.001[a]	0.041 ± 0.003[a,b]	0.048 ± 0.003[a]	**0.033 ± 0.004[a]**	0.004 ± 0.004[a]	0.030 ± 0.001[a]
Hap5	0.018 ± 0.001[a]	0.036 ± 0.002[b]	0.047 ± 0.003[a]	0.028 ± 0.004[a]	0.003 ± 0.004[a]	0.028 ± 0.001[a]
Hap6	0.020 ± 0.001[a]	0.037 ± 0.001[b]	**0.051 ± 0.002[a]**	0.030 ± 0.002[a]	0.004 ± 0.002[a]	0.029 ± 0.001[a]
Hap7	0.020 ± 0.001[a]	0.040 ± 0.001[a,b]	0.050 ± 0.002[a]	0.031 ± 0.002[a]	0.001 ± 0.002[a]	0.029 ± 0.001[a]

[a,b]Means within columns with different superscripts are significantly different ($p \leq 0.05$).

[1]ADG, average daily gain (kg) was estimated at different periods of the age. Least square means **±** SE. *$p \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

of 35 - 68 d, but not with other periods of age. During the period of 35 - 68 d, ADG of Hap3 was significantly higher ($p \leq 0.05$) compared to Hap5 and Hap6. Though not significant, Hap3 numerically appeared to be the advantageous haplogroup for ADG during the periods of age between hatch - 34 d, 232 - 309 d and 1-309 d, while the Hap6 and Hap4 had highest ADG during the periods of age between 69 - 159 d and 160 - 231 d respectively (Table 7). The FCR was calculated for four different periods of the age for turkeys. The statistical analysis showed that haplogroups were statistically associated with FCR for the periods of age between 160 - 231 d and 34 - 231 d ($p \leq 0.05$) (Table 8). The Hap3 had significantly lower FCR compared to Hap1, Hap2 and Hap5 for the period of 160 - 231 d, while Hap7 had significantly lower FCR than that of Hap1 for the period of 34 - 231 d ($p \leq 0.05$). However, Hap4 and Hap7 showed the lowest FCR for the periods of 34 - 68 d and 69 - 159 d, respectively though FCR was not significantly different among haplogroups ($p \geq 0.05$).

3.3 Associations of turCry1 haplogroups with reproductive parameters

The age at first egg (AFE), egg production, and average egg weight were compared within the haplogroups identified for the *turCry1* gene. According to the statistical analysis, results showed that haplogroups were significantly associated only with egg production ($p \leq 0.05$) but not with AFE and average egg weight (Table 9). The Hap5 and Hap4 had significantly higher egg production for the periods of 6 and 10 wks, respectively ($p \leq 0.05$). Though not significant, Hap2 had the lowest AFE while Hap3 had the highest average egg weight for both periods of 6 and 10 weeks compared to other haplogroups. The associations between the haplogroups and semen quality traits including ejaculate volume, sperm concentration, total number of sperm and sperm viability were analyzed. Results showed that haplogroups were significantly associated with ejaculate volume ($p \leq 0.05$), but not with other semen quality traits. The Hap3 had significantly higher volume of semen compared to Hap2 and Hap6 ($p \leq 0.05$). Though not significant, Hap1 and Hap4 had numerically higher value for sperm concentration and total number of sperm, respectively, while Hap5 numerically appeared to be advantageous haplogroup for sperm viability ($p \geq 0.05$) (Table 10).

The *turCry1* gene, as a candidate gene was selected to investigate associations of gene polymorphism with growth and reproductive parameters of the turkeys. In the present study, we described new genetic variants in the *turCry1* gene and used them to identify haplotypes and haplogroups. Haplotypes were constructed with the 7 SNPs and were used to develop the haplogroups. We analyzed the association among haplogroups of the *turCry1* gene with growth and reproductive parameters of turkeys. According to the association analyses of *turCry1* gene haplogroups, it showed a significant association of *turCry1* gene haplogroups with ADG, FCR, egg production and ejaculate volume. The potential associations of the haplogroups with ADG for the period of 35 - 68 d, FCR for the periods of 160 - 231 d and 34 - 231 d, egg production and ejaculate volume showed that

Table 8: Associations between haplogroups of *turCry1* gene and the feed conversion ratio (FCR) by periods of age for turkeys.

Haplogroups	FCE[1]			
	34 – 68 d	**69 – 159 d**	**160 – 231 d***	**34 – 231d***
Hap1	2.85 ± 0.16[a]	4.47 ± 0.13[a]	13.18 ± 0.66[a]	7.57 ± 0.21[a]
Hap2	3.02 ± 0.36[a]	4.66 ± 0.31[a]	12.08 ± 1.49[a]	7.51 ± 0.47[a]
Hap3	2.73 ± 0.26[a]	4.60 ± 0.22[a]	**10.12 ± 1.10[b]**	7.32 ± 0.35[a,b]
Hap4	**2.64 ± 0.31[a]**	4.71 ± 0.27[a]	11.21 ± 1.29[a,b]	7.19 ± 0.41[a,b]
Hap5	2.94 ± 0.28[a]	4.58 ± 0.24[a]	12.05 ± 1.16[a]	7.17 ± 0.37[a,b]
Hap6	2.96 ± 0.18[a]	4.46 ± 0.15[a]	10.99 ± 0.75[b]	7.22 ± 0.24[a,b]
Hap7	2.70 ± 0.15[a]	**4.45 ± 0.13[a]**	11.24 ± 0.64[a,b]	**6.92 ± 0.20[b]**

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1]FCR, feed conversion ratio was estimated at different periods of the age. Least square means **±** SE. *$P \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

Table 9: Associations between haplogroups of *turCry1* gene and the egg production traits for turkeys.

Haplogroups	Egg production traits[1]				
	AFE[4] (d)	Egg production[2]		Average egg weight[3] (g)	
		6 wks*	10 wks*	6 wks	10 wks
Hap1	229.99 ± 7.67[a]	9.51 ± 2.10[a,b]	14.96 ± 3.06[a,b]	77.92 ± 0.86[a]	77.77 ± 0.72[a]
Hap2	**219.11 ± 17.05[a]**	14.73 ± 4.62[a]	22.95 ± 6.73[b]	78.25 ± 1.71[a]	78.33 ± 1.58[a]
Hap3	249.35 ± 13.28[a]	15.09 ± 3.55[a]	23.26 ± 5.18[b]	**79.00 ± 1.44[a]**	**79.49 ± 1.24[a]**
Hap4	232.25 ± 19.86[a]	16.51 ± 3.95[a]	**25.60 ± 5.75[b]**	77.03 ± 1.78[a]	77.71 ± 1.65[a]
Hap5	233.45 ± 11.08[a]	**17.91 ± 3.63[a]**	24.68 ± 5.29[b]	78.36 ± 1.27[a]	78.02 ± 1.03[a]
Hap6	235.90 ± 8.99[a]	5.96 ± 2.41[b]	10.81 ± 3.52[a]	78.17 ± 0.93[a]	78.24 ± 0.82[a]
Hap7	226.96 ± 7.88[a]	15.81 ± 2.10[a]	24.31 ± 3.06[b]	78.10 ± 0.75[a]	77.85 ± 0.66[a]

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1]Least square means **±** SE. *$P \leq 0.05$.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks separately for each hen.

[4]AFE, age at first egg was recorded and given in days (d).

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

Table 10: Associations between haplogroups of *turCry1* gene and semen quality traits for turkeys.

Haplogroups	Semen quality traits[1]			
	Ejaculate volume (mL)*	**Sperm Concentration (x10^9/mL)**	**Total number of sperm (x10^8/ejaculate)**	**Sperm viability (%)**
Hap1	0.12 ± 0.01^{a,b}	**2.48 ± 0.18^a**	3.14 ± 0.40^a	84.18 ± 0.90^a
Hap2	0.07 ± 0.03^a	2.12 ± 0.41^a	2.24 ± 0.93^a	81.20 ± 2.08^a
Hap3	**0.14 ± 0.02^b**	2.00 ± 0.34^a	2.78 ± 0.77^a	83.01 ± 1.73^a
Hap4	0.13 ± 0.02^{a,b}	2.18 ± 0.36^a	**3.18 ± 0.82^a**	84.36 ± 1.83^a
Hap5	0.11 ± 0.02^{a,b}	1.89 ± 0.32^a	2.31 ± 0.72^a	**84.74 ± 1.61^a**
Hap6	0.09 ± 0.01^a	2.34 ± 0.20^a	2.15 ± 0.45^a	82.58 ± 1.00^a
Hap7	0.11 ± 0.01^{a,b}	2.42 ± 0.17^a	2.87 ± 0.38^a	83.01 ± 0.85^a

^{a,b}Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means **±** SE. *$P \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

the variations of turkey *Cry1* gene may be involved in regulation of growth, feed intake and egg production through a molecular mechanism. In general, haplogroups of *turCry1* gene were significantly associated with few growth and reproductive parameters suggesting that *turCry1* gene is a good candidate gene for association study in turkey.

The circadian clock mechanism in birds is composed of several proteins including CLOCK, BMAL1, PER2, PER3, CRY1 and CRY2. Many of these proteins are transcription factors (Yamamoto et al. 2001). These proteins work together to activate or inhibit its own transcription. In the transcriptional and translation feedback loop, CLOCK and BMAL1 proteins form a complex which acts as a positive feedback loop by activating the transcription of *Per* and *Cry* genes. Similarly, PER and CRY proteins form the protein complex which acts as a negative feedback loop by inhibiting the transcriptional activity of CLOCK/BMAL1 complex (Gekakis et al. 1998; Lee et al. 2001). These protein components are important to initiate circadian rhythms at the molecular level and lead for the proper functioning of the circadian clock. In addition, previous studies as discussed earlier have shown that genetic variants of clock genes are associated with reproductive traits, feeding, sleeping pattern and behavior patterns in various species of animals. Therefore, we selected *Cry1* gene out of the *Clock*, *Bmal1*, *Per3*, *Cry1* and *Cry2* genes for the current study by considering their connection in the molecular mechanism of the circadian clock and associations in other animals.

Most of the SNPs reported in the *turCry1* genes studied did not follow the HWE suggesting that gene and genotype frequencies of these variants change from one generation to another due to operational of some evolutionary forces (Cox and Kraft 2006). Most of the D´ values among SNPs were significantly different suggesting that these SNPs are in linkage disequilibrium though they are apart which shows the SNPs tend to be inherited together more often than expected by chance (Goodswen et al. 2010). The *Fst* is used to measure the genetic differentiation between turkey populations. *Fst* is a measure of population differentiation that ranges from 0 to 1 with 0 indicating no differentiation and 1 indicating highly differentiated populations. The strong genetic differentiation between two populations is confirmed by the large *Fst* values (Hayes et al. 2008). The candidate gene approach is a very powerful method to investigate associations of gene polymorphisms with economically important traits in farm animals. It is important to note that regulatory and coding SNPs are of particular interest to molecular association studies. Non-synonymous SNPs translate into amino-acid polymorphisms in the proteins they encode. Regulatory SNPs can also affect the expression, tissue-specificity or function of relevant proteins (Rothschild and Soller 1997). With the discovery of a large number of SNPs, it has been increasing interests in genetic associations with closely linked SNPs (Sha et al. 2007). In the present study, haplogroups of *turCry1* genes

were significantly associated with some of the growth and reproductive parameters suggesting that *turCry1* gene is one of the good candidate genes for further association studies in turkey. Haplotypes are naturally interpreted as genetic polymorphisms of SNP alleles on the same chromosome and tend to be conserved by evolutionary processes (Yang et al. 2008).

4. Conclusions

The *turCry1* gene is a good candidate gene for further association studies in turkey. DNA sequence variations of the *turCry1* gene may have some regulatory role in the molecular mechanism of the circadian clock which may affect the overall mechanism of the circadian clock. However, further association studies are required to show the value of our genetic data in genotype-phenotype correlations in turkey birds.

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