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In silico Methods for Modeling of Genomic Regions for Immunological and Metabolic Gene Modulating to Stress Response in Chicken: Where We Are?

Review Article

M.S. Ekhtiyari[1], A.S. Sadr[2*], M. Shirali[3] and A. Javanmard[4*]

[1] Department of Biochemistry, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[2] Aquaculture Research Center-South of Iran, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Ahvaz, Iran
[3] Agri-Food and Biosciences Institute, Hillsborough, BT26 6DR, United Kingdom
[4] Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

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* Correspondence E-mails: a.sadr@areeo.ac.ir; a.javanmard@tabrizu.ac.ir

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ABSTRACT

Traditionally, commercial broilers are not well adapted and currently subjected to a variety of environmental challenges. In recent years, researchers have shown an increased interest in stress as one of the greatest environmental challenges to the profitability of sustainable intensive poultry production. In this scenario, understanding the complexity of the molecular basis and genomics of the stress response is critical to successful breeding programs for climate-adapted chickens. Recently, numerous popular studies have attempted to identify candidate genes that control stress responses in chickens. However, a number of questions regarding the choice of stress response remain unanswered or inadequately answered regarding the number of lead candidate genes that control components of the non-infectious and infectious stress response. With this motivation, 89 journal articles were collected for the primary investigation and those with low validity were excluded from further analysis. In short, we used three types of information sources, namely: text-based systematic review, *in silico* modeling, and both network and pathway approaches, to introduce more effective and bio-indicators of gene-controlling stress responses in chickens through older literature. Gene ontology (GO) and pathway networking of candidate gene associated with stress was loaded into Cytoscape for analysis. The result provides additional evidence and highlights, including nearly 9 candidate genes. According to published studies, *CRYAB*, *HSP90AA1*, *IL6*, *HSPA2*, *HSF2*, *HSPB1*, *HSF3*, *PLK1*, *BAG3* are mostly associated with non-infectious and infectious stressors and may deserve further attention. String database analysis illustrated role of highlighted gene in multiple cellular task and functionally such as ATPase activity, cellular processes, including protection of the proteome from stress, folding and transport of newly synthesized polypeptides, activation of proteolysis of misfold proteins and the formation and dissociation of protein complexes. Obtained information from Animal QTL database indicated important role of chromosomes numbers 2, 3, 4, 5, 12, 14 and 24 associated with stress resistance and susceptibility. On this basis, this report attempts to find out which genomic regions control homeostasis and promote cell survival, molecular transport and cell signaling.

KEY WORDS controlling homeostasis, gene networking, genomics, stress response.

INTRODUCTION

Traditionally, commercial broilers are not well adapted and currently subjected to a variety of environmental challenges. One of the greatest environmental challenges to the profitability of sustainable intensive poultry production is stress. Stress can be defined as a state of threatened homeostasis (Virden and Kidd, 2009). Research into the etiology

of stress and environmental problems in poultry farming has a long history. Stress can have negative effects on the health and performance of chickens (Bogusławska-Tryk *et al*. 2012) as well as on human nutrition (Takeda *et al*. 2004). Stress is a huge umbrella term that can encompass a complex reaction of chicken cells in the chemical-immunological, metabolic and genetic response to threatening conditions (Liu *et al*. 2019).

A considerable amount of literature has recently appeared on environmental factors influencing physiological homeostasis, genomics of adaptation, and immunological and metabolic factors influencing stress response in chicken breeds (Soleimani *et al*. 2011; Sohn *et al*. 2015). There have been numerous surveys to study chickens' response to threatening conditions that highlighted environmental components of non-infectious and infectious stressors such as: physical factors (heat, cold, relative humidity, and changes in oxygen tension with altitude (Bessei, 2006), housing conditions, group sizes, nutritional factors (nutritional deficiency, feed restrictions, change in feed composition) (Waldenstedt, 2006), physiological factors (body pH, body temperature), glucose level and oxygen tension, muscle building (changes in the muscle composition of protein, fat, potassium, magnesium, calcium and sodium) as well as metabolic and infectious microbial and viral diseases (Nidamanuri *et al*. 2017).

There is a considerable body of literature on type responses and complex and multi-layered mechanisms of the chicken body's response to stressors, which can be briefly divided into: behavior, changes in the autonomic nervous system, abnormal behavior, physiological and endocrine systems, metabolic and assisted immunological responses can identify who is susceptible and resistant to stressful conditions (Khan *et al*. 2021). Upon heat stress, heat loss is increased through radiation, convection, conduction and evaporation. While it is generally accepted that poultry farming can be achieved between 10 and 27 ˚C, maximum yield is achieved in the narrower temperature range: around 18-22 ˚C for broilers in Growing at 19-22 ˚C for layers (Figure 1). Birds raised under heat stress conditions showed signs of panting and flapping wings, body temperature, lower feed consumption, higher feed conversion rate, and lower body weight gain (Hafeez *et al*. 2021). The link between quantitative and molecular genetics has provided invaluable advantages in identifying the molecular basis of the stress response and the gene networking of genomic regions for gene modulating the stress response in chickens. Although the molecular basis and polygenic genetic architecture of the stress response have been extensively researched, candidate gene polymorphism, gene expression profiling, QTL mapping, epigenetic programming, post-transcriptional changes in signal genes, GWAS and RNA-Seq studies of the stress response are few studies that systematically examine the contribution of key genes to networking alone in stress response variation, and more systematic and theoretical analysis is required to understand the complexity of cellular signaling pathways and associated cell signaling (Perini *et al*. 2020).

The regular review of articles

Systematic reviews can be seen as useful tools for developing concepts and identifying knowledge gaps and prioritizing areas for future research on the genomics of stress in chickens. For this reason, the PRISMA protocol has been used as a framework for a systematic study of key candidate genes that affect stress in chickens (Piray and Foroutanifar, 2021). A total of 124 articles were collected and later checked for duplicates, 89 articles were included for further analysis. The articles contain 24 different factors on different chromosomes that are most closely related to stress. The raw material for this report was provided using search engines, Science Direct, PubMed, SCIRUS, Oxford Journals, Cambridge Journals, Springer Journals and the Wiley Library, the NCBI website and Google Scholar. Most of the keywords were chosen to find the target papers: stress in chickens, candidate gene, QTL mapping, gene expression, networking and genetic ontology, GWAS and RNA-Seq profile. The search was limited to chicken species and most articles were in English and Endnote software was used to remove duplicates and sort papers. A brief list of the key genes for stress in chickens is then provided to readers. Criteria for networking were assessed: between centrality, attributes, co-expression, predicted genetic interactions, signaling pathways, physical interactions, common protein domains and co-locations. A brief list of the key genes for stress in chickens is then provided to readers. Criteria for networking were assessed: between centrality, attributes, co-expression, predicted genetic interactions, signaling pathways, physical interactions, common protein domains and co-locations. Finally, to better understand the relationship between the genes obtained in this study, a network was created using the STRING tool (http://string-db.org) and 24 candidate genes as input.

The *ACTC1* gene-gene ID: 423298

Actin Alpha Cardiac Muscle 1 (*ACTC1*) is a highly conserved protein that plays an important role in premature muscle and fetus growth (McNally and Dellefave, 2009), and the decreased expression of *ACTC1* is a potential biomarker that plays a key role in the innate heart. The disease and the atrial septum play a defect. This candidate gene was found in chromosome 5 in the chicken genome and has 7 exons and 377 amino acids (Jiang *et al*. 2010; Matsson *et al*. 2008).

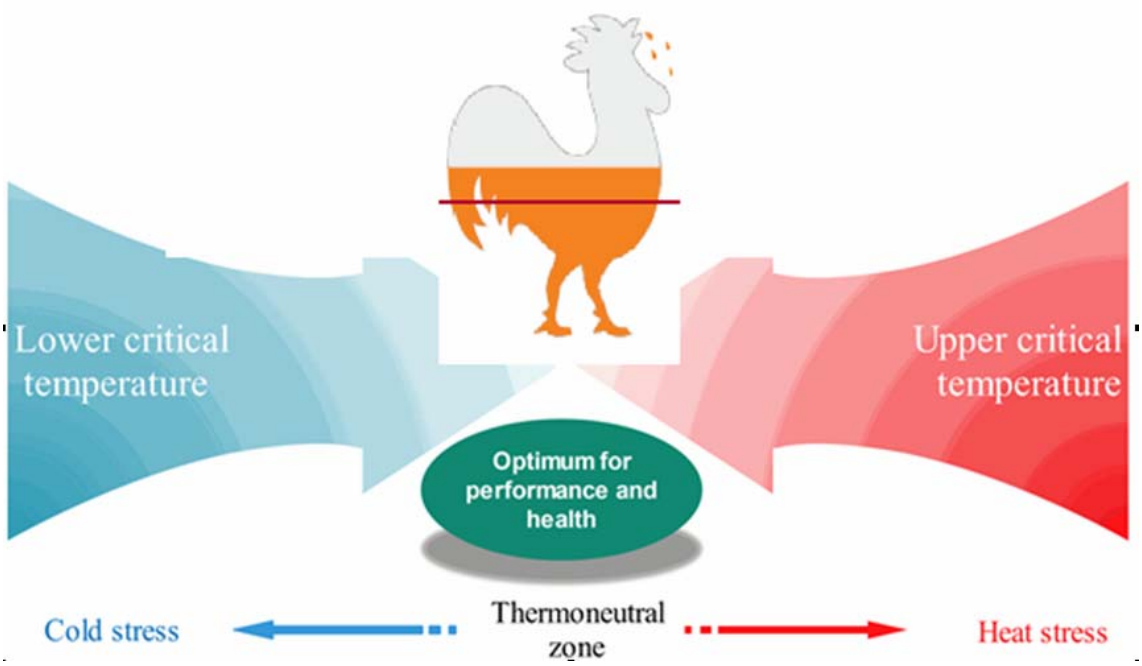

Figure 1 Optimum zone for performance and health in chicken physiology

Cheung *et al*. 2017 showed that the cardiac form of actin *ACTC1* is continuously regulated in the skeletal muscle of males and mice. This is most likely regulated by an intermediate androgen receptor pathway mediated as ACTC1 by dihydrotestosterone (*DHT*) in the gastrocnemius muscles of rodents (Cheung *et al*. 2017). *ACTC1* is also involved in small molecule biochemistry, molecular transport and molecular transport, lipid metabolism, and small molecule biochemistry (Nam *et al*. 2012; Wu *et al*. 2013). Many research studies (Nam *et al*. 2012) have shown that the *ACTC1* gene is involved in muscle growth and meat quality. *ACTC1* is upregulated under heat stress and during heat stress its convolution changed from 1.51 to 15.14 (Gaudet *et al*. 2011).

The *ADRA2C* gene-gene ID: 428799

The A-adrenergic receptor gene 2C (*ADRA2C*) is located in chromosome 4 chicken genome and consists of a single 1229 bp exon, starting at chromosome 4: 84590769, transcribed from 3 to 5, located at 10769 bp and with 446 amino acid residues. This gene is an effective gene to show stress-induced domestication behavior in animals and is fully associated with flight combat and reaction as well as stress-related behavior. This *ADRA2C* protein is absent in mammals and crocodiles. It is common in the central nervous system and kidneys and also plays a key role in regulating the release of adrenaline from the adrenal medulla. *ADRA2C* functions as an autoreceptor in presynaptic sympathetic nerve cells and is involved in the regulation of noradrenaline secretion with a negative feedback loop (Brede *et al*. 2003). *ADRA2C* is expressed in the CNS, where it cooperates with *ADRA2A* in a negative feedback loop prior to the synapse in releasing norepinephrine. *ADRA2A* controls potential excitation frequencies with high power and *ADRA2C* does the same for excitation frequencies with low potential. Low fears and high stress thresholds should be essential traits for animals during early domestication. *ADRA2C* is a major regulator of epinephrine secretion from the adrenal gland and can therefore be a good target for domestication remodeling (Elfwing *et al*. 2014).

The *ApoB* gene- gene ID: 396535

The apolipoprotein B (*ApoB*) gene plays a regulatory role in cholesterol homeostasis, cholesterol metabolism, cholesterol transport, lipid transport, lipoprotein transport pathways and was found in chromosome 3 chicken genome and exon number is 29 and also consists of 4631 amino acids. Chronic heat stress can increase fat synthesis in broilers, and excess triglycerides (TG) formed in the liver must be transported into the extrahepatic tissue through very low density lipoprotein tissue (VLDL). Otherwise, it will accumulate in the liver, which can even lead to liver steatosis. *ApoB* is an essential part of VLDL from the liver. One study reported that *ApoB* gene expression is stable without estrogen stimulation, and *ApoB* secretion is post-translational because it controls VLDL. By measuring the *ApoB* level using an immunoassay, it was found that exposure to chronic heat at the level of the *ApoB* level does not affect the liver (Lu *et al*. 2019). Therefore, broilers exposed

to chronic heat stress may be the main cause of fat accumulation in liver tissue, lack of stimulation of *ApoB* against heat stress (Ginsberg *et al.* 2005). Computer analysis showed that chickens 4/631 amino acids encoded by *ApoB* are predicted with 20 amino acids as a signal peptide with a molecular mass of 524 kDa and an isoelectric point of 8.49. A sequence comparison showed that the sequence of the amino acids from the *ApoB* gene from chickens is 49% identical to that of humans (Gen-Bank No. NM 000384) and 38% identical to the muscle of Mus (GenBank No.XM 137955). Analysis by SMART showed that N-terminal regions (45-597) of *ApoB* chickens included the N-terminal region of lipoprotein (LPD-N). The N-terminal domain of *ApoB* is structurally homologous to that of lipovitellin and the microsomal triglyceride transfer protein (Segrest *et al.* 1999). High expression levels were observed in the kidneys, liver and intestines, while low expression levels were observed in the heart, spleen, gizzard, belly fat, brain and leg muscles. *ApoB* has two isoforms in the bone marrow called Apo100 and Apo48, which are expressed in the liver and intestine, but so far only the Apo100 isoform has been described in chickens (Zhang *et al.* 2007).

The *ARRDC3* gene-gene ID: 427107

The arrestin domain, which contains the 3 (*ARRDC3*) gene, encodes a member of the arrestin family of proteins that regulate G-protein mediated signaling, and this gene is also a potent regulator of PPAR signal and the endosomal functions. In the chicken genome, this candidate gene was in chromosome? The Z and exon number for this gene is 8 and consists of 414 amino acids. Studies were performed with the arrestin domain-containing 3 (*ARRDC3*) gene located between 947 kb and 888 kb and below the 2nd and 3rd markers. This gene codes for the human protein arrestin domain-containing protein (www.uniprot.org). Writings show that the expression of the *ARRDC3* gene in the male amputum (intra-abdominal fat) increases with increasing body mass index and body volume. In this regard, the adipose tissue detached from the rat *ARRDC3*-NULL system shows that adrenergic visual acuity has increased dramatically. In fact, stimulating adrenaline by increasing CAMP signaling will increase fat loss. *ARRDC3* causes obesity resistance, which is usually associated with aging. However, the physiological poison of obesity resistance in the rat *ARRDC3*-NULL system has been adjusted by increasing the signal through the beta-3 adrenergic receptor (B3-AR) required for non-vibration and heat production. There is evidence that this effect is due to increased heat production in both white and brown adipose tissue at the molecular level through the interaction between *ARRDC3* and beta-adrenergic receptors, which limits the body's response to catecholamines. Therefore, with high *ARRDC3* values, the energy consumption is low, which reduces the heat production in the body. This evidence suggests that these signals, which regulate ARRDC3 expression, make the bird resistant to rising temperatures (Patwari *et al.* 2011). Based on the above, and since this gene has similar functions in humans, mice and chickens, it can be said that this gene can increase the birds' resistance to temperature rise by reducing body heat production and raising the temperature tolerance threshold in birds (www.uniprot.org).

The *BAG3* gene-gene ID: 423931

BCL2-associated athanogen 3 (*BAG3*) maybe candidate genes for thermotolerance in roosters and may be dramatically altered in heat-stressed chickens. This gene has a negative regulatory effect on apoptosis and can be used to reduce apoptosis in chickens exposed to heat stress. This candidate gene was in chromosome 6 chicken genome and consisted of 4 exons and 560 amino acids. *BAG3* is the only member of the BAG family that can be caused by heat-stressed. *BAG3* has been reported to be associated with apoptosis, development, protein breakdown, and skeletal organization. In heat-stressed chickens, the antibody-associated genes *BAG3* and *SERPINB2* are re-regulated. Adapted *BAG3* can be linked to the breakdown of antibodies and proteins to alleviate the damage caused by acute heat stress in chicken testicles. In the animal model, a deficiency or a mutation of *BAG3* leads to a myopathic phenotype (Wang *et al.* 2013). The *BAG3* gene responds to stress from heat shock factor 1. Although *BAG3* is widespread in many tissues, it is more pronounced in the skeletal and heart muscles, where it is located on the Z-disk of the sarcoma. The *BAG3* protein modulates the ATP turnover and the folding activity of the *HSP70* protein (induced) and the *HSC70* protein (constitutive) as a co-chaperone. It also interacts with a separate family of chaperones, small thermal shock proteins (*HSPB* genes), and combines the power of the *HSP70* and *HSPB* families. The *BAG3-HSP70-HSPB* complex regulates autophagy pathways in human cells and is also effective in the breakdown and regulation of ubiquitinated proteins by proteasomes (Judge *et al.* 2017).

The *BCL2* gene-gene ID: 396282

B-cell lymphoma 2 (*Bcl-2*) proteins exist at the height of apoptosis after the onset of cellular stress, and this candidate gene modulates ROS-mediated programmed cell death.

This gene was taken up in chromosome 2 chicken genomes and the exon number of this gene is 2 and consists of 233 amino acids. This genetic ontology and connection is B-cell homeostasis, commitment to the B-cell line, B-cell proliferation, B-cell receptor signaling pathway, CD8 positive, commitment to the alpha-beta T-cell line, T-cell differentiation into thymus, T-cell homeostasis, actin filament

organization, axon regeneration, axonogenesis, behavioral anxiety reaction, cell aging, cell-cell adhesion, cellular response to glucose deficiency, cellular response to hypoxia, cellular response to organic substances and defense reaction to viruses. Mitochondria play an important role in apoptosis and autophagy and are the main site of oxidative stress. Mitochondrial dynamics (fusion and splitting) play a key role in maintaining their shape, size, number, and function. Moll reported that p53 could interact with the X protein associated with *Bcl-2* (Bax) and *Bcl-2* at the mitochondrial level and has the ability to stop cell proliferation and stimulate apoptosis (Liu *et al*. 2019). Studies have shown that after exposure to the endoplasmic reticulum (ER), apoptosis can co-operate with autophagy (a mechanism that kills the second cell) (Mihara *et al*. 2003). Qin *et al*. (1997) report that during treatment for T lymphocyte leukemia with As2O3, they activated apoptosis and autophagy by upregulating Beclin-1, resulting in cell death and tumor remission. Beclin-1, a BH3 protein, can bind to *Bcl-2* to form the Beclin-1-Bcl-2 complex, which inhibits Beclin-1-mediated autophagy. However, the high expression of the Bcl-2-associated X protein (Bax) can separate the Beclin-1-Bcl-2 complex, resulting in autophagy and apoptotically sensitive cells. The results of the analysis, which according to previous studies show that the interaction between *Bcl-2* and Beclin-1 could be between autophagy and intermediate apoptosis (Huang *et al*. 2013; Liu *et al*. 2019). *Bcl-2* protects cells from apoptosis by various stress factors and is mainly expressed in the outer membrane of the mitochondria. The *Bcl-2* family can be divided into two groups: antiapoptotic (similar to the *Bcl-2* proteins such as *Bcl-2* and *Bcl-X L*) and apoptotic stimulants (such as Bax-like and BH3-only protein). Proteins that promote apoptosis release cytochrome C from the mitochondria into the cytoplasm. However, Bcl-2-like proteins can prevent Bax-induced cell death by preventing the release of the cytochrome C content, which causes the release of cytochrome C and the onset of apoptosis in the spleen (Figure 2) (Huang *et al*. 2013).

The *CALB1* gene-gene ID: 396519

Calbindin 1 (*CALB1*) is a 27 kDa protein and belongs to the superfamily of calcium-binding proteins. It was found that the *CALB1* transcript level in the heat stress group of the hens was increased by 34.2-fold compared to the controls. This candidate gene was in chromosome 2 and consists of 11 exons and genetic ontology and pathway analysis show that this gene has calcium ion transport, cellular calcium ion homeostasis, cellular response to organic substances, locomotor behavior, long-term memory, metanephric manifold development, metanephric connection, tubular development, anephric distal tubule development and sensory pain perception. This gene consisted of 262 amino acids. Calcium transfer in uterine fluids includes *TRPV6* or other calcium channels to penetrate the cells of the uterine gland, calbindin D 28k (*CALB1*: function= Ca+2 transporter (intracellular)) for intracellular transport, and calcium excretion occurs through calcium converters Na^{+}/Ca^{+2} or Ca^{+2}/H^{+}. As an intracellular transporter, calbindin 1 (*CALB1*) is able to transport Ca^{+2} through the cell and acts as a Ca^{+2} sensor and buffer for cytoplasmic Ca^{+2} buffers, causing the intracellular Ca^{+2} concentration to be toxic level is prevented. *CALB1* is commonly expressed in various tissues such as the uterus, retina, brain, kidneys, intestines, bones, and pancreas. This protein is an important factor in egg formation and significantly increases uterine expression during egg shell calcification. However, *CALB1* maintains low intracellular calcium ions in various cells to prevent cell death and apoptosis (Adji *et al*. 2019). An increase in *CALB1* in prostate cancer cells has been observed during heat stress (Li *et al*. 2011a), which may be related to its compensatory and protective effects against abnormal mineralization and apoptosis. A positive association between egg quality and *CALB1* was confirmed in the uterine cavity. Studies have shown that the release of many hormones such as estrogen, glucocorticoids, and catecholamines is associated with changes in heat stress. These hormones can differentiate the expression of the *CALB1* gene in tissues (Ebeid *et al*. 2012).

The *PLK1* gene-gene ID: 416575

Serine / threonine protein kinase *PLK1*, also known as polo-like kinase 1 (*PLK-1*) or serine / threonine protein kinase 13 (*STPK13*), is an enzyme that temporarily interacts with mitotic structures such as the spindle apparatus, kinetochores, and centrosomes. This candidate gene was located in the chromosome 14 chicken genome and has a number of 10 exons and also consisted of 595 amino acids. PLK1 protein activity is required for cell cycle progression regulate several aspects of cell division including centrosome maturation (Javadi Esfehani, 2014). *PLK* proteins are important regulators of the cell cycle. This gene plays an important and key role in activating and regulating the activity of these proteins by stimulating and interacting with enzymes of the *PLK* family, particularly *PLK1* and *PLK4* (Casenghi *et al*. 2003). *PLK1* substrates contain several proteins involved in mitosis, including *APC*, *CDC25C*, and *CLYNB*, which regulate the rate of dephosphorylation of tyrosine by CDKs (Asteriti *et al*. 2010). Studies have shown that through the phosphorylation of *HSF-1* in serine 419 by *PLK1* and the interaction between *HSF-1* and *PLK1* under thermal stress, an interaction between *PLK1* and *HSF-1* proteins exists in the body and this is strengthened and effective at the regulation of the thermal load.

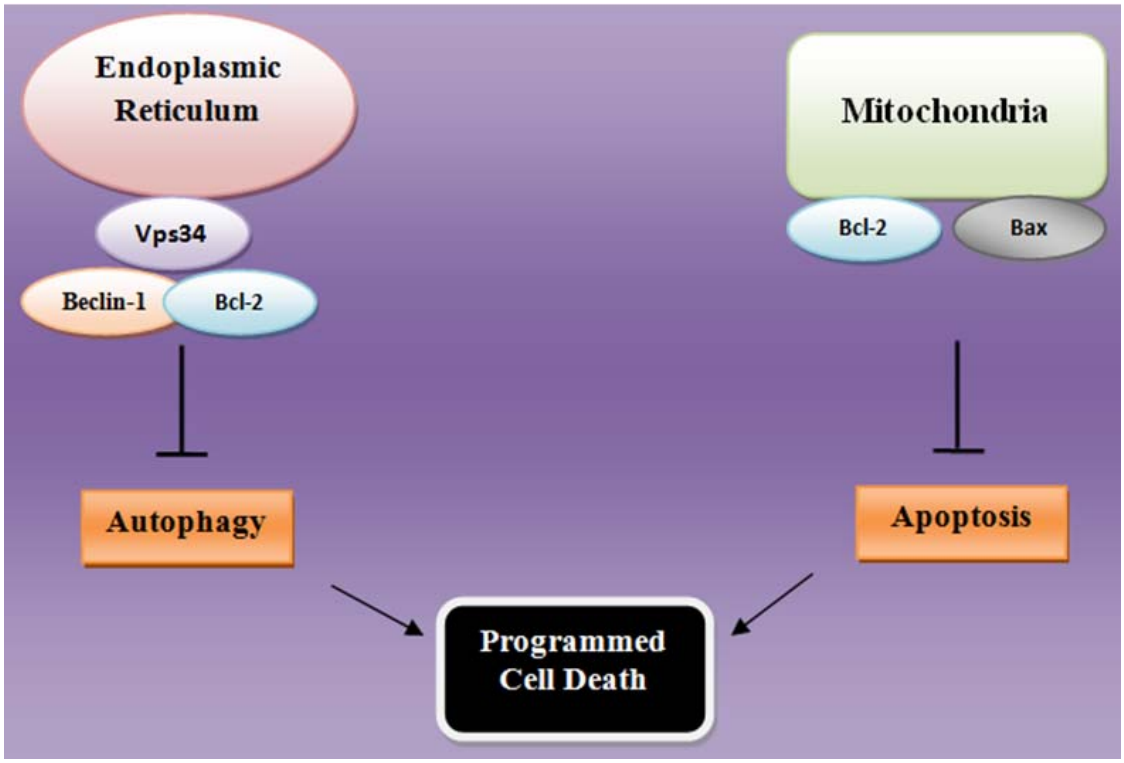

Figure 2 Beclin 1 complex: multiple, mechanisms regulating autophagy/apoptosis toggle switch (inhibition: ┤, activation: →)

The results show that mutations in serine 419 to alanine and the inhibition of thermal stress are due to *HSF-1* nuclear transfer and that *HSF-1* phosphorylation by PLK1 is an essential step for *HSF-1* activity under thermal stress (Kim *et al.* 2005). As a result, *PLK1* plays an important role in the activation and regulation of *HSF-1* protein activity under thermal stress. In normal cells, *HSF-1* is largely concentrated in the cytoplasm as an inactive monomer. Hemothyroidism (essential for *HSF-1* activity) is altered and transferred into the nucleus to bind to DNA. Thereafter, *HSF-1* binds to the elements or elements of thermal shock (HSES), which are repetitive reverse structures of 5 nucleotide motifs in the promoter region of *HSP* genes, thereby increasing the expression of *HSP* genes, including *HSP70* and *HSP90*. In various organisms, the regulation of *HSP* protein expression in response to stress is primarily dependent on the HSF-1 transcript (Kim *et al.* 2005). In fact, the activity of HSF-1 proteins increases the cellular level of heat shock proteins (*HSPs*), which are used as molecular protection to denature and reduce these proteins as well as protect the cell (Fink, 1999).

The *CRH* gene-gene ID: 404297

Corticotropin-releasing hormone (*CRH*) affects resistance or susceptibility to heat stress, and a peptide hormone involved in the stress response has the main function of stimulating pituitary synthesis of ACTH as part of the HPA axis. This gene was found in chromosome 2 *Gallus gallus* and has two exons and 167 amino acids. In fish, the corticotropin-releasing hormone (*CRH*) is the dominant hypothalamic hormone that controls the stress axis. *CRH* is derived from a 160 amino acid of the preprohormone, which is broken down into a mature bioactive peptide of 41 amino acids. With recent efforts at the full genome sequence, two family members such as *CRH* have been identified to date, urocortin-II and -III. These peptides signal via seven-helix G protein-coupled receptors. UII appears to use its own receptor, but the two receptors for *CRH* and *UcnI* (*CRH-R1* and *CRH-R2*) overlap the ligand properties. *CRH-R1* has the same affinity for *CRH* and *UcnI/UI* in catfish and mammals, while *CRH-R2* has a higher affinity for Ucn than *CRH*. *CRH*, *CRH-R1*, and *CRH-BP* are some of the key molecules that regulate the response to vertebral stress. For each of the three proteins described there are two very similar genes (0.95% amino acid identity) (Huising *et al.* 2004). In the pituitary gland of chickens, *CRH-R2* was clearly expressed by thyrotropes, indicating that it is involved in the secretion of *CRH* stimulant for *TSH* release. *ACTH* after binding to *CRH-R1*, while CRH-induced TSH secretion is mediated by CRH-R2 on thyrotropies. This mechanism allows the pituitary action of *CRH* to be fine-tuned. Depending on requirements, *CRH* can preferentially release *ACTH*, *TSH* or both via various regulated receptors (Figure 3) (De Groef *et al.* 2003).

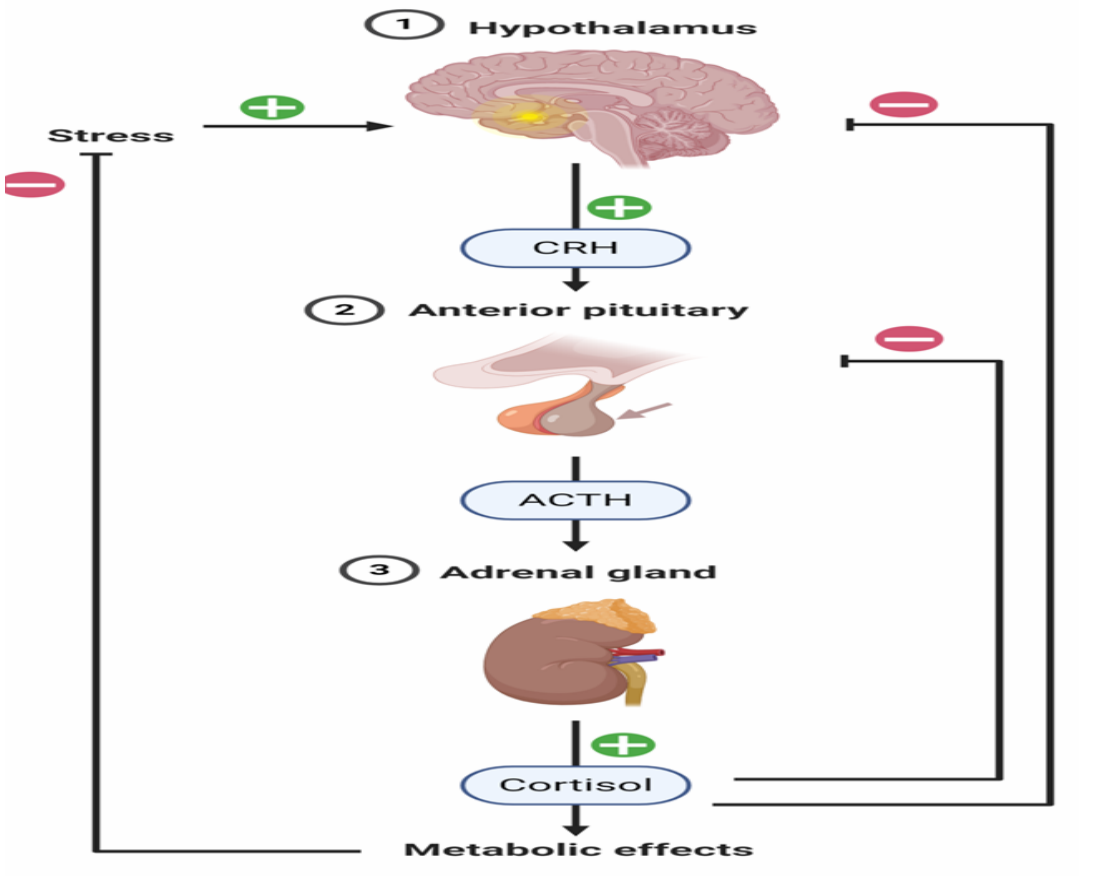

Figure 3 *CRH* pathway (the stress response begins in the brain; the function of the hypothalamus is very similar to that of the command center, which triggers a cascade that eventually leads to the secretion of cortisol, which in turn stimulates the immune system)

The *CRYAB* gene-gene ID: 396089

Crystallin Alpha B (*CRYAB*) belongs to the family of small heat shock proteins (*sHSP*) and lowers the cellular *CRYAB* level through heat stress. *CRYAB* is a member of the *sHSP* family (*HSPB1HSPB10*) that acts as a chaperone and occurs in several organs and could also play an important role in heart cells. This candidate gene was in chromosome 24 the genome of chickens, and their exon numbers are 3 and consist of 174 amino acids. Gene ontology and signal path analysis showed their regulatory role in the following signal pathways: apoptotic process that is involved in morphogenesis, cellular reaction to gamma radiation, lens development in the camera eye, muscle organ development, negative regulation of intracellular transport, negative regulation of the protein complex structure, negative regulation of transcription, DNA Template, protein stabilization, response to hydrogen peroxide, response to hypoxia and tubulin complex formation. The small heat shock protein B-crystallin (*CryAB, HSPB5*, 20 kg Dalton) belongs to the family of *sHSP* and reacts in most organisms to different types of stress (e.g. cell protection (Li *et al.* 2011b). *CryAB* has also been shown to have anti-apoptotic properties because in conditions such as stroke it can prevent cell death by preserving the skeletal structure of cells. Mechanically, *CryAB* is localized in the I-band and M-line region in myofibrils and plays a stabilizing role *in vitro* for myofibrils in cardiomyocytes (Wieske *et al.* 2001). It is now believed that the pleiotropic functions of *CryABs* are the result of their multiple interactions with a variety of different proteins. Previous research has shown the expression and localization of *CryAB* and has shown that it plays a protective role against heat stress in mice *in vivo* and *in vitro*. However, the performance of *CryABs* is poor compared to other members of the *sHSP* family. The cytoskeleton plays a key role in maintaining the body shape of heart cells, protecting against stress from the endoplasmic reticulum (ER), and maintaining mitochondrial function *in vivo* and *in vitro* (Figure 4) (Tang *et al.* 2016; Zhang *et al.* 2019).

The *FABP1* gene-gene ID: 374015

Fatty acid binding protein (*FABP1*) is highly transcribed in liver cells and this protein affects lipid metabolism by regulating PPARalpha and L-BABP in chicken hepatocytes. This gene was located in the chromosome 4 chicken genome and consisted of 4 exons and 127 amino acids. This suggests that free radical production was linked to L-FABP

production during cold stress. Cold stress provokes the body to produce additional free radicals, which are suppressed by unsaturated fatty acids from the biomembrane membranes. Hence, it destroys the integrity of the cell membrane and causes severe damage to the cell membrane. It has been reported that L-FABP has a high affinity for unsaturated fatty acids and lipid peroxides, which can support cell membranes by preventing the oxidation of unsaturated fatty acids and damage to cell membranes by lipid peroxides. In addition, by simply releasing it, L-FABP can transport fatty acids into the mitochondria or endoplasmic reticulum so that homeostasis regulates the liver's lipid metabolism. Lipid synthesis for energy storage can be the first response to prolonged cold stress. In addition, the binding of L-FABP to fatty acids can generate energy through oxidation. It's esterified to produce phospholipids to resist cell membrane damage or to produce triglycerides to complete fat loss (Chen *et al.* 2015).

The *HSF1* gene-gene ID: 420362

Heat shock factor 1 (*HSF1*) is a stress-inducible and DNA-binding transcription factor that plays a central role in the transcriptional activation of the heat shock response. *HSF1* is the main regulator of heat shock genes. *HSF1* is activated at lower temperatures than *HSF3*, while *HSF3* binds and is activated at higher and longer temperatures. The heat shock factors (*HSF*) and heat shock proteins (*HSP*) are the main mechanisms of cell protection against environmental heat stress. This candidate gene was found in the chr. 2 chicken genome and has 11 exons and 491 amino acids (aa). The genetic ontology and pathway analysis of this candidate gene showed its role in the following pathways: DNA binding, DNA binding, DNA binding transcription factor activity, RNA polymerase II promoter sequence-specific DNA binding, chromatin-DNA binding, core-promoter sequence-specific DNA binding, heat shock protein binding, promoter-specific chromatin binding, protein homodimerizing activity, protein self-association and sequence-specific DNA binding. The *HSF1* trimmer and *HSF3* dimmer can be connected to the *HSPs* heat shock element (*HSE*) to make *HSPs*. *HSF1* was involved in the further synthesis of *HSPs*, while *HSF3* was only required in avian species for *HSR* (the heat shock reaction). In addition, some research suggests that *HSF3* plays an important role in the induction of *HSPs* 70 in chickens compared to *HSF1*. The results showed that the expression of the *HSF1* and *HSF3* genes can be adjusted by heat stress in the chicken heart. These results suggest that *HSF1* and *HSF3* may play an important role in the re-regulation of *HSP70* (Fujimoto *et al.* 2010). Both *HSF1* and *HSF3* are available as a monomer form under normal conditions, and when the cell is under stress, the *HSF1* trimmer and *HSF3* dimmer can be combined with the *HSE* to mediate expression of thermal shock proteins. Therefore, the formation of *HSF* polymers was a prerequisite for the expression of thermal shock proteins, the results on the native side showed that the expression of total *HSF1* and *HSF3* was regulated under thermal stress, *HSF1* trimmer formation and HSF3 dimmer also decreased under heat stress (Figure 5) (Xu *et al.* 2019a).

The *HSF2* gene gene ID: 421724

Heat shock transcription factor 2 (*HSF2*) modulates the expression of heat shock genes by interacting directly with HSF1 or heat shock factor 4 (*HSF4*). This gene was found in chromosome 13 chicken genome and has 13 exons and 563 amino acids. In vertebrates, four members of the *HSF* family (*HSF1*, *HSF2*, *HSF3* and *HSF4*) are bound to the thermal shock element (*HSE*), which is the inverse repeat of the consensus nGAAn sequence. *HSF1* and *HSF3* retain the DNA binding activity, both are transferred into the cell nucleus during thermal shock, while *HSF2* does this only to a small extent. In addition, *HSF1* mammals or *HSF3* birds, but not *HSF2*, are necessary and sufficient to express *HSP*. Consequently, *HSF1* significantly suppresses mouse models of protein misbinding diseases such as Huntington's disease and prion disease (Steele *et al.* 2008), e.g. single *HSF* in *Caenorhabditis elegans*. Since *HSF2* is expressed abundantly and ubiquitously so that it can spread to vertebrate cells to express *HSP*, its growth patterns have been extensively analyzed. *HSF2* has been shown to be important for brain development and reproduction (Chang *et al.* 2006). *HSF2* is a short-term protein that is heavily regulated by the ubiquitin-proteasome pathway. Blocking the protease activates *HSF2* and *HSF1*. *HSF1*, but not *HSF2*, is necessary for the upregulation of *HSP* expression during proteinase inhibition. Therefore, *HSF2* is unlikely to be an important factor in the regulator of *HSP* expression. Instead, *HSF2* is partially involved in the inductive expression of HSPs by interacting directly with *HSF1* or by tagging the *HSP* gene during mitosis. *HSF2* is turned on during heat shock, and its deficiency increases the sensitivity of vertebrate cells to heat shock. Heat shock leads to cell death through apoptosis and necrosis. However, the path of cell death and the primarily imperfect factors can vary depending on the temperature and duration of the heat shock. Indeed, *HSF2* deficiency in mouse embryonic fibroblast (MEF) cells, unlike *HSF1*, has little effect on survival rates when exposed to extreme temperatures or heat. This significantly reduces the survival rate in the event of heat shock at high temperatures. Similarly, stress-related cell death phenotypes have been shown in *HSF2*-deficient mice. A unique need for *HSF2* for cell survival, particularly under stable heat shock conditions, that is evolutionarily sustained in mammalian and poultry species.

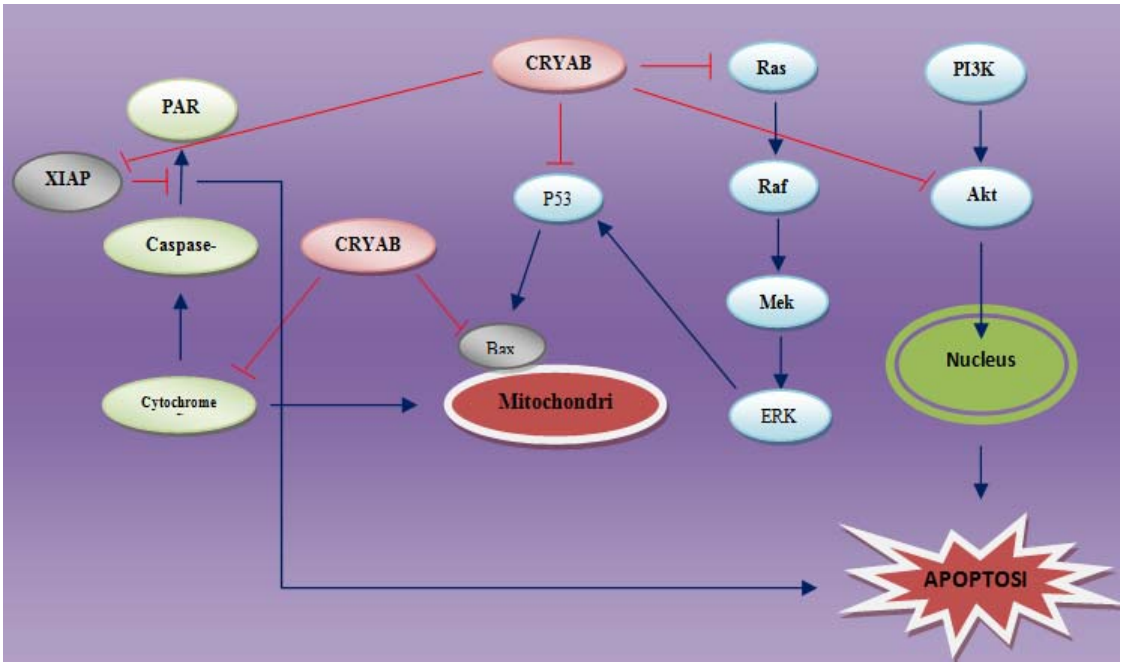

Figure 4 Schematic diagram of CRYAB protein involved in the regulation of apoptosis (inhibition: ┤, activation: →)

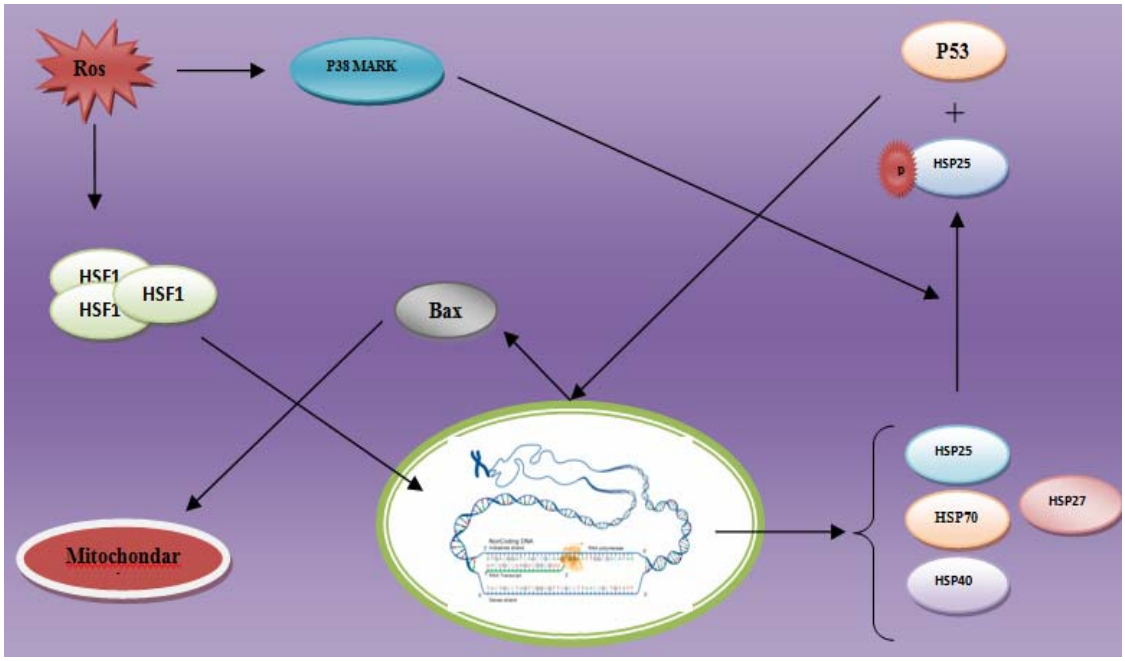

Figure 5 *HSP25* overexpression in Dox-treated failing hearts of mice

HSF2 deficiency increases the accumulation of cellular proteins during sustained mild heat shock, suggesting that *HSF2* reduces protein misfolding or destroys misfolded proteins. *HSF2* regulates both folding and degradation pathways via B-crystallin expression. *HSF2* deficiency, required for the expression of non-HSP proteins, increases the expression of *HSP*s by accelerating the activation of *HSF1* rats or *HSF3* chickens in mild hours (Åkerfelt *et al.* 2007).

The *HSF3* gene-gene ID: 422169

The heat shock transcription factor 3 (*HSF3*) is stronger than *HSF1* for the thermally induced expression of *HSP* genes. This candidate gene was in chromosome 4 and has

14 exon counts and 551 AA protein. The genetic ontology and signal path analysis of this gene showed that its key roles lie in the following signal pathways: DNA binding, DNA-binding transcription factor activity, RNA polymerase II promoter sequence-specific DNA binding, nuclear promoter sequence-specific DNA binding, Protein binding, protein homodimerizing activity, and sequence-specific DNA binding. *HSP*s are regulated by transcription factors called heat shock factors, 4 of which (*HSF1*, *HSF2*, *HSF3* and *HSF4*) play key roles in stress biology (Zhang *et al.* 2014). Fujimoto and Nakai (2010) found that *HSF1* is the most important *HSP* gene in mammals, while *HSF3* is the most important *HSP* gene in poultry (Fujimoto *et al.* 2010).

The binding of *HSF1* to the *HSP70* gene shock promoter is important in *HSP70* transcription. *HSP*s are regulated by *HSF1* during the stress response (Yasuhara *et al.* 2011). In chickens, at high temperatures, *HSF1* affects the nucleus of the brain cells, causing them to express *HSP*. However, *HSF3* is expressed in mild, moderate, and severely elevated temperatures in the brain and blood. The mRNA expression of *HSF3* gene expression tissue is associated with different tissue requirements. 6 hours after the start of heat treatment, *HSF*3 mRNA expression in the heart increased further, while *HSP*70 mRNA expression decreased. *HSP*70 and *HSF*3 mRNA expression levels in Lingshan chicken tissue (LSC) are different from those of White Recessive Rock (WRR) chicken tissue under heat treatment, which reflects species characteristics (Zhang *et al.* 2014).

The *HSPA2* (*HSP70*) gene-gene ID: 423504

Heat Shock Protein Family A (*HSP70*) Member 2 (*HSPA2*) is a 70 kDa protein that has been correlated with levels of mRNA expression and heat tolerance, which have been considered an effective marker for the selection of heat tolerant chickens. This gene was in chromosome 5 chicken genome and has 1 exon and has 634 aa. Genetic ontology and pathway analysis of this gene functionally indicated: ATP binding, ATPase activity, heat shock protein binding, misfolded protein binding, progesterone receptor binding, protein binding, protein binding involved in protein folding and unfolded protein binding. Some genes of the *HSP* family or their cochaperones are regulated in the testes of heat-stressed roosters, for example *HSP70*, *HSP90AA1*, *HSPB8*, *HSPA5*, *DNAJB6*, *HSPA8*, *HSPB1*, *HSPA4*, *AHS2*. *FKBP4 and ST13* (Wang *et al.* 2013). The *HSP70* family binds to newly synthesized polypeptides, preventing aggregation and promoting folding, while *HSP90* interacts with customer proteins in the end-stage of folding and modifies their configuration. The duration and intensity of heat stress can also affect the expression pattern of *HSP*s. In broilers, acute heat stress leads to the expression of the *HSP70* gene in the liver, lungs, heart, kidney, blood vessels and *HSP 90* in the heart, liver and kidney (Mazzi *et al.* 2003). Increased expression of the mRNA expression of the *HSP70* gene is associated with the simultaneous induction of the *HSP70* protein, indicating that the induction of *HSP70* is active at both the translational and transcriptional levels. High testosterone and corticosteroids appear to affect the protein *HSP70* and *HSP70* mRNA during heat stress. Among these *HSP*s, *HSP70* is the best association with acquired thermometer, and this was confirmed by observations that the competitive inhibition of the expression of the *HSP70* gene increased with increasing temperature. Mammalian cells cannot survive extreme heat stress when anti-*HSP*70 antibodies are used. In contrast, microinjection of *HSP70* mRNA increases thermal cell resistance in mammalian oocytes (Wang and Edens, 1994; Ciocca *et al.* 2015). The expression of *HSP70* varies between different genotypes or combinations of different haplotypes under normal conditions and heat stress. Heterozygous genotypes appear to be associated with a higher base level of homozygous genotypes than *HSP70* in both liver and muscle tissue, but this does not apply to stress caused by stress in liver and brain tissue (Zhen *et al.* 2006). The *HSP70* and *HSP90* genes are the best-studied family of *HSP*s, and each has different functions. *HSP70* binds to newly synthesized proteins, prevents their accumulation and helps with wrinkles.

The *HSP90AA1* gene-gene ID: 423463

Heat shock protein *HSP* 90-alpha. The gene (*HSP90aa1*) is the stress-inducible isoform of the molecular chaperone *HSP90* and this protein is a rich and ubiquitous molecular chaperone that plays an essential role in many cell biological processes. This gene was found in chromosome 5 chick genome and has 10 exon counts and consisted of 728 AA protein. Gene ontology and signal path analysis This gene showed contributions to the following signal pathways: *HSP90* protein binding, TPR domain binding, chaperone binding, disordered domain-specific binding, estrogen receptor binding, heat shock protein binding, identical protein binding, nitric oxide synthase-regulatory activity, progesterone terminator binding and protein binding, protein C-terminal binding and protein binding. Some reports exposed broilers to heat stress and recorded large differences in *HSP90* expression. The mechanism of *HSP90* is such that it preserves the function of proteins through the formation of certain complexes including kinase proteins and steroid receptors (Csermely *et al.* 1993). In the presence of stressors, these proteins are more strongly expressed and synthesized than other proteins (Zhao *et al.* 2017). The anti-stress performance of *HSP*90 is achieved in two ways: 1- Support of the refolding structure 2- Suppression of non-specific protein accumulation.

In addition, the function of *HSP90* in hepatitis B has been shown to be related to reverse transcription. Hence, *HSP90* control mechanisms in various tissues may be important as they are related to stressors. For example, there is a special relationship between *HSF1* and this protein under stress conditions that can change, and *HSF1* is separated from the mass and forms trimmers and invades the nucleus and controls the expression of *HSP*. More recent studies have shown that the expression of *HSP90* is influenced by the interleukin-6 and Interferon-G signaling pathways, multifunctional cytokines.

During physiological stress, the level of catecholamines secreted by the hypothalamic-pituitary-adrenal axis increases and increases the expression of *HSP90*. When stressed, calcium builds up in the muscles to which both alpha and beta 90 *HSP* are bound. It is noteworthy that in region B there is no diversity in the balance of the different species and the greatest difference is between fish and poultry compared to mammals. This region is very important for *HSP90* mechanisms, mechanisms such as the regulation of the N-terminal chaperone functions and the tendency towards N-terminal binding to denatured proteins (Pepin *et al.* 2001; Åkerfelt *et al.* 2010).

The *HSPB1* gene-gene ID: 396227

Heat shock protein beta-1 (also called heat shock protein 27) plays a role in stress resistance and actin organization. This gene was found in chromosome 19 in the chicken genome and has exon number 3 and consisted of 193 aa. The genetic ontology and pathway analysis of this gene revealed its key regulatory role in the following cellular signaling pathways: identical protein binding, protein binding involved in protein folding, protein homodimerization activity, protein kinase C-binding protein kinase C, and inhibitory activity. *HSPB1* or *HSP27* has been shown to be implicated in a variety of stress resistance without limb features. *HSPB1* has also been reported to provide greater protection when attached to the cytoskeleton as large oligomers or by phosphorylation as monomers, thereby further protecting *HSP*s cells. As long as there is heat stress in this experiment, there are stronger signals in the core of *HSPB1* than in the cytoplasm of myocardial cells (De Graauw *et al.* 2005). As an oligomer, *HSPB1* has two different approaches to stressors (Rogalla *et al.* 1999), and it is believed that non-phosphorylated *HSPB1* could stabilize skeletal components such as actin with its chaperone function. Under certain conditions, this *HSPB1* oligomer is separated into phosphorylated diameters. Phosphorylated *HSPB1*, which may be involved in cell death signaling pathways, is a potential anti-apoptotic molecule. Heat stress or ATP depletion cause *HSPB1* to form insoluble structures in the core of large detergents (6106 kDa), which are visible as granules and also contain heat-repellent proteins. Therefore, the protective role of *HSPB1* is also regulated by phosphorylation and transfer (Creagh *et al.* 2000; Paulat, 2016; Tang *et al.* 2016)

The *HSPB9* gene-gene ID: 428310

Heat Shock Protein Family B (Small) Member 9 (*HSPB9*) is a constitutive protection against environmental pollution during blastoderm dormancy. This gene was in chromosome 27 chicken genome and has 1 exon and 171 aa. Luo *et al.* (2014) investigated the gene expression of chickens under heat stress with the profile chip technology and found significant differences in the expression of the *HSPB9* (*HSP25*) gene in various tissues such as the brain, liver and leg muscles. The *HSPB9* gene is located on chromosome 27, which has 993 open pairs, and its mRNA sequence consists of 194 amino acids (the NCBI *Gallus gallus* genome database). Normally, *HSPB9* is not expressed and is only induced in all embryonic tissues of the chicken under thermal stress. These *HSP*s behave differently than other *HSP*s and bind to non-native proteins, protecting them from heat stress and preparing them for use with the *HSP70* system (Lee and Vierling, 2000). In simulation studies on the expression of *HSPB9*, it was found that moderate amounts of this *HSP* play a repressive role against *HSPB1* and *HSPA2*, which could explain why *HSPB9* is preferentially associated with non-native proteins. One of the properties that causes the rapid expression of this *HSP* under stressful conditions is the lack of internogenesis.

The *IL6* gene-gene ID: 395337

Interleukin (6*IL6*) is a protein that is a candidate gene for stress because it shows the protective immunological response to heat stress by increasing the expression of interleukin-6 (IL-6) and modulating the expression of genes. This gene was found in chromosome 2 chick genome and has 3 exon counts and consisted of 241 aa. Genetic ontology and pathway analysis highlighted their key roles in the following cellular pathways: cytokine activity, growth factor activity, and interleukin-6 receptor binding. In response to chronic heat stress (CHS), an increase in the expression of pro-inflammatory cytokine interleukin 6 (IL-6) and heat shock proteins of 70 KDa (*HSP70*) has been reported (Al-Zghoul *et al.* 2019). Cytokines are immune signal proteins that strengthen the cellular protective mechanisms against heat stress-induced inflammation (Hietbrink *et al.* 2006). IL-6 and IL-1 are anti-inflammatory cytokines that are produced during periods of stress and regulate the acute phase response (APR) to stress. IL-1 produces IL-6 (Weber *et al.* 2010). IL-6 has proven to be a very important regulator of systemic and localized acute inflammation, mainly through the modulation of non-inflammatory cytokines (Al-Zghoul

et al. 2019). Activation of IL-6 improves tissue repair by activating the cytokine IL-8, which plays an important role in wound healing (Wigley and Kaiser, 2003). During stressful periods, IL-6 expression can be induced in various ways. First, damaged cells release *HSP70* when exposed to heat stress or when secreted by specialized cells. *HSP70* in combination with TLR2 and TLR4 triggers the signal waterfall and peaks when the core factor transcription factor Kappa-B (NF-B) is activated, the latter causing IL-6 expression. The second way of IL-6 induction involves activation of NF-B by regulating the regulation of TNF and IL-1 cytokines. Finally, *HSF3* was identified as the third IL-6 induction mechanism due to exposure to environmental stressors. Of course, it should be noted that final IL-6 activation occurs only in poultry. According to some reports, IL-6, which is activated by *HSF*3 due to heat stress, acts as a heat shock gene in chickens (Al-Zghoul *et al*. 2019).

The *KLF2* gene-gene ID: 420148

Kruppel-Like Factor 2 (*KLF2*), a flow-responsive gene, is a known member of this family that is activated by fluid shear stress in cultured endothelial cells, where it regulates a large number of vasoactive endothelial genes. This gene is placed in chromosome 28 chicken genome and exon counts are 3 and 354 aa. In the venous clip model, there is close communication between the activation of phosphatidylinositol-3-kinase (PI3K), histone esterification, *KLF2* transcription and NOS-3 induction (Groenendijk *et al.* 2007). *KLF2* can induce NOS-3 expression and enzymatic activity as described by Sen Banerjee *et al*. (2004) who also showed that *KLF2* regulates endothelial activation in response to proinflammatory stimuli. In addition, some reports showed that *KLF2* reduced ET-1 expression and *KLF2* also induced NOS-3 expression. It recognizes *KLF2* as an inductive molecule between NO and ET-1 production in places with high shear stress. In addition, *KLF2* deficiency can be avoided through the use of phenylephrine, which increases vascular tone. *KLF2* contains a MEF2 compound in its promoter. The MEF2 family is one of the best properties of extracellular signal regulated kinase 5 (ERK5) and a potent flow-induced factor. In this way, *KLF2* expression can be induced.

The *MEF2C* gene-gene ID: 769007

Myocyte Enhancer Factor 2C (*MEF2C*) is a protein that belongs to the myocyte-specific Enhancer-Binding Factor 2 (MEF2) protein family, which is involved in the development and differentiation of skeletal muscles in vertebrates. This large gene was in the chromosome Z chicken genome and has 17 exon counts and 473 aa. The human *MEF2C* gene codes for the myocyte-specific enhancer factor 2C protein (Leifer *et al*. 1993).

This protein is a transcription factor in the MEF2 family (Molkentin *et al.* 1996). Nebreda and Porras (2000) has shown that this gene affects MAPK activity and that phosphorylation and regulation of *MEF2C* protein activity are influenced by MAPK protein kinases. These kinase proteins are specific for the amino acids serine, threonine and tyrosine and control and direct cellular responses to various stimuli, such as mitogen (mitogen is a chemical that stimulates the cell to initiate cell division and mitosis), osmotic stress, thermal shock and pre-inflammatory cytokines are involved as well as the regulation of cell function, including proliferation, gene expression, differentiation, lung cell survival and apoptosis (Pearson *et al*. 2001).

Typically, two specific events of cellular responses to different types of stress include activation of heat shock drugs (*HSF*s) and transcriptional activators of these genes and the other activation of JNKs and P38-MAPKinase, known collectively as protein kinases, to be active during stress (Pearson *et al*. 2001).

The *SYK* gene-gene ID: 427272

Spleen-associated tyrosine kinase (*SYK*) is essential for platelet activation, which is based on the immune receptor tyrosine-based activation motif (ITAM). This gene was in chromosome Z-Chicken and its exon number is 15 and consisted of 613 aa. Genontology and signaling pathway analyzes showed a remarkable role of this candidate gene in the following cellular signaling: B-cell receptor signaling pathway, B-cell receptor signaling pathway, adaptive immune response, adaptive immune response, blood vessel morphogenesis, cell differentiation, cellular response to hydrogen peroxide, cellular response to Molecules of fungal origin, defense reaction against bacteria, innate immune response, integrin-mediated signaling pathway and leukocyte-cell-cell adhesion. Specific cellular stress such as oxidative stress are powerful activators of the PTKs of the splenic tyrosine kinase (*SYK*) family in lymphocytes. Stimulus stress can also activate *SYK* in B cells as well as in humans and chickens (Qin *et al.* 1997). Spleen Tyrosine Kinase (*SYK*) (72 kDa protein tyrosine kinase) is an N-terminal tandem pair of SH2 domains separated by a C-terminal catalytic domain.

The *TRH* gene-Gene ID: 414344

The thyrotropin-releasing hormone (*TRH*) synthesized and secreted by the hypothalamus not only regulates the corresponding hormonal secretion of the pituitary, adrenal and thyroid glands, but also plays an important role in many physiological regulations. The gene architecture for this protein consisted of 7 exons and this candidate gene was located in the chr.12 chicken genome and comprised 260 aa.

Table 1 Lists the genes that affect stress and their involved biological pathways

Chromosome	Gene symbol	Genomic region	Pathway	Reference
2	*BCL2*	67837316-67924872	Mitochondrial- and death receptor-dependent pathways Intrinsic and extrinsic apoptotic pathways p53 signaling pathway	Liu *et al.* (2019); Mihara *et al.* (2003)
2	*CALB1*	124313483-124331355	Calcium ion transport Cell apoptotic pathway MAPK pathway	Li *et al.* (2011a); Li *et al.* (2011b)
2	*CRH*	115011152-115012145	Paracrine pathway cAMP-PKA pathway	Huising *et al.* (2004); De Groef *et al.* (2003)
2	*HSF1*	130889950-131021873	Expression(HSP27, HSP70) Ubiquitin proteolytic pathway	Xu *et al.* (2019a); Xu *et al.* (2019b)
2	*IL6*	30862822-30873241	NF-κB pathway p38 MAPK pathways IL-1 signaling pathway.	Hietbrink *et al.* (2006); Weber *et al.* (2010)
3	*APOB*	102199405-102233660	Cholesterol metabolic pathway Lipid transport LXR pathway	Lu *et al.* (2011)
3	*HSF2*	61387823-61409039	Protein phosphatase 2A pathway p35–Cdk5 pathways	Chang *et al.* (2006)
4	*ADRA2C*	81565509-81578375	Adrenergic receptors Gi/Go pathway GPCR Pathway	Brede *et al.* (2003)
4	*FABP1*	85982308-85986038	Lipid anabolism pathway Anti-oxidative response pathway Nrf2/ARE signaling pathway	Chen *et al.* (2015)
4	*HSF3*	282366-297374	c-myb pathway	Zhang *et al.* (2014)
5	*ACTC1*	32480624-32485436	Cardiac myocyte Calcium signaling pathway Sarcoplasmic Reticulum pathway JNK pathway	Cheung *et al.* (2017); Wu *et al.* (2013)
5	*HSPA2*	53058059-53060378	Apoptosis signaling pathway p38/MAPK pathway Lysosomal death pathway	Ciocca *et al.* (2015)

Genontology and pathway analysis have highlighted their key roles in the following processes: eating behavior, histamine metabolism, hormone-mediated signaling pathway, negative regulation of glutamate secretion, positive regulation of gamma-aminobutyric acid secretion and positive regulation of insulin secretion.

The results show that cold stress can alter *TRH* mRNA levels in the hypothalamus of broilers (Joseph-Bravo *et al.* 2016).

Cold exposure significantly activates the HPT axis, with increased thyrotropin-releasing hormone (TRH) synthesis, thyroid-stimulating hormone (Rage *et al.* 1994; Wu *et al.* 2013).

This suggests that cold stress can regulate *TRH* gene expression at the translational level or after translation. In regulating the expression of *TRH* gene mRNA expression in the hypothalamus, most studies have shown that negative feedback from thyroid hormones is the most important regulatory mechanism (Kim *et al.* 1996).

The *VPS13A* gene-gene ID: 427050

The human *VPS13A* gene codes for the vacuolar protein sorting-associated protein 13A (Rampoldi *et al.* 2001). The gene also codes for chorein as a key regulator of platelet secretion and accumulation and plays an important role in the production and regulation of platelets (Schmidt *et al.* 2013). This gain gene was in chromosome Z chicken genome and exon number is 74 exons and 3206 AA. The chorein protein is obtained from a large number of human tissue cells as well as from primary skin fibroblasts and red blood cells. In humans, the platelet count also affects the overall viscosity of the blood. Because thermal stress increases total platelet counts and blood viscosity, which in turn increases physiological damage and the risk of brain and coronary thrombosis, the *VPS13A* gene may play an important role in coding for chorein. It plays a role in the production and regulation of platelets during heat stress, thereby increasing the body's resistance to heat (DobsonStone *et al.* 2004).

Continued Table 1 Lists the genes that affect stress and their involved biological pathways

Chromosome	Gene symbol	Genomic region (bp)	Pathway	Reference
6	*BAG3*	31229092-31247001	Proteasome and autophagy pathways	Judge *et al*. (2017)
12	*TRH*	20087096-20111713	Protein kinase C (PKC)- phosphophatidylinoshitol- and Ca2+ Mediated pathways The PKA pathway The mitogen-activated protein kinase (MAPK) pathway	Ohba *et al*. (2011)
14	*PLK1*	7296538-7302070	Cell cycle pathway	Javadi Esfehani (2014)
19	*HSPB1*	4403293-4405185	FAS pathway and Stress induction of HSP regulation MAPK Signaling Pathway p38 MAPK Signaling Pathway Inflammatory pathway	Creagh *et al.* (2000); Paulat (2016)
24	*CRYAB*	6242317-6246235	Calcium-activated Raf/MEK/ERK signaling pathway Akt signaling pathway NF-κB signaling pathway	Tang *et al*. (2016); Zhang *et al*. (2019)
27	*HSPB9*	7654562-7655554	p53/Bax pathway c-JNK pathway	Krishnamurthy *et al.* (2011)
28	*KLF2*	4485518-4487126	ET-1 pathway NF-$_{K}$B pathway	Groenendijk *et al.* (2007); Sen Banerjee *et al.* (2004)
z	*ARRDC3*	59536896-59550459	β-adrenergic signaling pathway Aerobic pathways	Patwari *et al*. (2011)
z	*MEF2C*	60508107-60645113	p38 Pathways MAP kinase pathways The ERK1/2 pathway The JNK/SAPK pathways The high osmolarity glycerol (HOG) pathway The Gi pathway The PI3K/Akt pathway	Pearson *et al*. (2001); Koul *et al.* (2013)
z	*SYK*	44075480-44127404	MAPK4 pathway The JNK pathway NFκB and PI3-K survival Pathways STAT3 pathway the PI3-K/AKT pathway	Qin *et al.* (1997); Uckun and Qazi, (2014)
z	*VPS13A*	37613751-37719217	ERM ES-redundant pathways The ERM ES-bypass pathway	John Peter *et al*. (2017)

Exposure to acute heat stress also affects birds' behavior by affecting the secretion of stress hormones.

Gene ontology (GO) and networking

Gene ontology (GO) and pathway networking of candidate gene associated with stress was loaded into Cytoscape for analysis. String database analysis illustrated role of highlighted gene in multiple cellular task and functionally and pathways.

CONCLUSION

Previous research has shown that the effects of stress are observed in a variety of biological pathways. For example, in one of the test chickens, the heat stressed chickens, three hundred and nine different genes were expressed in the testes of chickens with acute heat stress. These various factors were mainly involved in the response to stimuli or stress, metabolism, signal transmission, transcription, transport, and protein metabolic processes. An application network and a qRT-PCR analysis showed that *HSP* genes and BAP3 antibodies, co-cochaperones (*HSP25*, *HSP90AA1*, *HSP70* and *DNAJA4*), *SERPINB2* and *BAG3* genes are regulated in pressure testicular chicks). Another study used global gene expression analysis to examine the genetic structure of the hypothalamus of chickens exposed to natural temperatures, heat stress and temperature recov-

ery. The expression of heat shock protein families has also shown some changes, particularly in *HSPCB* and *DNAJC13* (Sun *et al*. 2015). Also, a study in the rate of change in expression of heat shock protein transcription factors after induction of acute and chronic heat stress in laying hens showed that acute and chronic heat stroke causes various oxidative damage and changes in the *HSP* and *HSF* genes in the liver, heart and muscles of laying hens. Some work has shown changes in *HSP* expression in the heart, liver, kidneys, blood, and fleshy muscles. It is important that, in addition to the genes directly or indirectly related to thermal stress in chickens (Table 1), further studies are conducted with these genetic groups in order to establish molecular profiles related to thermal stress and other genes of the *HSF* and *HSP*. According to published studies, *CRYAB*, *HSP90AA1*, *IL6*, *HSPA2*, *HSF2*, *HSPB1*, *HSF3*, *PLK1*, *BAG3* are mostly associated with non-infectious and infectious stressors and may deserve further attention. String database analysis illustrated role of highlighted gene in multiple cellular task and functionally such as ATPase activity, cellular processes, including protection of the proteome from stress, folding and transport of newly synthesized polypeptides, activation of proteolysis of misfolded proteins and the formation and dissociation of protein complexes. Obtained information from Animal QTL database indicated important role of chromosomes 2, 3, 4, 5, 12, 14 and 24 associated with stress resistance and susceptibility. Since these genes are expressed at the beginning of stress-related metabolic pathways, genes play a key role in the development of stress and protective pathways. On this basis, this report seeks to examine most of the genomic regions that control homeostasis and promote cell survival, molecular transport, and cell signaling. Future research is needed to validate the kind of conclusions that can be drawn from this study.

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