Genome-wide scan for selection signatures and genes related to heat tolerance in domestic chickens in the tropical and temperate regions in Asia

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ABSTRACT Heat stress is one of the major environmental stressors challenging the global poultry industry. Identifying the genes responsible for heat tolerance is fundamentally important for direct breeding programs. To uncover the genetic basis underlying the ambient temperature adaptation of chickens, we analyzed a total of 59 whole genomes from indigenous chickens that inhabit South Asian tropical regions and temperate regions from Northern China. We applied F_{ST} and π-ratio to scan selective sweeps and identified 34 genes with a signature of positive selection in chickens from tropical regions. Several of these genes are functionally implicated in metabolism (*FABP2, RAMP3, SUGCT*, and *TSHR*) and vascular smooth muscle contractility (***CAMK2***), and they may be associated with adaptation to tropical regions. In particular, we found a missense mutation in thyroid-stimulating hormone receptor (41020238:G>A) that shows significant differences in allele frequency between the chicken populations of the two regions. To evaluate whether the missense mutation in *TSHR* could enhance the heat tolerance of chickens, we constructed segregated chicken populations and conducted heat stress experiments using homozygous mutations (**AA**) and wild-type (**GG**) chickens. We found that GG chickens exhibited significantly higher concentrations of alanine aminotransferase, lactate dehydrogenase, and creatine kinase than AA chickens under heat stress (35 ± 1°C) conditions ($P < 0.05$). These results suggest that *TSHR* (41020238:G>A) can facilitate heat tolerance and adaptation to higher ambient temperature conditions in tropical climates. Overall, our results provide potential candidate genes for molecular breeding of heat-tolerant chickens.

Key words: chicken, heat tolerance, TSHR, adaptation, genome sequencing

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INTRODUCTION

With the development of society and the increase in the human population, the need for protein resources and food is increasing. Domestic chickens are one of the most economically important poultry species, providing the primary protein resource for humans in most areas of the world (Liu et al., 2020). However, chickens are sensitive to high ambient temperatures due to their feather coverage and the lack of functional sweat glands (Loyau et al., 2013). Under an environmental temperature of 18 to 21°C, chickens have optimal production performance (Kumari and Nath, 2019), but above 25°C, chickens may suffer from heat stress, which could lead to death in severe heat stress situations (Donkoh, 1989). Exposure of chickens to heat stress results in a series of physiological dyshomeostatic processes, including systemic immune dysregulation, endocrine disorders, respiratory alkalosis, and electrolyte imbalance, which affect health and performance (Liu et al., 2020). Heat stress reduces feed intake and weight gain (Ma et al., 2021), resulting in impairment of growth performance (Beckford et al., 2020). Heat stress also decreases egg production by inducing apoptosis of follicular cells (Li et al., 2020) and reduces meat quality by changing aerobic metabolism and glycolysis (Zaboli et al., 2019), which results in pale meat color, a decrease in muscle pH, reduced water-binding capacity, and increased cook and drip losses (Zaboli et al., 2019). In addition, heat stress affects carcass traits and animal well-being (Cândido et al., 2020; Huang et al., 2020) and impairs the function of the digestive (Quinteiro-Filho et al.,

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2012) and immune systems (Sugiharto et al., 2017). With the rising severity of global warming, heat stress triggered by high ambient temperature is the main environmental factor causing huge enormous economic losses (St-Pierre et al., 2003), challenging the poultry industry, especially in tropical and subtropical regions (Gregory, 2010). Thus, uncovering the genetic basis underlying the heat stress response has both theoretical importance and practical applications in facilitating breeding programs to address global warming.

Since domestication, chickens have been translocated across the globe and developed into thousands of breeds with remarkable phenotypic characteristics in morphology, physiology, and behavior (Guo et al., 2016; Ulfah et al., 2016; Wang et al., 2020). These chicken breeds have adaptations to diverse environmental conditions spanning torrid and subtropic environments (Wang et al., 2015; Ulfah et al., 2016), providing excellent models with which to study the genetic mechanisms underlying the temperature adaptations. Recently, using a comparative genomic strategy, Tian et al. (2020) suggested that 12 genes found to be under selection are involved in adaptations to both tropical desert and tropical monsoon island climates of Saudi Arabian and Sri Lankan indigenous chickens. Additionally, based on 600k chicken genotyping array data of Sri Lankan, Brazilian, and Egyptian chickens, Walugembe et al. (2019) identified several genes, including *TRMT1L, SOCS2*, and *NFKB1,* with a signal of positive selection and concluded that these genes may be involved in the survival of chickens in hot conditions. However, the aforementioned studies were restricted to a single chicken breed or small sample size, and the results, particularly the selective sweeps analysis, may be biased by genetic drift. Despite the many efforts that have been made, our understanding of the genetic basis adaptation of chickens to high ambient temperatures remains limited.

In the present study, we analyzed 59 genomes of indigenous chickens from the tropical and temperate zones to evaluate the potential genomic footprints responsible for environmental heat adaptation. Our results add to the growing knowledge on the genetic mechanisms of temperature adaptation and may benefit future breeding design.

MATERIALS AND METHODS

Ethical Approval of the Study

The study protocol was approved by the Animal Care and Use Committee of Anhui Agricultural University (approval no: SYXK2016-007).

Samples, Read Mapping, and SNP Calling

Fifty-nine genomes from our previous studies (Wang et al., 2020), including 2 Red Jungle Fowls, 26 village chickens from northern China, 18 indigenous Indonesian chickens, 7 individuals from Sri Lanka, and 6 indigenous chickens from Thailand, were analyzed in the present study. The geographic distribution of these samples is provided in Figure 1A, and the sample information is available in Supplementary Table S1.

We removed low-quality bases using Btrim (version 0.2.0) (Kong, 2011) with parameres '-s -a 20 -q'. The high-quality sequence reads were mapped onto the chicken reference genome (GRCg6a) using BWA-MEM (Li, 2014) with default parameters. The BAM file produced for each individual was sorted using the 'SortSam' program in the Picard package (version 1.119) (http://picard.sourceforge.net). PCR duplicates were identified and removed using the 'MarkDuplicates' program of Picard (version 1.119). We then used the function index from Samtools (version 1.9) (Li et al., 2009) to create index files and used the 'UnifiedGenotyper' function implemented in Genome Analysis Toolkit (GATK v3.5) to call SNPs with default parameters (McKenna et al., 2010). SNPs were filtered with VariantFiltration tools in GATK (version 3.5) with the same parameters used previously (Wang et al., 2017;) ("QUAL < 40.0 MQ < 25.0 MQ0 >= 4 & ((MQ0/(1.0*DP)) > 0.1) -cluster 3 -window 10").

Population Structure, Diversity, and Relationships

For the estimation of population structure, PCA was performed using GCTA (Yang et al., 2011). Our analysis was based on a SNP dataset that was pruned with PLINK with the settings "−indep-pairwise 50 10 0.1". First, we calculated the genetic relationship matrix with the "−make-grm" option and then estimated the three principal components with the "−pca 3" option. The genome-wide nucleotide diversity (π) of each population was calculated using VCFtools (Danecek et al., 2011) with a nonoverlapping window size of 50 kb. To reveal the phylogenetic relationships of chickens, neighbor-joining (**NJ**) and maximum-likelihood (**ML**) trees were constructed using MEGA7 (Kumar et al., 2016) and FastTree (Price et al., 2010), respectively. To reduce potential bias arising from putatively missing and/or unreliable genotypes, the SNP dataset used for phylogenetic reconstruction was filtered using VCFtools (version 0.1.16) (Danecek et al., 2011), with the parameters "−maf 0.01 −max-missing 0.5".

Screening for Selective Sweeps

The allele frequency of variable sites was used to identify regions with signatures of positive selection. We calculated the population fixation statistics (F_{ST}) and π-ratio $_{(Temperate/Tropical)}$ across the chicken genome using a genome-wide sliding window strategy (50-kb windows with 25-kb steps). The putatively positively selected genes (**PSGs**) with the top 1% of both the π-ratio and F_{ST} values were extracted within the windows. A gene enrichment analysis was performed with g:profiler (Reimand et al., 2016). Benjamini−Hochberg FDR (false discovery rate) was used for correcting the P values. The enrichments for the Gene Ontology categories "molecular function", "biological process" and

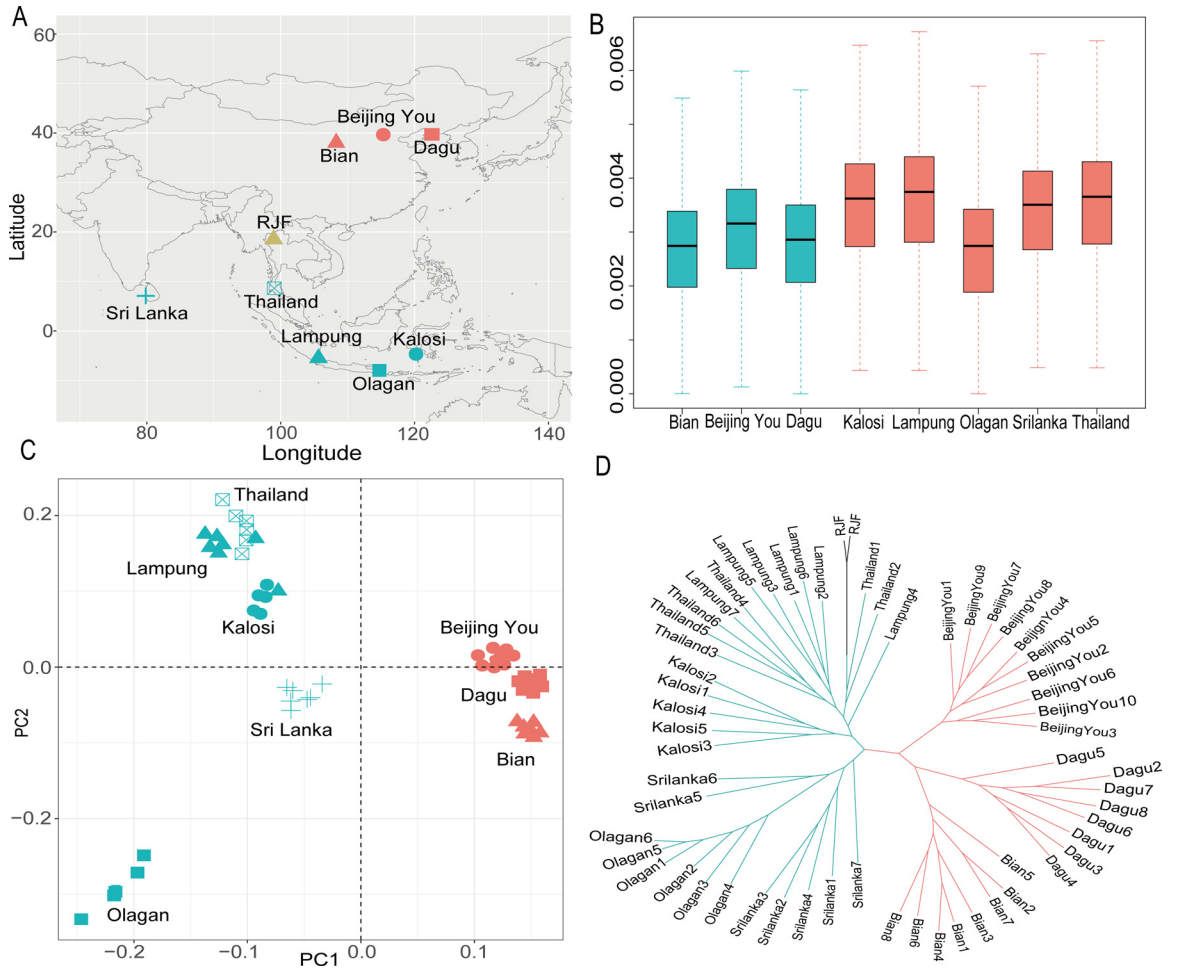

Figure 1. Geographic distribution, population relationships, and genetic diversity of chickens. (A) Geographic distribution of samples used in this study. (B) Population nucleotide diversity. (C) Principal component analysis. (D) ML tree constructed based on autosomal data.

"cellular component" and human phenotype (**HP**) categories were analyzed.

To survey advantageous alleles for chickens living in the tropical region, we further extracted allele counts for the non-synonymous variants of the PSGs among the 57 domestic chickens and used the chi-squared test to filter variants that showed significant differences in allele frequencies between the tropical and temperate populations. After filtration, 15 variants located within chromosomes 2 to 5 were retained (Supplementary Table S2).

Prediction of gga-mir-7468 Target Genes and Functional Annotation

The target genes of gga-mir-7468 were predicted using TargetScan (Agarwal et al., 2015) and miRDB (Chen and Wang, 2020). Based on the combination of the methods, only those supported by 2 programs were selected for further study. Enrichment analyses of target genes were performed with g:profiler (Reimand et al., 2016).

Sequence Retrieval and Structural Analysis

The protein sequences of thyroid-stimulating hormone receptor (**TSHR**) and carboxypeptidase Z (**CPZ**) were downloaded from Ensembl (https://asia.ensembl.org/index.html). Their protein architecture (i.e., domain organization) was examined using the Pfam sequence search engine (Mistry et al., 2021).

SNP Validation

A total of 142 individuals from 18 chicken breeds, including 7 breeds (Hetian chicken, Shouguang chicken, Luxi gamefowl, Gushi chicken, Nanjiang gamefowl, Hetian black chicken, and Tulufan gamefowl) from temperate regions and 11 breeds (Wenchang chicken, Huiyang chicken, Black Java chicken, Black Sumatra chicken, Kedu Hitam chicken, indigenous Sumatran breed, indigenous Yunnan breed, Merawang, indigenous Guangxi breed, indigenous Vietnamese breed, and Balinese fighting chicken) from tropical regions, were used to measure the allele frequency of 15 SNPs. Detailed information is listed in Supplementary Table S3. We only mapped sequencing reads to chicken chromosome 2 to chromosome 5 and called SNPs using the aforementioned methods and extracted allele counts of the 15 SNPs in each chicken population. A chi-squared test was used to measure the differences in allele frequencies between the tropical and temperate populations.

TSHR (41020238: G/A) Segregation Resource Population

The TSHR (41020238: G/A) segregation population was constructed by crossing between the 20 heterozygous Huainan roosters and 100 heterozygous Huainan hens (a Chinese indigenous breed with dual purpose). In total, 600 eggs were collected and incubated. After hatching, the chickens were wing-banded. Water and commercial chicken feed were supplied ad libitum. The diet contained 12.59 **MJ**/kg of metabolizable energy (**ME**) and 20.5% crude protein (**CP**) at 1 to 3 wk of age, 12.98 MJ/kg **ME** and 18.5% CP at 4 to 7 wk of age, and 11.75 MJ/kg ME and 17.0% CP thereafter. At 9 wk of age, blood samples from each individual were collected via the wing vein. Genomic DNA was extracted from blood using the phenol−chloroform method. The DNA concentration and quality were examined using gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Genotyping was performed using the PCR-Sanger sequencing method. Individual PCR amplification was performed with the primer F: 5'-TGGACCTCTACACCAGGTCA-3'&&R: 5'ACAGCCATCCTCTTGGCAAT-3'. The PCR conditions were as follows: 5 min at 94°C for one cycle and 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C for 35 cycles. The PCR products were sequenced on an ABI 3730 sequencer (Applied Biosystems, Forster City, CA). From the PCR results we found 64 homozygous mutations (**AA**), 128 heterozygous mutations (**AG**), and 54 wild-type (**GG**) male chickens.

Thermal Stress of Chickens

At 140 d of age, 30 males Huainan chickens with similar body weights were selected from the AA (1796 ± 123 g) and GG groups (1,789 ± 130 g). Fifteen birds for each genotype were assigned to the control group and placed under normal temperature (20 ± 1°C). Fifteen birds from the 2 groups were assigned to the heat stress group and exposed to high temperatures (35 ± 1°C) for 12 h.

Serum Parameters

Blood samples of 60 birds were collected via the wing vein before and after the heat stress experiment and centrifuged at 4,000 × *g* for 15 min to separate the serum. The serum samples were stored at −20°C until analysis. The levels of serum total protein (**TP**), albumins (**ALB**), alanine aminotransferase (**ALT**), alkaline phosphatase (**ALP**), lactate dehydrogenase (**LDH**), and creatine kinase (**CK**) were measured by the methods of biuret, bromocresol green, alanine substrate, 2-amino-2-methyl-1-propanol (**AMP**) buffer, lactic acid substrate, and N-acetylcysteine, respectively, using a Toshiba 120 automated biochemical analyzer (Toshiba, Tokyo, Japan).

Statistical Analysis

Serum biochemical parameters were analyzed by two-way ANOVA using R software (version 4.04) with stress temperature and genotype as the main effects, and their interactions were also analyzed. The Tukey method was used for comparisons among multiple means, and $P < 0.05$ was considered statistically significant.

RESULTS

Genomic Variants and Diversity

A total of 59 genomes were analyzed in this study (Supplementary Table S1). After quality filtering and mapping the reads to the chicken reference genome, we counted the genome sequencing depth for each sample, which ranged from 4.7 to 12.0 × . We identified ∼12.99 million biallelic SNPs. A total of 93.04% of the SNPs were located in intronic regions and intergenic regions (Supplementary Table S4), and 3.59% were located within regions upstream or downstream of the transcription start or end sites. Only 1.84% was assigned to exonic regions (Supplementary Table S4). To measure the genetic diversity for each population, we estimated the nucleotide diversity. We found that the chickens from the tropical regions (except Olagan chicken) had higher levels of nuclear diversity than the chickens from the temperate regions (Figure 1B).

Population Relationship and Structure

To investigate the genetic structure of these chicken populations, we performed a principal component analysis (**PCA**). The first principal component (**PC1**) explained more than 30.3% of the total variance and clearly distinguished tropical chickens from temperate chickens (Figure 1C). This analysis suggests genetic differentiation between the two groups of chickens. The genetic clustering analysis using ADMIXTURE also corroborates the pattern found with PCA (Supplementary Figure S1). When K = 2, we found a division between the tropical and temperate chickens, although tropical chickens appear to harbor a component that is found predominantly in temperate chickens (Supplementary Figure S1). To infer the phylogenetic relationships of these chickens, we further used autosomal SNPs to construct ML and NJ phylogenetic trees. Both the ML and NJ trees showed that chickens from the temperate region clustered together, and chickens from the tropical regions grouped separately (Figure 1D and Supplementary Figure S2).

PSGs in Tropical Chickens

The above analysis showed a clear genetic differentiation between the tropical region and temperate chickens, which might be partially driven by location environments. To identify potential mechanisms for the adaptation to tropical temperature, we used F_{ST} and π-ratio to

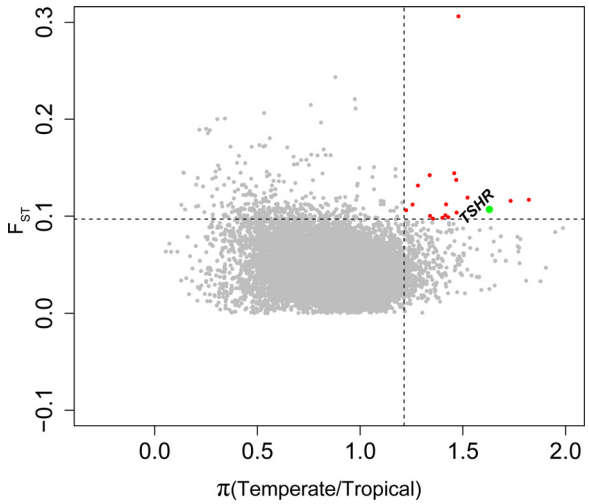

Figure 2. The putative selected genomic regions in tropical populations were identified using both fixation statistics (F_{ST}) and π-ratio (Temperate/Tropical) approaches with a sliding window strategy (50-kb windows with 25-kb steps).

scan for footprints of positive selection in tropical region chickens, and a total of 301 and 389 PSGs were identified, respectively (Supplementary Tables S5 and S6). Thirty-four genes, including one minRNA (gga-mir-7468) and 6 long noncoding RNAs (lncRNAs), were detected by both statistics (Figure 2 and Supplementary Table S7). The functional enrichment analysis using protein-coding PSGs showed that these genes were associated with 'sphingosine N-acyltransferase activity' and 'thyroid-stimulating hormone receptor activity' (Supplementary Table S8). Among these PSGs, several genes (*FABP2, RAMP3, SUGCT, CAMK2,* and *TSHR*) are functionally related to metabolism and vascular smooth muscle contractility and could potentially be involved in the adaptation of chickens to hot environments.

We further predicted target genes of gga-mir-7468. A total of 319 genes were identified (Supplementary Table S9). There were no overlapping genes between the 319 target genes and 34 PSGs. The enrichment analyses showed that these target genes were involved in 'metabolic process' and 'regulation of signal transduction' (Supplementary Table S10).

Potential Causative Mutations in PSGs

We screened for nonsynonymous variants among the PSGs across the tropical and temperate populations. After filtration, 15 variants remained, and they had significant differences in allele frequencies between the 2 populations (Supplementary Table S2). We further genotyped the 15 SNPs in 142 chickens from 18 breeds and found that 2 variants, TSHR (41020238:G>A, resulting in a Gly558Arg mutation) and CPZ (81175728:G > A, resulting in an Ala34Thr mutation), showed significant differences in allele frequencies between the tropical and temperate populations (Figure 3 and Supplementary Table S11). The protein domain prediction analysis revealed that the missense mutation in the *TSHR* gene c.41020238:G>A (p.Gly558Arg) is located in the 7tm_1 domain (Figure 4).

Blood Chemistry Parameters

We examined components of serum from both AA and GG group chickens that are both under normal temperature (20 ± 1°C) and high temperatures (35 ± 1°C). The effects of heat stress on the blood biochemistry parameters of AA and GG chickens are presented in Table 1.

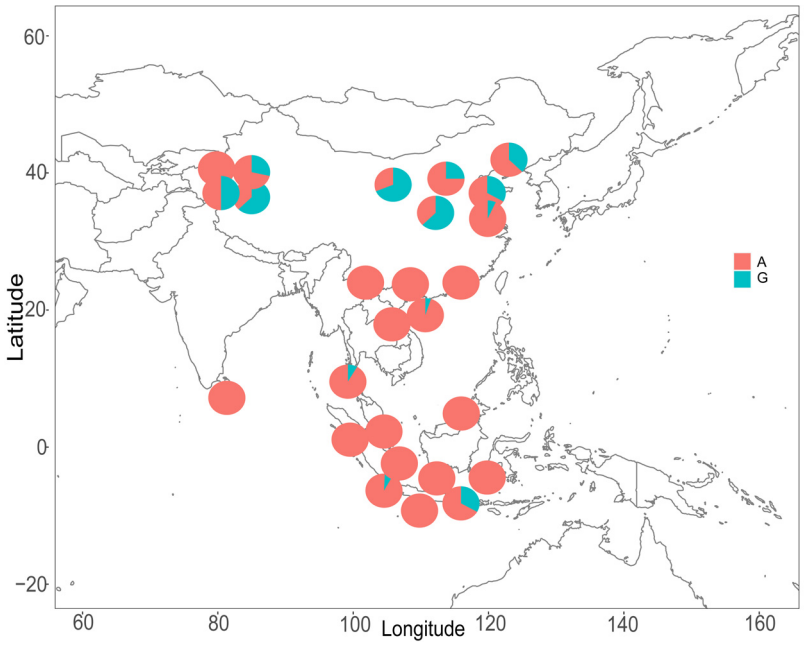

Figure 3. Allele frequency distribution of TSHR (41020238:G>A) in domestic chickens from temperate (10 breeds) and tropical regions (16 breeds).

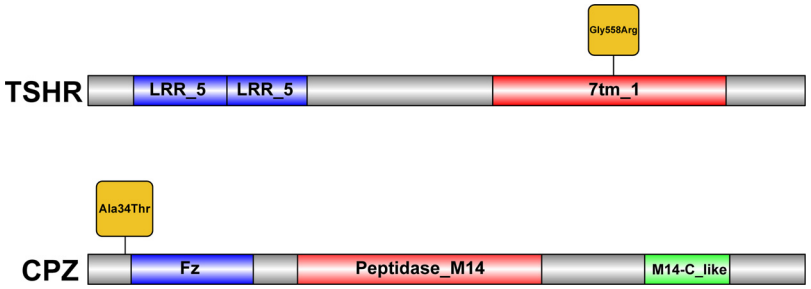

Figure 4. Schematic representation of the thyroid stimulating hormone receptor (TSHR) and carboxypeptidase Z (CPZ) domain organization. LRR_5: leucine-rich repeat domain; 7tm_1: seven-(pass)-transmembrane domain receptors, also known as G protein-coupled receptors (GPCRs); Fz: cysteine-rich domain of CPZ; Peptidase_M14:peptidase M14-like domain of CPZ; M14-C_like: CPZ regulatory-like domain.

Neither heat stress nor genotype had an effect on the TP, ALB, or ALP concentrations ($P > 0.05$). Genotype had no effects on the ALT, LDH, or CK concentrations. However, heat stress significantly elevated the ALT, LDH, and CK concentrations in each group ($P < 0.01$). Moreover, after heat stress, wild-type chickens exhibited significantly higher concentrations of ALT, LDH, and CK than homozygous chickens ($P < 0.05$ or $P < 0.01$).

DISCUSSION

With rising global temperatures, heat stress triggered by high ambient temperature is the crucial factor challenging the development of poultry production. Genetic selection for heat-tolerant chicken breeds is an effective strategy for alleviating heat stress in the poultry industry. Since domestication, chickens have been translocated across the world and have adapted to a variety of local environments, such as hot and temperate conditions. To study the tropical adaptive mechanism of chickens, 3 indigenous Chinese chicken breeds from temperate zones and 5 indigenous chicken breeds from tropical regions were analyzed. We calculated π-ratio ($_{\text{Temperate/Tropical}}$) and F_{ST} to identify the PSGs in chickens from tropical climates.

We found that *CAMK2D* (calcium/calmodulin-dependent protein kinase II delta) showed a signal of positive selection in tropical chickens. *CAMK2* is expressed as 4 different isoforms (*CAMK2α, CAMK2β, CAMK2d, and CAMK2γ*) (Marganski et al., 2005; Saddouk et al., 2017) and plays an important role in the control of vascular smooth muscle contractility (Rokolya and Singer, 2000). The balance between vascular contraction and relaxation plays a pivotal role in body temperature homeostasis (Ai et al., 2015; Tian et al., 2020). Vasoconstriction has been linked to a reduction in peripheral blood flow leading to an increase in internal body temperature and vasodilatation. Increased cutaneous blood flow has been reported to increase heat loss (Klotz et al., 2016). The positively selected *CAMK2D* gene suggested that dissipating excessive heat through regulation of blood pressure to maintain the core body temperature is a crucial way for chickens from tropical regions to respond to heat stress.

We found several genes, including *FABP2* (fatty acid-binding protein 2), *RAMP3* (associated receptor-activity modifying protein 3), *SUGCT* (succinyl-CoA: glutarate-CoA transferase), and *TSHR,* which are functionally implicated in metabolism and energy production, to be potentially involved in adaptation to hot environments. This result is in agreement with a previous study (Tian et al., 2020) and in other animals (Ai et al., 2015; Kim et al., 2016). For example, energy metabolism-related genes (*MYH1, MYH3, MYH8, MYH10*, and *MYH13*) showed a signal of selection in sheep that may play an important role in their adaptation to hot arid environments (Kim et al., 2016). Similarly, Ramírez-Ayala et al. (2021) reported that positively selected genes involved in thermogenesis (*ATP9A, GABBR1, PGR, PTPN1*, and *UCP1*) may have crucial roles for French Charolais cattle in adaptation to Cuban tropical conditions. *FABP2* is highly abundant in intestinal enterocytes (Agellon et al., 2002), which play a major role in the

Table 1. Effects of heat stress on serum biochemical parameters of homozygous mutations of TSHR and wild-type chickens.

Parameter	AA[1]		GG[1]		*P*value		
	Standard[2]	HS[2]	Standard[2]	HS[2]	G[2]	T[2]	G*T
TP (g/L)	48.32 ± 3.94	47.00 ± 4.77	48.07 ± 4.67	48.30 ± 6.65	0.30	0.22	0.20
ALB (g/L)	21.10 ± 1.23	20.22 ± 1.54	21.87 ± 1.26	22.12 ± 2.25	0.19	0.17	0.87
ALT (U/L)	3.20 ± 0.72^{c}	7.10 ± 1.89^{b}	3.65 ± 0.65^{c}	12.20 ± 2.44^{a}	0.42	0.006	0.007
ALP (U/L)	798.44 ± 110.13	814.89 ± 134.94	811.56 ± 148.95	815.89 ± 134.83	0.27	0.30	0.41
LDH (U/L)	1,800.56 ± 259.5^{c}	2,043.22 ± 234.94^{b}	1,894.22 ± 248.94^{c}	2,832.56 ± 248.32^{a}	0.07	0.01	0.02
CK (U/L)	1,550.00 ± 261.7^{c}	2,379.22 ± 257.12^{b}	1,373.56 ± 142.75^{c}	2,968.33 ± 100.12^{a}	0.15	0.005	0.001

^{abc}Values of each parameter in a row with different superscripts indicate significance.

Abbreviations: ALB, albumin; ALT, alanine transaminase; ALP, alkaline phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase; TP, total protein.

[1]AA: chickens with the TSHR (41020238:A/A) genotype; GG: chickens with the TSHR (41020238:G/G) genotype.

[2]Standard: ambient temperature was 20 ± 1°C; HS: ambient temperature was 35 ± 1°C; G: genotype; T: temperature.

intracellular transportation of long-chain fatty acids and whole-body energy homeostasis (Gajda et al., 2013). *SUGCT* encodes an enzyme that synthesizes glutaryl-CoA from glutarate in tryptophan and lysine catabolism. A previous study showed that *SUGCT*-null mice exhibited imbalanced lipid and acylcarnitine metabolism (Niska-Blakie et al., 2020). *RAMP3* is a critical member of the amylin receptor (Christopoulos et al., 1999) that plays an important role in glucose and energy homeostasis (Coester et al., 2020). *TSHR* belongs to the G protein-coupled receptor (**GPCR**) superfamily. Usually, TSH combines with TSH receptors to regulate growth and proliferation and plays essential roles in thyroid development and function (Zhou et al., 2018). Recently, many studies have demonstrated that *TSHR* may be involved in regulating energy balance, metabolism, and thermoregulation (Warner et al., 2013; Jiang et al., 2015; Lundbäck et al., 2020; Zhang et al., 2020). In particular, Wang et al. (2021) constructed chicken TSHR (Gly558Arg) knock-in mice and found that TSHR (Gly558Arg) homozygous mice had significantly lower energy expenditure and lower metabolism rates than wild-type mice, especially when fed under high-temperature conditions. Thus, we hypothesize that these energy metabolism-related genes may contribute to the chicken response and adaptation to hot temperature environments. Moreover, other mechanisms, such as epigenetic regulation, may also be involved in the tropical adaptation of chickens. A miRNA (gga-mir-7468) showed a signal of positive selection in tropical chickens. We further predicted target genes of gga-mir-7468 by using TargetScan (Agarwal et al., 2015) and miRDB (Chen and Wang, 2020) and identified 319 genes. There were no overlapping genes between the 319 target genes and 34 PSGs. Enrichment analyses showed that these target genes were involved in the "metabolic process", suggesting that gga-mir-7468 is potentially involved in chicken adaptation to hot environments.

LncRNAs can be not only cis-acting lncRNAs that regulate the expression of target genes that are located at or near the same genomic locus but also trans-acting lncRNAs that can either inhibit or activate gene transcription at independent chromosomal loci (Fatica and Bozzoni, 2014). Among the 6 lncRNAs under selection in chickens from tropical climates, the location of ENSGALG00000047413 overlaps with *TSHR*. However, the function of both gga-mir-7468 and 6 lncRNAs is still unknown, and the role of these noncoding RNAs needs to be studied in the future.

We further searched for nonsynonymous variants among the PSGs found in the 57 sequenced chicken genomes. After filtration, 15 missense mutations in 6 genes remained. We next calculated the allele frequency in another 142 chickens representing both temperate and tropical populations and found only 2 variants with significant differences in allele frequency between the temperate and tropical populations: a missense mutation in TSHR (41020238:G>A, resulting in a Gly558Arg mutation) and a missense mutation in CPZ (81175728:G>A, resulting in an Ala34Thr mutation). CPZ is a member of the carboxypeptidase E subfamily of metallocarboxypeptidases that functions as a regulator in the development of skeletal elements in chickens (Moeller et al., 2003). CPZ was also reported to be associated with the aggressive behavior of gamecock chickens (Luo et al., 2020).

Based on the function of these candidate genes, we decided to evaluate whether the missense mutation in *TSHR* (41020238:G>A) is associated with heat tolerance in chickens because the gene has been well established to have biological significance in TSHR signaling for metabolism and thermoregulation (Warner et al., 2013; Lundbäck et al., 2020, Wang et al., 2021). We constructed *TSHR* (41020238:G/A)-segregating chicken populations and used AA and GG chickens to conduct heat stress experiments. Under thermoneutral conditions, there was no significant difference in blood biochemistry parameters between the 2 groups. However, when chickens were exposed to high temperature, GG chickens exhibited significantly higher concentrations of ALT, LDH, and CK than AA chickens. ALT enzymes were normally expressed in the liver. When the liver is damaged, ALT enzymes are released into the blood (Kim et al., 2015); therefore, the ALT level in the serum is used as a reliable and specific marker of liver damage (Chang et al., 2020). Heat stress is known to induce liver damage (Zeng et al., 2014) and increase serum ALT levels (Chang et al., 2020; Roushdy et al., 2020). Similarly, under normal conditions, LDH and CK were mainly present inside cardiomyocytes but could be released when the cardiomyocytes were damaged (Yin et al., 2020); thus, the contents of serum can be used as important indicators for myocardial damage (Tang et al., 2018; Xu et al., 2019). Previous studies have revealed that heat stress increases the activities of serum CK and LDH (Xie et al., 2015; Luo et al., 2018). In the present study, the ALT, LDH, and CK levels in chickens with homozygous TSHR mutations were lower than those in the wild-type group, indicating less cell damage in homozygous chickens under exposure to heat stress conditions.

CONCLUSIONS

In conclusion, our results suggest that the TSHR (41020238:G>A) variation might regulate the metabolic rate of chickens to enhance heat tolerance and contribute to chicken adaptation to high ambient temperature conditions in tropical climates. However, further studies are necessary to uncover the molecular basis and networks of TSHR (41020238:G>A) in the regulation of the heat stress response of chickens. Overall, our study provides insight into the tropical climates of chickens and will contribute to the molecular breeding of heat-tolerant chicken breeds.

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DISCLOSURES

The authors declare that they have no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101821.

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