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Research Article
Association of Polymorphisms in the *Period3* (*turPer3*) Gene with Growth and Reproductive Traits in Turkeys (*Meleagris gallopavo*)

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Abstract

Background and objective: Biological clock controls behavioral, physiological and biochemical circadian rhythms of animals. Circadian clock genes including period3 are involved in the circadian clock mechanism. The present study was conducted to test the hypothesis that differences in DNA sequence variations of the turkey *period3* (*turPer3*) gene may be associated with performance traits including growth and reproduction. **Methodology:** The *turPer3* gene was screened for DNA sequence variations and evaluated the relationships among haplogroups with performance traits. The DNA sequences of *turPer3* (16.6 kb) gene were screened using 290 turkey birds by re-sequencing the individual amplicons. **Results:** Seven SNPs, including one each in exon 18 and intron 5, two SNPs in exon 19 and three SNPs in intron 6, were detected. The SNPs detected in the exon 19 were non-synonymous, which changed the amino acids from methionine to threonine and serine to phenylalanine at 953rd and 955th positions, respectively. Linkage disequilibrium (D') among SNPs ranged from 0.03-1.00. Pairwise F_{ST} ranged from 0.01-0.43. Haplogroup frequencies of the *turPer3* ranged from 0.02-1.00, were significantly associated with body weight (BW) at 231 days of age, average daily gain (ADG) for the period of 160-231 d of age, FCR for the periods of 69-159 d and 160-231 d, egg production and semen quality traits ($p \leq 0.05$). **Conclusion:** The DNA sequence variations of turPer3 gene are significantly associated with BW, ADG, FCR, egg production, egg weight and semen quality traits. *turPer3* gene may seem to have some regulatory role in the molecular mechanism of the circadian clock. Genomic reagents reported in the present study would be valuable for future genotype: phenotype evaluation studies in the turkey using a candidate gene approach.

Key words: *Period3* gene, nucleotide polymorphism, growth performance, reproductive traits

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The biological clock controls behavioral, physiological and biochemical circadian rhythms of animals. Molecular components of the circadian clock have been identified in diverse animal species[1]. The transcriptional and translational feedback loop characterize the basic molecular mechanism of the circadian clock[2]. Several genes such as *Clock*, *Bmal1*, *Period1* (Per1), *Period2* (*Per2*), *Period3* (*Per3*), Cryptochrome1 (Cry1) and Cryptochrome2 (Cry2) are involved in molecular mechanism of the circadian clock[3]. The Clock, one of the core genes involved in generating endogenous rhythms, encodes the transcription factor CLOCK which dimerizes with BMAL1 protein to activate the transcription of target genes including Per and Cry. The CLOCK and BMAL1 produce rhythmic transcription activation that serves as the basic driving force for the circadian clock. The Bmal1 gene encodes BMAL1 protein, which plays as a positive element along with CLOCK to activate the *Per* and *Cry* genes in the molecular mechanism of the circadian clock[4].

The *Period* (*Per*) gene is a central component of the molecular mechanism of the circadian clock and provides a role as the negative element in the transcriptional and translation feedback loop. In mammals, *Period* gene has three paralogues, *Per1*, *Per2* and *Per3*[5]. In human, two SNPs have been reported in exon 18 of the Per1 gene. Of the two SNPs, the first SNP in exon 18 of the *Per1*, which is an A to G synonymous substitution at position 2548, is not associated with diurnal preference in normal adults[6]. But, the T2434C polymorphism, a synonymous substitution is associated with extreme diurnal preference[5]. The first mutation (A2106G) of the *Per2* has been reported by Toh *et al*.[7] and linked with pathologically extreme morningness (DSPT). In addition, Carpen et al.[8] also reported three more polymorphisms in the *Per2* gene, one downstream of the transcription start site (C1228T), one in the 5'-untranslated region (C111G) and one missense mutation (G3853A) located in exon 23. In human Per3 gene, Ebisawa *et al*.[9] reported 20 sequence variations of which six variants changed amino acids. Of the total variants, two missense mutations (T1940G and C2590G) were significantly associated with DSPT. Johansson *et al*.[10] also reported T1940G mutation is associated with morning preference in a mixed European populations. Only two paragons of *Per* gene have been identified in the quails and chicken, *Per2* and *Per3*[11]. Avian *Per2* and *Per3* are rhythmically expressed in the pineal gland, retina and hypothalamus in both light and dark conditions with peak at early light phase[2]. It is believed that *Per2* gene in birds appears more similar to mammalian *Per1* than *Per2*.

Even though many studies have been carried out in human and wild birds on polymorphisms of clock genes, association studies of circadian clock genes are limited in poultry. It is believed that turkey (*Meleagris gallopavo*) genome sequence and genomic resources provide a tool that is required to improve the growth and reproductive traits in turkey production. In addition, emerging turkey genome sequence provides a unique opportunity to understand the DNA sequence variations of the clock genes. In the present study, we hypothesized that differences in DNA sequence variation of turkey clock genes may be associated with performance traits including growth and reproductive traits. The objectives of the current study, therefore, were to screen the *period3* gene (*turPer3*) for DNA sequence variations and to evaluate the relationships among its haplogroups with growth and reproductive traits in turkey birds.

MATERIALS AND METHODS

Genotyping and screening the population: A total of 290 birds including hybrid turkeys (CC) and seven different varieties of 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 for geno in the present study. The management and measurement of growth and reproductive traits were described by Adikari *et al*.[12]. Genomic DNA from 290 turkey birds were isolated using a standard salting out procedure[13]. The *turPer3* gene was used to design primers using Primer 3 software[14]. 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*.[13].

Statistical analyses: Allele, genotype and haplotype frequencies were determined by standard counting. The computer program, Arlequin ver3.5[15] 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.

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

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, (LxG) is the interaction between the turkey variety and genotype, (GxS) 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 Body Weight (BW) at each measurement day, Average Daily Gain (ADG) and Feed Conversion Ratio (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 $p \leq 0.05$.

RESULTS AND DISCUSSION

Genetic variation of *turPer3* gene: The amplicons produced by the four primer-pairs spanned the *turPer3* gene (16.6 kb), are presented in the Table 1. A total of 7 SNPs were detected in the sequences scanned and validated. The complete list of SNPs, sequence contexts, alleles and GenBank identification (*dbSNP*) are presented in the Table 2. Of the 7 SNPs identified in the present study, one and two SNPs were detected in the exon 18th and 19th while one and three SNPs were detected in the intron 5th and 6th, respectively. The putative SNPs discovered in the current study, have not been published in the *dbSNP*, NCBI and these SNPs represent novel nucleotide variants in the turkey genome. The SNP detected in the exon 18, was synonymous while two SNPs detected in the exon 19, were non-synonymous. Of non-synonymous SNPs, one SNP changed an amino acid from methionine to threonine at 953rd position while other changed the amino acid from serine to phenylalanine at 955th position of the protein transcribed from the *turPer3* gene.

Most of the SNPs showed C-T/A-G transitions. Within the 290 birds screened, the frequency of minor alleles ranged from 0.03-0.49 with the observed heterozygosity of 0.07 and 0.50, respectively. About 42% the reported SNPs was in HWE ($p \geq 0.05$) (Table 2). Across all SNPs, Linkage disequilibrium (D') ranged from 0.03-1.00. The correlation coefficient (r^2) for the SNPs ranged from 0.002-0.89 (Table 3). The pairwise F_{ST} estimated for the eight turkey varieties ranged from 0.01-0.43. The highest F_{ST} (0.43) reported between BR and CC turkeys while lowest (0.01) reported between RP and NA, CC and NA and CC and RP turkeys. Most of the F_{ST} 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 six haplogroups. The frequency of haplogroups ranged from 0.02-1.00 in the turkey varieties (Table 5). Most common haplogroup identified in BS, CC, NA and RP turkeys was Hap6, with the frequencies of 0.44, 0.76, 0.43 and 0.56, respectively. Hap4 was predominant in MW and WH turkeys with the frequencies of 0.42 and 0.41, respectively. The Hap2 and Hap3 were the leading haplogroups in SB turkey with the frequency of 0.32. The BR turkey had only one haplogroup (Hap1).

The turkey *period3* gene is located over a region of 15.84 kb of the chromosome 23 and contains 25 exons. Only one transcript of the *turPer3* gene has been identified up to now. The length of the transcript has been estimated the 3447 bp, which translate into a protein of 1149 aa

Table 1: Primer sequences, the expected sizes of amplicons and PCR characteristics for turPeri3 gene

Primer ID	Primers[1]	Sequences	Tm[2] (°C)	Amplicon length[3] (bp)
Per-1	For(281658)	5'-GTGTCTGACTACAGATAGCATTAAAACAAAT-3'	62.0	4800
	Rev(276851)	5'-AATAGTTTACGACTGTTTACAGCTGCATTA -3'	61.9	
Per-2	For(277117)	5'-CTGTAATTTTGTAATGTCTCTTTGAGTTACTG-3'	62.1	4000
	Rev(273122)	5'-CAATTACGTTACTTTACCTTCTAGTACTTCTG-3'	63.4	
Per-3	For(273542)	5'-TACGAGAGTGGTGTTATTAACTTCTGTAGG-3'	64.6	4800
	Rev(268757)	5'-GGGAGACAGAGGGAGTAATTAGAAAAGTAT-3'	64.6	
Per-4	For(269236)	5'-GCTAAATGTGTAAGTAACAGAGATTTTTAGTG-3'	62.1	3000
	Rev(266155)	5'-CTTTAGAAAGGAACAAGGACATATCATTTA-3'	60.5	

[1]For, forward primer; Rev, reverse primer. Primer-binding sites in the turkey genome (GenBank accession No: LOC100008578)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 turPeri3 gene in eight divergent turkey varieties

SNP ID	Location	Nucleotide position[1]	Sequence context[2]	dbSNP identification[3]	Genotype	Genotype frequency (%)	MAF[4]	HWE[5]
sPer3-1	Exon 19	269002	ATAAsA(A/G)AAAGC	rs271791607	A/A	25.17	0.49	NS
					A/G	48.28		
					G/G	26.55		
Per3-2	Exon 19	269008	AAAGC(A/G)TTGCC	rs271791608	A/A	91.38	0.05	0.00*
					A/G	6.90		
					G/G	1.72		
Per3-3	Exon 18	269119	TCAAA(C/T)GTAGT	rs271791609	C/C	91.38	0.05	0.00*
					C/T	6.90		
					T/T	1.72		
Per3-4	Intron 6	276755	CACTA(A/T)CAAAT	rs271791610	A/A	87.93	0.07	0.01*
					A/T	10.34		
					T/T	1.73		
Per3-5	Intron 6	276883	CACAC(A/T)CCTCA	rs271791611	A/A	1.03	0.04	0.01*
					A/T	6.56		
					T/T	92.41		
Per3-6	Intron 6	276891	CTCACA(A/G)TCCTT	rs271791612	A/A	90.34	0.05	NS
					A/G	8.96		
					G/G	0.70		
Per3-7	Intron 5	277055	ACTTA(A/G)TTCTG	rs271791613	A/A	93.79	0.03	NS
					A/G	5.52		
					G/G	0.69		

[1]Position of the SNP in Ensembl on the forward strand of chromosome 23 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, [5]Significance of deviation from HWE for the 7 SNPs. NS indicates non-significant ($p>0.05$) while *refers to significant at $p<0.05$

Table 3: Linkage disequilibrium as measured by D′ and r^2 between the 7 segregating SNPs in the turPer3 gene

SNPs[1]	Per3-1	Per3-2	Per3-3	Per3-4	Per3-5	Per3-6	Per3-7
Per3-1		0.69	0.68	0.66	0.76	NS	NS
Per3-2	0.03		0.96	NS	0.12	0.37	0.34
Per3-3	0.03	0.89		NS	0.11	0.37	0.34
Per3-4	0.04	NS	NS		NS	NS	NS
Per3-5	0.03	0.01	0.01	NS		0.15	0.13
Per3-6	NS	0.13	0.13	NS	0.02		0.91
Per3-7	NS	0.09	0.09	NS	0.02	0.66	

[1]SNP identification (Per3-1 to Per3-7), NS in the table indicates non-significant ($p\geq0.05$) D′ and r^2 values, 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 among turkey varieties using turPer3 SNPs

Turkey varieties[1]	BR	BS	NA	MW	SB	WH	RP	CC
BR	0.0							
BS	0.41	0.0						
NA	0.40	0.06	0.0					
MW	0.39	0.37	0.23	0.0				
SB	0.39	0.03	0.15	0.40	0.0			
WH	0.37	0.18	0.05	0.07	0.25	0.0		
RP	0.41	0.06	0.01*	0.22	0.15	0.04	0.0	
CC	0.43	0.05	0.01*	0.24	0.15	0.06	0.01*	0.0

[1]Seven different varieties of heritage turkeys including BR: Bourbon Red, BS: Blue Slate, NA: Narragansett, RP: Royal Palm, SB: Spanish Black, WH: White Holland and MW: Midget White and CC: Commercial, turkeys were used for the present study *$p\geq0.05$

(www.ensembl.org). However, nucleotide variants of *turPer3* gene have not been published yet and the SNPs that we detected in the present study were novel genetic variants of the *turPer3* gene. We compared the genetic structure of *turPer3* gene with chicken and zebra finch genomes to identify the sequence identity between these species. We found that the genetic structure of the turkey *period3* gene has 90 and 83% sequence similarity with chicken and zebra finch genome respectively. According to BLAST result (http://blast.ncbi.nlm.nih.gov/), it suggests that most of the nucleotides of the *period3* gene are conserved within these birds. Here, we investigated the genetic relatedness between CC and seven heritage turkey varieties using Fst. Small Fst values between RP and NA, CC and NA and CC and RP turkeys

Table 5: Haplogroup frequencies of turPer3 gene in eight turkey populations

	Haplogroups						
Turkey varieties	N	Hap1	Hap2	Hap3	Hap4	Hap5	Hap6
BR	23	1.00	0.00	0.00	0.00	0.00	0.00
BS	36	0.03	0.22	0.31	0.00	0.00	0.44
CC	50	0.00	0.04	0.10	0.08	0.02	0.76
MW	31	0.00	0.03	0.03	0.42	0.39	0.13
NA	33	0.00	0.00	0.27	0.21	0.09	0.43
RP	39	0.00	0.03	0.15	0.18	0.05	0.59
SB	37	0.00	0.32	0.32	0.03	0.08	0.25
WH	37	0.00	0.00	0.05	0.41	0.30	0.24

[1]Seven different varieties of heritage turkeys including BR: Bourbon Red, BS: Blue Slate, NA: Narragansett, RP: Royal Palm, SB: Spanish Black, WH: White Holland and MW: Midget White and CC: Commercial turkeys were used for the present study, N: No. of birds from each variety used for calculation of haplogroup frequencies

Table 6: Associations between haplogroups of turPer3 gene and the body weight (BW) at different ages of turkeys

	BW[1] (kg)					
Haplogroups	1 d	34 d	68 d	159 d	231 d*	309 d
Hap1	0.046±0.004[a]	0.63±0.12[a]	1.97±0.32[a]	6.45±0.85[a]	8.65±0.99[a,b]	8.79±0.98[a]
Hap2	0.046±0.001[a]	0.68±0.03[a]	1.98±0.09[a]	6.66±0.25[a]	8.99±0.28[a]	9.12±0.28[a]
Hap3	0.046±0.001[a]	0.71±0.03[a]	2.02±0.07[a]	6.56±0.19[a]	8.42±0.22[b]	8.76±0.22[a]
Hap4	0.046±0.001[a]	0.69±0.03[a]	2.05±0.08[a]	6.75±0.20[a]	8.91±0.20[a]	9.10±0.23[a]
Hap5	0.045±0.001[a]	0.70±0.03[a]	2.01±0.09[a]	6.39±0.23[a]	8.52±0.27[a,b]	8.83±0.26[a]
Hap6	0.047±0.001[a]	0.73±0.03[a]	2.07±0.06[a]	6.56±0.16[a]	8.78±0.18[a,b]	9.14±0.18[a]

[a,b]Means within columns with different superscripts are significantly different ($p \leq 0.05$)1BW, body weight (kg) was measured at 1, 34, 68, 159, 231 and 309 days (d), Least square Mean±SE. *$p \leq 0.05$, Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

Table 7: Associations between haplogroups of turPer3 gene and the average daily gain (ADG) by period of age for turkeys

	ADG[1] (kg)					
Haplogroups	1-34 d	35-68 d	69-159 d	160-231 d*	232-309 d	1-309 d
Hap1	0.018±0.003[a]	0.039±0.007[a]	0.049±0.009[a]	0.031±0.011[a,b]	0.002±0.012[a]	0.028±0.003[a]
Hap2	0.019±0.001[a]	0.038±0.002[a]	0.051±0.003[a]	0.033±0.003[a]	0.002±0.003[a]	0.029±0.001[a]
Hap3	0.020±0.001[a]	0.039±0.002[a]	0.050±0.002[a]	0.026±0.002[b]	0.004±0.003[a]	0.028±0.001[a]
Hap4	0.019±0.001[a]	0.040±0.002[a]	0.052±0.002[a]	0.029±0.002[a,b]	0.002±0.003[a]	0.029±0.001[a]
Hap5	0.020±0.001[a]	0.039±0.002[a]	0.048±0.002[a]	0.030±0.003[a,b]	0.004±0.003[a]	0.029±0.001[a]
Hap6	0.021±0.001[a]	0.039±0.001[a]	0.049±0.002[a]	0.031±0.002[a,b]	0.004±0.002[a]	0.030±0.001[a]

[a,b] Means within columns with different superscripts are significantly different ($p \leq 0.05$), 1ADG, average daily gain (kg) was estimated at different periods of the age, Least square Mean±SE. *$p \leq 0.05$, Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

indicated that closer relatedness between those turkeys. Smith *et al.*[16] also showed the closer relatedness between RP and NA turkeys which we also observed in the present study. In addition, we found that CC was more closely related with NA and RP turkeys which was inconsistent with the previous findings reported by Kamara *et al.*[17] and Smith *et al.*[16].

Associations of *turPer3* gene haplogroups with growth, reproductive parameters and plasma melatonin: The statistical analysis revealed that there was a significant association of haplogroups with some of the growth and reproductive traits of the turkeys. Haplogroups were significantly associated with BW at 231 d of age ($p \leq 0.05$) where Hap2 had significantly higher BW than that of Hap3. Though not significant, Hap6 numerically appeared be the advantageous haplogroup at 1, 34, 68 and 309 days of age while Hap2 was numerically greater at 159 days of age (Table 6). As shown in Table 7, haplogroups were significantly associated with ADG ($p \leq 0.05$) during the period of 160-231 d but not with other periods of age. The Hap2 had significantly higher ADG during the period of 160-231 d compared to Hap3. The Hap6 numerically appeared to be advantageous haplogroup for the periods of 1-34 d, 232-309 d and 1-309 d. Hap4 had higher ADG during the periods of 35-68 d and 69-159 d though not significant ($p \geq 0.05$) (Table 7).

The FCR was calculated for the different periods of age. The statistical analysis showed that haplogroups were statistically associated with FCR for the periods of 69-159 d and 160-231 d ($p \leq 0.05$) (Table 8). The Hap4 had significantly lower FCR compared to Hap5 for the period of 69-159 d while

Table 8: Associations between haplogroups of turPer3 gene and the feed conversion ratio (FCR) by periods of age for turkeys

	FCE[1]			
Haplogroups	34-68 d	69-159 d*	160-231 d*	34-231
Hap1	2.77±0.88[a]	4.54±0.75[a,b]	10.65±3.64[a]	6.99±1.16[a]
Hap2	2.85±0.25[a]	4.44±0.22[a,b]	11.72±1.05[a]	7.06±0.33[a]
Hap3	2.87±0.20[a]	4.57±0.17[a,b]	13.36±0.81[b]	7.61±0.26[a]
Hap4	2.81±0.21[a]	4.39±0.18[a]	11.49±0.86[a]	7.31±0.27[a]
Hap5	2.80±0.23[a]	4.77±0.20[b]	12.58±0.98[a]	7.58±0.31[a]
Hap6	2.88±0.17[a]	4.52±0.14[a,b]	11.61±0.68[a]	7.27±0.22[a]

[a,b] Means within columns with different superscripts are significantly different ($p \leq 0.05$). 1FCR, feed conversion ratio was estimated at different periods of the age. Least square Mean±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 turPer3 gene and the egg production traits for turkeys

	Egg production traits[1]				
		Egg production[2]		Average egg weight[3] (g)	
Haplogroups	AFE[4] (d)*	6 weeks	10 weeks*	6 weeks*	10 weeks*
Hap1	243.13±13.01[a]	5.91±9.43[a]	10.30±5.15[a]	76.01±3.01[a]	76.05±2.27[a]
Hap2	240.00±16.46[a,b]	13.00±3.84[a]	12.30±5.15[a]	77.69±3.01[a]	77.68±2.87[a]
Hap3	227.55±8.23[a,b]	14.58±2.48[a]	20.07±3.08[a,b]	77.81±1.51[a]	77.86±1.37[a]
Hap4	238.25±8.23[a,b]	12.81±2.82[a]	19.87±3.40[a,b]	77.27±1.55[a]	76.79±1.40[a]
Hap5	231.33±9.50[a,b]	15.78±3.30[a]	27.94±4.08[b]	76.16±1.87[a]	76.07±1.66[a]
Hap6	217.58±5.06[b]	12.91±1.97[a]	19.34±1.91[a]	82.90±0.96[b]	82.89±0.89[b]

[a,b]Means within columns with different superscripts are significantly different ($p \leq 0.05$). [1]Least square Mean±SE. *$p \leq 0.05$, [2]Egg production was individually recorded for a period of 10 wks starting from 30-40 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 turPer3 gene and semen quality traits for turkeys

	Semen quality traits[1]			
Haplogroups	Ejaculate volume (mL)*	Sperm concentration (x10^9 mL^{-1})*	Total number of sperm (x10^8/ejaculate)*	Sperm viability (%)
Hap1	0.05±0.01[a]	1.81±0.18[a]	1.00±0.44[a]	82.65±1.04[a]
Hap2	0.12±0.01[b]	2.19±0.17[a]	2.66±0.42[b,c,d]	83.00±1.00[a]
Hap3	0.11±0.01[b]	2.18±0.16[a]	2.58±0.38[c]	83.88±0.91[a]
Hap4	0.14±0.01[b]	2.65±0.14[b]	3.68±0.34[d]	84.18±0.80[a]
Hap5	0.12±0.01[b]	2.30±0.16[b]	2.63±0.39[c]	83.19±0.94[a]
Hap6	0.12±0.01[b]	2.35±0.12[b]	2.79±0.28[c]	82.89±0.67[a]

[a,b,c,d]Means within columns with different superscripts are significantly different ($p \leq 0.05$), Least square Mean±SE. *$p \leq 0.05$, bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

Hap1 had significantly lower FCR than that of Hap3 for the period of 160 - 231 d ($p \leq 0.05$). However, Hap1 had the lowest FCR for the periods of 34-68 d and 34-231 d though not significant ($p \geq 0.05$). In general, Hap1 numerically appeared to be advantageous haplogroup of FCR.

The AFE, egg production and average egg weight were compared with the haplogroups of *turPer3*. Haplogroups were significantly associated with AFE, egg production of 10 wks and average egg weight ($p \leq 0.05$) (Table 9). The Hap 6 had significantly lower AFE compared to Hap1. Hap5 produced more eggs for the both periods (6 and 10 wks) compared to Hap1 but, it was significantly different only for the egg production at 10 wks ($p \leq 0.05$). The average egg weight was significantly different among haplogroups where Hap6 had significantly greater average egg weight compared to Hap1 ($p \leq 0.05$). In general, Hap5 appeared to be most advantageous haplogroup of egg production while Hap6 was advantageous for AFE and average egg weight.

We also analyzed the association between haplogroups and semen quality traits including ejaculate volume, sperm concentration, total number of sperm and sperm viability. There was a significant association of haplogroups with ejaculate volume, sperm concentration and total number of sperm ($p \leq 0.05$). The Hap4 had significantly higher values for ejaculate volume, sperm concentration and total number of sperm ($p \leq 0.05$) compared to Hap1 (Table 10). Overall, Hap4 appeared to be most advantageous haplogroup of semen quality traits of the turkeys.

According to our assumption, we selected *period3* gene as a candidate gene to investigate associations of gene polymorphism with growth and reproductive traits in turkeys. In the present study, we described new genetic variants in the *turPer3* gene and used them to identify haplotypes and haplogroups. Haplotypes were constructed with the reported SNPs and were used to categorize the haplogroups. We analyzed the association among haplogroups of the *turPer3* gene with growth and reproductive traits. According to the association analyses of *turPer3* gene haplogroups, it revealed the significant association of *turPer3* gene haplogroups with some of the BW, FCR and AFE, egg production and semen quality traits. The potential associations of the haplogroups with BW at 231 d of age, ADG for the period of 160-231 d, FCR for the periods of 69-159 and 160-231 d, AFE, egg production and semen quality traits, showed that the variations of turkey period3 gene seems to be involved in regulation of growth, feed intake, reproduction and semen quality traits through a molecular mechanism of the circadian clock.

The results indicated that haplogroups of *turPer3* gene was statistically associated with most of the growth and reproductive traits. This shows that genetic variants of the *turPer3* gene is closely associated with growth and reproductive traits suggesting that *turPer3* gene is a good candidate gene for association studies for growth and reproductive traits in turkeys.

Most of the SNPs reported in the turPer3 genes studied in the present study did not follow the HWE due to operational of some of the evolutionary forces. A deviation from HWE may be due to natural selection, population admixture, inbreeding, experimental errors and duplication[18]. Most of the D' values among SNPs were significantly different. These SNPs are in linkage disequilibrium though they are apart. This confirms that SNPs tend to be inherited together more often than expected by chance[19]. Fst is used to measure the genetic differentiations between turkey populations. Fst is a measure of population differentiation that ranges from 0-1. The strong genetic differentiation between two populations is confirmed by the large Fst values[20]. 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[21]. The association studies have been used to find any association between the genetic variants and phenotype of the animals. With the discovery of large number of SNPs, genetic associations with closely linked SNPs are widely studies[22]. In the present study, haplogroups of *turPer3* gene were significantly associated with most of the growth and reproductive traits. Thus, it is suggested that *turPer3* gene is a good candidate gene for further association studies in turkey.

CONCLUSION

The *turPer3* gene is a good candidate gene for further association studies in turkey. The DNA sequence variations of the turPer3 gene may seem to 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 will be needed to show the value of our genetic data in genotype:phenotype correlations in the turkey .

SIGNIFICANCE STATEMENT

This study discovers seven SNPs, including one SNP in the exon 18 and the intron 5, two SNPs in the exons 19 and three SNPs in the introns 6 of *turPeriod-3* gene that can be beneficial for future genotype:phenotype studies. The reported SNPs represent novel nucleotide variants in the turkey genome. The SNPs detected in the exon 19 are non-synonymous which change from methionine to threonine and serine to phenylalanine at 953rd and 955th amino acid positions, respectively. The information gathered from genetic and association analyses of *turPeriod-3* gene would be useful for the breeder to select the best birds at the early stage of their life. This study will help the researcher to uncover the correlation of genotype:phenotype studies between other clock genes and traits in the turkey using a candidate gene approach.

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REFERENCES

1. Bailey, M.J., N.W. Chong, J. Xiong and V.M. Cassone, 2002. Chicken's cry2: Molecular analysis of an avian cryptochrome in retinal and pineal photoreceptors. FEBS Lett., 513: 169-174.

2. Helfer, G., A.E. Fidler, D. Vallone, N.S. Foulkes and R. Brandstaetter, 2006. Molecular analysis of clock gene expression in the avian brain. Chronobiol. Int., 23: 113-127.
3. Froy, O., 2007. The relationship between nutrition and circadian rhythms in mammals. Front. Neuroendocrinol., 28: 61-71.
4. Yu, W., M. Ikeda, H. Abe, S. Honma and T. Ebisawa *et al*., 1999. Characterization of three splice variants and genomic organization of the mouse BMAL1 gene. Biochem. Biophys. Res. Commun., 260: 760-767.
5. Carpen, J.D., M. von Schantz, M. Smits, D.J. Skene and S.N. Archer, 2006. A silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. J. Hum. Genet., 51: 1122-1125.
6. Katzenberg, D., T. Young, L. Lin, L. Finn and E. Mignot, 1999. A human period gene (HPER1) polymorphism is not associated with diurnal preference in normal adults. Psychiatric Genet., 9: 107-109.
7. Toh, K.L., C.R. Jones, Y. He, E.J. Eide and W.A. Hinz *et al*., 2001. An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. Science, 291: 1040-1043.
8. Carpen, J.D., S.N. Archer, D.J. Skene, M. Smits and M. von Schantz, 2005. A single nucleotide polymorphism in the 5 untranslated region of the hPER2 gene is associated with diurnal preference. J. Sleep Res., 14: 293-297.
9. Ebisawa, T., M. Uchiyama, N. Kajimura, K. Mishima and Y. Kamei *et al*., 2001. Association of structural polymorphisms in the human period3 gene with delayed sleep phase syndrome. EMBO Rep., 2: 342-346.
10. Johansson, C., M. Willeit, C. Smedh, J. Ekholm and T. Paunio *et al*., 2003. Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. Neuropsychopharmacology, 28: 734-739.
11. Fukada, Y. and T. Okano, 2002. Circadian clock system in the pineal gland. Mol. Neurobiol., 25: 19-30.
12. Adikari, A.M.J.B., W.A.D. Nayananjalie, J. Xu and E.J. Smith, 2016. Phenotypic Variations of growth and reproductive performances among Turkeys (Meleagris gallopavo). Asian J. Poult. Sci., 10: 86-95.
13. Guan, X., T. Geng, P. Silva and E.J. Smith, 2007. Mitochondrial DNA sequence and haplotype variation analysis in the chicken (Gallus gallus). J. Heredity, 98: 723-726.
14. Rozen, S. and H. Skaletsky, 2000. Primer3 on the WWW for General Users and for Biologist Programmers. In: Bioinformatics Methods and Protocols, Misener, S. and S.A. Krawetz (Eds.). Humana Press, Totowa, NJ., pp: 365-386.
15. Excoffier, L. and H.E.L. Lischer, 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and windows. Mol. Ecol. Resour., 10: 564-567.
16. Smith, E.J., T. Geng, E. Long, F.W. Pierson, D.P. Sponenberg, C. Larson and R. Gogal, 2005. Molecular analysis of the relatedness of five domesticated turkey strains. Biochem. Genet., 43: 35-47.
17. Kamara, D., K.B. Gyenai, T. Geng, H. Hammade and E.J. Smith, 2007. Microsatellite marker-based genetic analysis of relatedness between commercial and heritage Turkeys (Meleagris gallopavo). Poult. Sci., 86: 46-49.
18. Cox, D.G. and P. Kraft, 2006. Quantification of the power of Hardy-Weinberg equilibrium testing to detect genotyping error. Hum. Heredity, 61: 10-14.
19. Goodswen, S.J., C. Gondro, N.S. Watson-Haigh and H.N. Kadarmideen, 2010. FunctSNP: An R package to link SNPs to functional knowledge and dbAutoMaker: A suite of Perl scripts to build SNP databases. BMC Bioinformat., Vol. 11. 10.1186/1471-2105-11-311
20. Hayes, B.J., S. Lien, H. Nilsen, H.G. Olsen and P. Berg *et al*., 2008. The origin of selection signatures on bovine chromosome 6. Anim. Genet., 39: 105-111.
21. Rothschild, M.F. and M. Soller, 1997. Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe Newslett. Agric. Genomic, 8: 13-20.
22. Sha, Q., H.S. Chen and S. Zhang, 2007. A new association test using haplotype similarity. Genet. Epidemiol., 31: 577-593.