Genomics of heat stress in chickens

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ABSTRACT: Global climate change, increasing human population and improved economic status in populous developing countries are causing an expansion of poultry production under hot ambient temperatures. High environmental temperatures have a negative impact on productivity and health in poultry. There is evidence for a substantial genetic component to the bird's response to high temperature, including single genes of major effect as well as complex multigenic control. Therefore, it is feasible and desirable to identify such genes and genomic regions, and to breed chickens that can adapt to high temperatures. Contemporary genomic approaches will yield insight into the genetic mechanisms that contribute to a bird's ability to cope with a hot environment.
Keywords: chickens; heat stress; genomics

Introduction

Poultry production in hot climates is increasing in response to multiple factors. As the economic status improves in populous, developing countries such as China and India, consumer demand for high-quality animal protein in those regions increases. As climate changes, the weather affecting the major centers of chicken production is expected to become warmer (Meehl and Tebaldi. (2004)). Elevated temperatures and the increased incidence of heat waves will cause stress in poultry, resulting in reduced productivity, anorexia, heat stress and mortality (Turnpenny et al. (2001)). Breeding of birds that are genetically more resistant to heat stress will lessen these effects.

Both meat type and egg laying chickens respond negatively to high ambient temperatures. Temperatures exceeding 35 ^{o}C (Yalcin et al. (2005)) can cause heat stress in chickens, reducing nutrient utilization (Yalcin et al. (2005); Deeb et al. (2002); Yahav and Plavnik (1999)). In meat birds, this leads to reduced protein production and increased fat deposition (Temim et al. (2000)), whereas in layers there is reduced total egg production, reduced egg mass and decreased shell strength (Mashaly et al. (2004)). In both broilers and layers, heat stress provokes synthesis of reactive oxygen species in chickens (Tan et al. (2010)).

Genetic factors clearly influence the bird's physiological response to heat. In layer-type chickens, divergent selection for tolerance to heat produced two lines that differed in survival time in heat (Wilson et al. (1975)). Also, different lines of chickens exhibit different, heritable responses to heat stress (Lu et al. (2007)).

Many specific genes have been shown to respond to heat stress in the chicken. Several, including the heat shock proteins (Lei et al. (2009); Yan et al. (2009)) and the glucose transporter SGLT1 (Garriga et al. 2006)) increases transcription in response to heat stress. In contrast, some genes, such as amylase (Osman and Tanios. (1983)) and several genes involved in steroid metabolism decrease transcription at elevated temperatures (Rosenboim, et al. (2007)).

Early thermal conditioning allows increased transcription of the BDNF gene in response to heat stress later in the bird's life. In broilers, exposure to elevated temperatures in ovo or during the 1st week post-hatch (thermal conditioning) can reduce susceptibility to heat later in life (Yahav and Plavnik (1999)); Meiri, (2008)). However, to date, thermal conditioning appears to have relatively little effect on improving performance in layers (Star et al. (2009); Yalcin et al., 2005)).

Heat resistance correlates with reduced feather density and lower growth rate. These observations indicate that identifying alleles with different effects on heat stress tolerance is a reasonable goal. They also suggest that some of the genes controlling thermal tolerance have pleiotrophic effects on a variety of performance traits. With the possible exception of the Naked Neck gene (Cahaner et al. (1993); Yunis and Cahaner (1999); Cahaner et al. (2008)), however, it is still uncertain which specific genes or epigenetic effects play a direct role in the chicken's ability to tolerate or succumb to heat stress.

Collectively, these observations indicate that identifying alleles with different effects on resistance to heat stress is a feasible and attainable goal. The studies also suggest that some of the genes controlling thermal tolerance have pleiotropic effects on a variety of performance traits, so those relationships must be defined to accomplish overall improvement in important traits.

Current Genomic Research

Two powerful tools to elucidate the genomics underlying response of poultry to heat include genome-wide association studies (GWAS) and transcriptomic analyses. We have incorporated these tools into a study on the genomics of heat stress in chickens, with the long-term goal to adapt chickens to climate change through breeding. Some of our initial results are summarized here.

Genome-wide association study. To date, few genome-wide searches for regions controlling response to the stressor of high ambient temperature have been conducted. We initiated a genome-wide association study (GWAS) using a novel, highly advanced intercross line (AIL) of chickens. The two founder lines of this AIL were

a commercial broiler breeder male line and the Fayoumi breed. The Fayoumi breed originated in Egypt and was imported to the U.S.A. for research in 1954. Subsequent to its importation, the research line of Fayoumis was inbred by decades of full-sib matings. The long history of natural selection in a hot climate is hypothesized to aid the evolution of genetic adaptation to high ambient temperatures in the Fayoumi breed, and the recent process of inbreeding in the research line is assumed to fix most of the alleles that were in the highest frequency in the founder birds of the population. The broiler line was typical of the genetics and phenotype used in the U.S. broiler industry at the time that the birds were sourced. To initiate the intercross, a single broiler male was mated to multiple inbred Fayoumi hens. Birds were intermated to produce each subsequent generation. The GWAS was conducted on 456 AIL birds of the G_{18} and G_{19} generations that were genotyped using a 600K chicken SNP panel (Kranis et al. (2013)). Over 200K SNPs passed quality control with a minor allele frequency > 0.05, and were used for the analyses. GWAS was conducted using GenSel software (Fernando and Garrick (2012)).

The AIL birds were exposed to daily cycles in ambient temperature from day 22 to 28 of age, with 7 hours daily at 35-37 ^{o}C and the remainder of the day at 24-25 ^{o}C. Recorded phenotypes included body temperature (BT), body weight (BW), breast yield, and digestibility (Bjorkquist et al (2014)) and blood parameters (data not shown). Phenotypes were evaluated in three stages: pre-heat, acute heat (first day of high temperature exposure) and chronic heat (after one week of daily fluctuating temperatures). Additionally, the changes over time were considered as additional phenotypes for GWAS.

The heritability estimate (see Bjorkquist et al. (2014)) of BW pre-heat was 0.25 and after chronic heat was 0.36. The heritability of the BW gain over the time of fluctuating ambient temperature was 0.21. Thus, the BW traits are all moderately heritable and should respond to genetic selection, including the important economic trait of body weight gain under the stress of high ambient temperature. The BT heritability estimates at pre-heat, acute heat and chronic heat phases were 0.27, 0.17 and 0.20, respectively. These moderate heritabilities again indicate the feasibility of genetic selection to modify these phenotypes. Several genomic regions, tested as 1-Mb windows, were identified to be associated with each of the phenotypes, with the most variation explained by quantitative trait loci (QTL) on chromosomes 1, 2, 6, and 7.

Because of the relatively high economic value of the breast muscle, total breast muscle yield is a trait of importance in meat-type birds. The breast yield (pectoralis muscle as a percentage of body weight) in the current study was measured after harvest at the end of the experiment, at the chronic heat exposure phase. The heritability estimate of breast yield, measured after chronic, fluctuating heat exposure, was 0.19. This value suggests that improvement in meat yield under hot conditions can be successful. Interestingly, a single large QTL on chromosome 1 explained 8.6% of the total phenotypic variation in breast yield in this AIL test population (Figure 1).

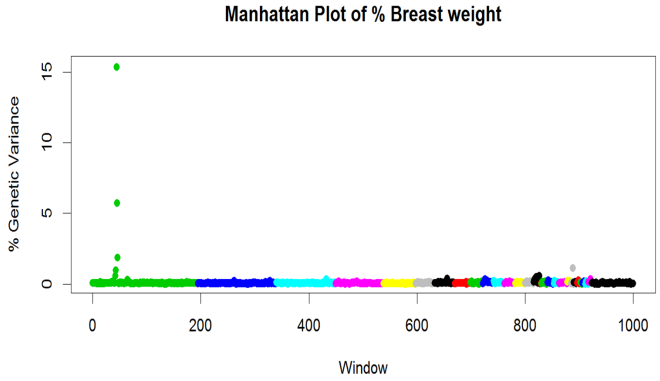

Figure 1: Manhattan plot of percentage of genetic variance in breast yield under heat stress explained by 1-Mb SNP windows in an advanced intercross line, showing one major QTL on chromosome 1.

Potentially important as an indicator of resistance to the stress of high ambient temperature is the change in BT that occurs as a result of heat stress. The heritability of the change in temperature from pre-heat to acute or chronic heat phases was 0.06 and 0.10, respectively. Thus, it will prove more difficult to genetically select birds for resistance to heat-induced changes in body temperature. The QTL explaining the most phenotypic variation for the BT traits are on chromosomes 14, 15 and 22.

Transcriptome analysis. Poultry often respond to high ambient temperature with reduced feed consumption and digestive inefficiency, thus altering major metabolic pathway. Transcriptome analysis can yield insights into the many signaling pathways and interactions that occur with changes in gene expression. To more fully understand the impact of heat on the metabolic functions of broilers, we analyzed the transcriptome of liver tissue of broilers. RNA-seq was conducted using four full sib pairs in which one bird of each pair was exposed to daily, 7-hour cycles of heat (35 – 37 C) for one week, while the other was maintained at a thermoneutral temperature (24 – 25 C).

Nearly 14 gigabases of total RNA sequence was generated, with an average of over 17 million reads per bird. Forty genes were significantly differentially expressed in response to heat, with twice as many genes down-regulated in response to high temperature than up-regulated. Two gene networks were created from the use of the function-based Ingenuity Pathway Analysis (IPA) of the differentially expressed genes: “Cell Signaling, Molecular Transport, Small Molecule Biochemistry” and “Endocrine System Development and Function” (Figure 2). Genes from the MAPK-signaling pathway or with related functions were prominent in the networks, and have also been

detected in studies of the transcriptome response to biotic stressors such as bacterial infection (Coble et al. (2012)).

Genes associated with cellular proliferation and differentiation, inflammation- and stress-related signaling, and apoptosis-associated genes were down-regulated in birds exposed to heat. In contrast, genes that inhibit feed intake and sphingolipid-related signaling were up-regulated. Genes that regulate inflammation, stress, thyroid hormone levels, and body temperature were either up- or down-regulated.

Transcriptome analysis, therefore, demonstrated that broiler response to heat is characterized by gene expression changes aimed toward decreasing internal temperature, reducing hyperthermia-induced apoptosis, and promoting tissue repair. Additionally, genes that regulate the perturbed cellular calcium levels that result from heat exposure were differentially expressed. Obtaining this deeper understanding of the genetic mechanisms that birds use in response to heat will aid in the development of future intervention strategies that target the pathways of response to heat, including genetic selection programs based upon the key identified genes.

Summary Points

- It is increasingly important to address heat stress for production of chickens in both the developed and the developing world.
- A variety of approaches to genetic improvement for heat stress resistance exist, including genomic selection.
- Utilization of native ecotypes may serve as a source of novel, beneficial alleles that are adapted to the local environment.
- Genome-wide association analyses identify regions with significant effects on heat-resistance related traits.
- Transcriptomic analyses identify genes and signaling pathways associated with response to heat.
- Opportunities exist to apply genomic approaches to improve heat resistance in large-scale and small-scale production using highly developed commercial lines as well as relatively undeveloped local ecotypes.

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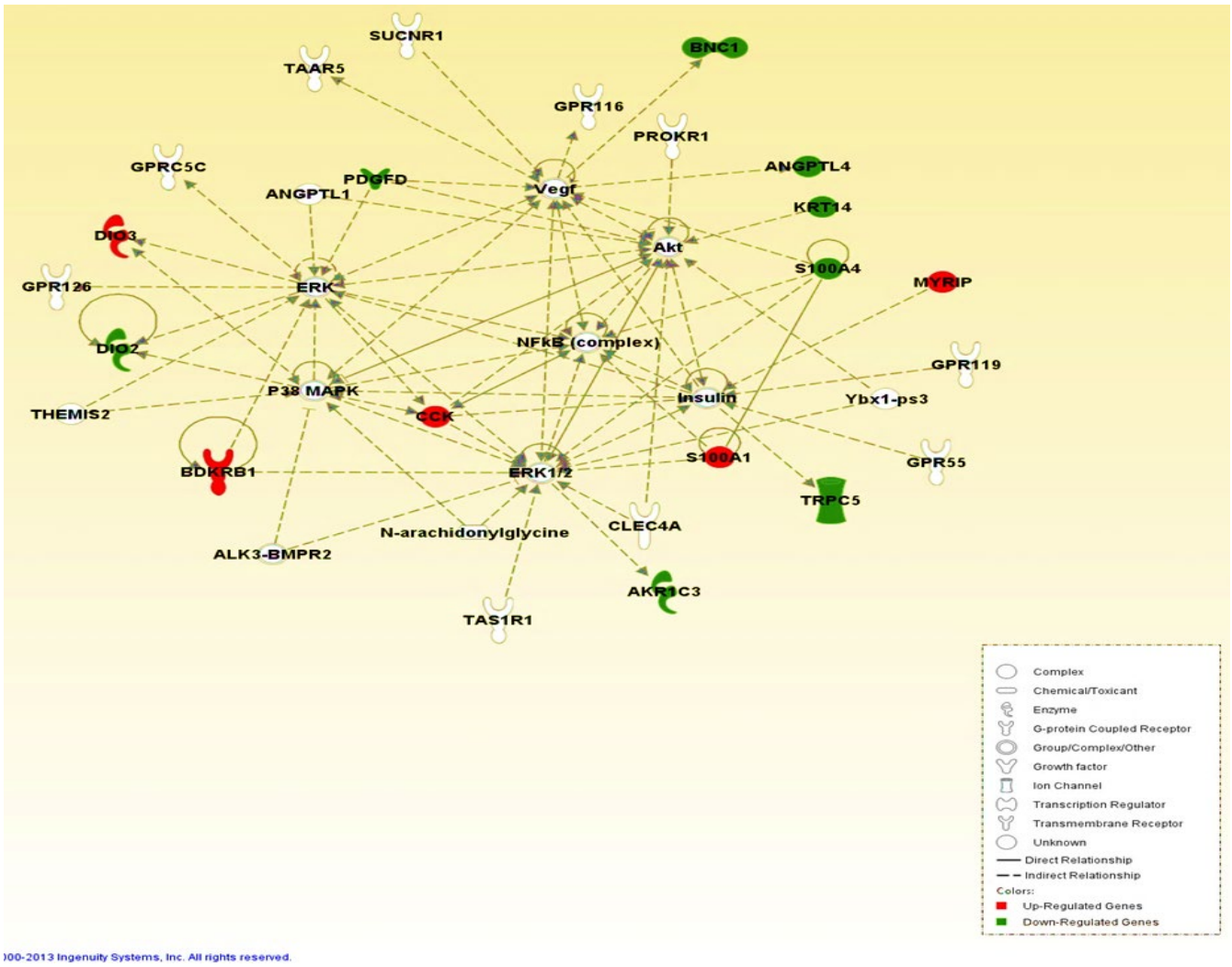

Figure 2: Pathway diagram showing upregulation of genes involved with inhibition of feed intake, inflammation, organelle motility, and endosomal recycling; downregulation of genes involved with cell proliferation, angiogenesis regulation, inhibition of epithelial tumor growth, stress induced-cellular signaling and inhibition of cell differentiation; and both up- and downregulation of genes involved with thyroid hormone regulation, thermal control, Ca2+ regulation, inflammation regulation, and cell growth in livers of broilers exposed to heat.