RESEARCH ARTICLE

Haplotype structure and variation of telomerase reverse transcriptase (*tur*TERT) gene in turkeys (*Meleagris gallopavo*)

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Abstract:
Telomerase is a specialized ribonucleoprotein enzyme complex that adds telomere repeats (5'-TTAGGG-3') to the end of chromosomes. Mutations in telomerase reverse transcriptase (TERT) gene leads malfunction of telomerase enzyme which causes short telomeres and associated with age-related diseases. A total DNA sequence of 28 kb including the *tur*TERT gene was screened by re-sequencing for structural variation based on single nucleotide polymorphisms (SNPs) and haplotypes using a diversity panel of turkeys. Seven SNPs, including four and three SNPs were identified in the introns 7 and 8 respectively. The minor alleles ranged in frequency from 0.05 to 0.30 with the observed heterozygosity from 0.09 to 0.42.Most of the SNPs did not follow the Hardy Weinberg Equilibrium ($P<0.05$). Linkage disequilibrium among the SNPs ranged from 0.46 to 1.00. A total of 15 haplotypes were identified and assembled into 4 haplogroups. The haplogroups frequencies ranged from 0.10 to 0.38 in the diversity panel of turkeys. The most frequent haplogroup was turTERTHap3 with a frequency of 0.38. Of the haplogroups, Royal Palm had a unique haplogroup of *tur*TERTHap1 as expected while most of Commercial, Bourbon Red, Blue Slate and Spanish Black birds had another unique haplogroup of *tur*TERTHap4. Most of wild turkeys were laid within the haplogroup of *tur*TERTHap3. The haplotype groupings of TERT gene variations of turkeys confirmed that Royal Palm and wild turkey birds possess unique genetic groups.The genomic reagents gathered in the present study will be useful for future genotype: phenotype evaluation studies between *tur*TERTand traits in the turkey using a candidate gene approach.

Key words: Telomerase reverse transcriptase, SNPs, haplogroups, diversity panel

Introduction

Telomeres, located at the end of eukaryotic chromosome, are composed of tandem DNA repeats of the sequence 5'-TTAGGG-3' in vertebrates[1]. Telomere plays a critical role in protecting chromosome integrity by differentiating chromosome ends from DNA breaks and also overcome the end replication problem[2]. Telomerase is an enzyme that adds telomere repeats onto the telomere, thereby it controls the cellular replicative capacity and senescence[3]. Telomerase also has other important functions such that maintenance of telomere length [4]. Telomere length is maintained for the given organism at a constant length. There are many processes including end replication and nuclease activity, which shorten telomeres. This shortening process is balanced by adding *de novo* telomere repeats from telomerase[5]. The primary mode of telomere restoration is through an enzyme, telomerase which consists of two essential components, a catalytic protein subunit telomerase reverse transcriptase (TERT) and a template RNA subunit (TR). TERT and TR together reconstitute telomerase activity[6]. Mutations in TERT gene lead to malfunction of telomerase enzyme which causes short telomeres and which is associated with age-related diseases including heart disease, hypertension and dementia as well as risk factors like insulin resistance and obesity in humans[7].

In humans, numerous reports suggest that single nucleotide polymorphisms (SNPs) located in human TERT (hTERT) locus are associated with exceptional longevity[7], coronary artery disease[8], pulmonary fibrosis[9], several cancers[10]and breast cancer[11].

Though many studies of TERT gene mutations and its association with aging and diseases have been carried out in humans, very few studies have been done in poultry. Therefore, it is important to understand the mechanism of TERT gene and its association with aging and diseases in poultry.

Moreover, the recently released turkey genome sequence offers an opportunity to characterize and define the role of some genes that affect turkey performance and productivity. As a proof of concept, we evaluated the structural variation of TERT gene in turkeys (*tur*TERT). The objective of the study was to screen the *tur*TERT gene for structural variation based on SNPs and haplotypes using a diversity panel of turkeys consisting of birds from heritage (native), commercial and wild varieties.

Materials and Methods

Animals and DNA Extraction

A diversity panel including 42 birds from heritage, commercial and wild turkey varieties were used for the analysis.Blood samples were used to isolate genomic DNA using a salting out procedure [12]. Purity and quantity of the DNA was checked using a NanoDrop 1000.

Gene Sequences and Primer Design

The *tur*TERTgene sequences in the genomic database were amplified and re-sequenced from a diversity panel of 42 turkeys. Four overlapping primer pairs to scan the *tur*TERTgene, were designed for polymerase chain reaction (PCR) using Primer3 software (Table 1)[13]. Each amplicon was purified using DiffinityRapidTips, and sequenced using an ABI Genetic Analyzer 3730 with BigDye Terminator Version 3.1. Sequences for *tur*TERT gene from 42 birds included in the study were analyzed with Phrap (for assembly of the sequences), Polyphred (for scanning the traces), and Consed (for viewing the analysis) to detect nucleotide variation as described by Guan *et al*[14].

PCR Amplification

Long range PCR using Takara Taq Polymerase was performed. The reaction parameters were as follows: 30 s at 940C, 1 min at 60 – 63.30C, and 6 min at 720C for a total of 30 cycles in a GeneAmp, PCR System 9700.

Statistical Analysis

Pairwise linkage disequilibrium (LD) among loci was evaluated and Hardy-Weinberg Equilibrium (HWE) was tested with locus by locus option using the software package Arlequin ver3.5[15]. Haplogroups were manually determined based on the output from Visual Haplotype (VH1) software (http://pga.gs.washington.edu/VH1.html).

Results and Discussion

Amplicons produced by the four primer-pairs spanned a 28kp region that included the *tur*TERT gene (Table 1). A total of 7 SNPs were detected 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 detected in introns 7 and 8 respectively. The seven putative SNPs discovered in the current study have not been published earlier in the dbSNP, NCBI. Though as expected, most of the SNPs were C - T transitions while two were transversions (A-T/ A-C). Within the 42 birds screened, the minor allele frequency (MAF) for 7 SNPs ranged from 0.05 to 0.30 with the observed heterozygosity from 0.09 to 0.42. A significant fraction of the SNPs deviated from HWE ($P<0.05$) (Table 2). Table 3 summarizes the extent of linkage disequilibrium (D´) among SNPs in turTERT gene for a diversity panel of turkeys. Across all SNPs, D´ ranged from 0.46 to 1.00. The correlation coefficient (r^2) for the SNPs ranged from 0.02 to 0.84. The average gene diversity over loci was 0.36 ± 0.22 while overall gene diversity was 0.82 ± 0.02. A total of 15 haplotypes were identified and assembled into 4 haplogroups (Figure 1). The haplogroups ranged in frequency from 0.10 to 0.38 in the diversity panel of turkeys (Table 4). The most frequent haplogroup identified in the studied group was *tur*TERT Hap3 with a frequency of 0.38. In addition, *tur*TERT Hap1 and *tur*TERT Hap4 had frequencies of 0.24 and 0.28, respectively (Table 4).

Of the haplogroups, Royal Palm turkey had a unique haplogroup of *tur*TERT Hap1 as expected while most of Commercial, Bourbon redand Spanish black turkeys had another unique haplogroup of *tur*TERTHap3. Most of wild turkeys were placed within the haplogroup of *tur*TERTHap4 (Figure 1). When haplogroups are compared, Hap1 where Royal palm belonged, consisted of four different rare alleles which were absent in other haplogroups.

Similarly, Hap4 where most of the wild turkeys placed, had onerareallele which was not observed in other haplogroups (Figure 1). The BLAST analysis of *tur*TERT gene sequence against the chicken and Zebra finch genome revealed 93 and 86 % sequence identity respectively. SNPs have been reported in the TERT gene of chicken and Zebra

Table 1. Primer sequences, the expected sizes of amplicons and PCR characteristics for the *tur*TERT-gene.

Primer ID	Primers[1]	Sequences	Tm[2](⁰C)	Product size (bp)[3]
TERT1	For(34986462)	5′-GGCTTTTAAGTTTGTTAAAACTCCTTTATT-3′	60.0	8500
	Rev(34977884)	5′-GCTATTTTGTTAGACTAATATCATTAAGACAG-3′		
TERT2	For(34978396)	5′-CCAAGTTCTGTAGAGATTAGTATTTGTAGT-3′	61.0	8000
	Rev(34970119)	5′-GTTTAACTACCGTAAAATAAAGTTAGTCTC-3′		
TERT3	For(34970502)	5′-GTAGATACATAACCTTCATTTAGAGCTTCAG-3′	63.3	2500
	Rev(34968006)	5′-CCAATAAATCCTGTTAAGAGACAGATCATAG-3′		
TERT4	For(34968271)	5′-GAACTTAAGAAACCAAGATTCAGAAGAG-3′	61.0	9000
	Rev(34956117)	5′-ATCTTTATCCCATATATCTTGCTAGACG-3′		

[1]For, forward primer; Rev, reverse primer. Primer-binding sites in the turkey genome are presented in parentheses.
[2]The optimized annealing temperature at which a single amplification of the expected size was obtained.
[3]Length in base pairs (bp) of the expected amplification based on the binding sites of the forward and reverse primers.

Table 2. Characteristics of single nucleotide polymorphisms (SNPs) identified in the *tur*TERT gene in a diversity panel of turkeys comprising heritage, commercial and wild birds.

SNP ID	Location	Nucleotide position[1]	Sequence context of SNP[2]	dbSNP Identification[3]	Genotype	Genotype Frequency (%)	MAF[4]	HWE[5]
turT-1	Intron 8	34968046	CTTTT(*T*/A)AAAGC	*rs*271791588	A/A A/T T/T	73.8 4.8 21.4	0.19	0.00*
turT-2	Intron 8	34968178	AAAGG(*A*/C)CAAAT	*rs*271791589	C/C C/A A/A	69.0 7.2 23.8	0.27	0.00*
turT-3	Intron 8	34968454	GCCTC(*T*/C)CAAAA	*rs*271791590	C/C C/T T/T	76.2 0.0 23.8	0.24	0.00*
turT-4	Intron 7	34970042	CTAAA(*C*/T)GCTAG	*rs*271791591	T/T C/T C/C	64.3 11.9 23.8	0.30	0.00*
turT-5	Intron 7	34970289	CTCCT(*C*/T)TGTAA	*rs*271791592	T/T C/T C/C	90.5 9.5 0.0	0.05	NS
turT-6	Intron 7	34970355	TTATA(*T*/C)TTTTA	*rs*271791593	C/C C/T T/T	66.7 7.1 26.2	0.30	0.00*
turT-7	Intron 7	34970401	AGTTT(*T*/C)ATTTT	*rs*271791594	C/C C/T T/T	66.7 7.1 26.2	0.30	0.00*

[1]Position of the SNP in Ensembl on the forward strand of chromosome 3 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 here and assigned numbers in dbSNP, NCBI.
[4]Minor allele frequency (MAF) of 7 SNPs markers.
[5]Significance of deviation from Hardy-Weinberg Equilibrium (HWE) for the 7 SNPs. NS indicates non-significant (P> 0.05) while * refers to significance at P< 0.05.

finch. However, SNPs in *tur*TERT gene have not been reported yet and these 7 SNPs which are located in the TERT gene of turkeys became the novel SNPs.In Summary, the haplotype groupings in terms of TERT gene variations of turkeys confirmed that Royal Palm and wild turkeys possess

Table 3. Linkage disequilibrium as measured by D´ and r² between the 7 segregating SNPs in the *tur*TERT gene.

SNPs[1]	*turT-1*	*turT-2*	*turT-3*	*turT-4*	*turT-5*	*turT-6*	*turT-7*
turT-1		1.00	0.46	0.48	0.67	0.57	0.50
turT-2	0.12		1.00	0.86	1.00*	1.00	1.00
turT-3	0.19	0.13		1.00	0.66	1.00	1.00
turT-4	0.15	0.13	0.75		0.63*	0.82	0.82
turT-5	0.07	0.02*	0.06	0.04*		0.64	0.64
turT-6	0.24	0.16	0.83	0.60	0.05		0.89
turT-7	0.18	0.16	0.84	0.61	0.05	0.79	

[1]SNP identification (see Table 2).
* indicates non-significant (P> 0.05) D´ and r2 values.
D´ values are listed in upper right section, and r2 values are listed in lower left section.

Table 4. *tur*TERT haplogroups diversity and their frequencies.

ID	N[1]	Haplogroups	Frequency[2]
*tur*TERT Hap1	10	-A*-C-T-C-T*-T-T-	0.24
*tur*TERT Hap2	04	-A*-C-C-T*-T*-C*-C*-	0.10
*tur*TERT Hap3	16	-A-A*-C-T*-T-C-C-	0.38
*tur*TERT Hap4	12	-A*-A*-C-T-T-C-C-	0.28

[1]Number of haplotypes within the haplogroups.
[2]Frequency was calculated in a total of 42 diversity panel of turkeys.
The variant nucleotide within each haplogroup is represented by

*Alternate alleles are shown in Table 2.

unique genetic groups. The information gathered in the present study, would be useful for future genotype:phenotype evaluation (association mapping) studies between *tur*TERT and economically important traits in the turkey using a candidate gene approach.

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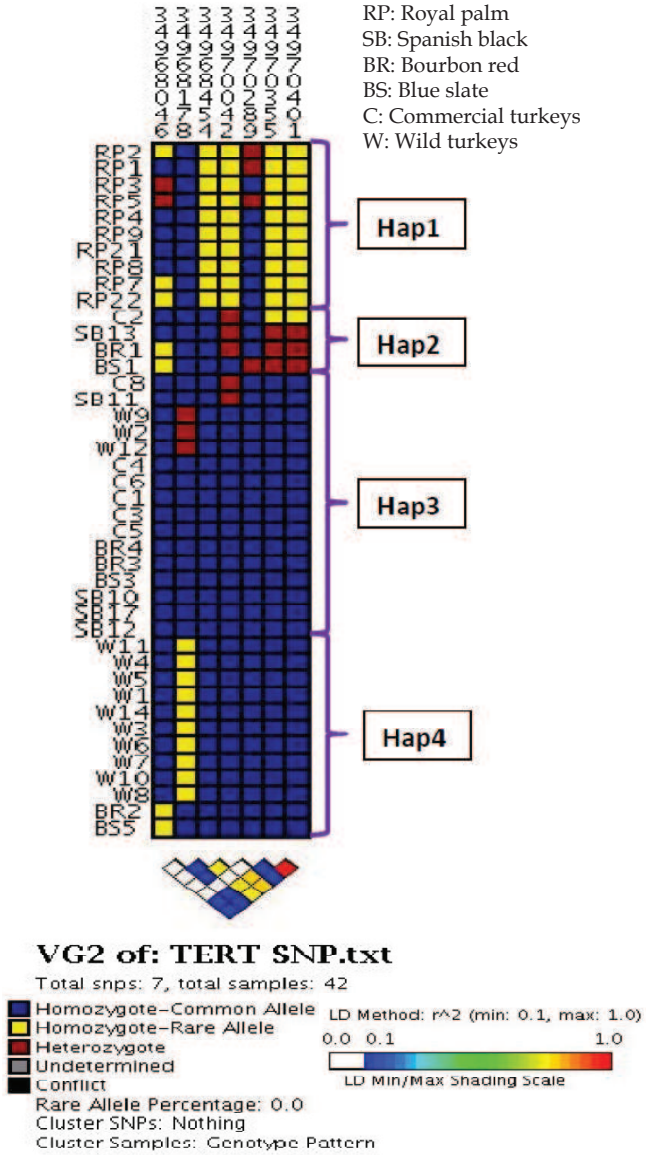

Figure 1. Visualization of haplogroups using Visual Haplotype (VH1) software. (http://gvs.gs.washinton.edu/VH1.html).

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