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Characterization of intron 7 to exon 8 of heat shock protein 90-aa1 (hsp90aa1) gene in dominant brown layer chicken using some bioinformatic tools

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RESEARCH ARTICLE

Abstract

HSP90AA1, an isoform of HSP90 has been characterized to indicate it plays important roles in basic cellular events. It is activated in chicken in response to heat stress. This study was aimed at the computational analysis of the biochemical cum structural features and an evolutionary relationship study on the HSP90AA1 gene in Dominant brown layers (DBL) and some selected avian species using bioinformatics tools. ProtParam for physicochemical properties. Scanprosite for post-translational modification sites, Netphos-3.1 for phosphorylation sites, BDM-PUB program for Ubiquitination sites, PDBSUM for Secondary structure and homology modelling with SWISS-model. The findings revealed that intron 7 and exon 8 of HSP90AA1 protein in DBL had a molecular weight of 24681.19Da and an instability index of 27.60, contains N-myristoylation, Protein-kinase-C-phosphorylation and Tyrosine-kinase-phosphorylation site-2 post-translational modification sites, 4-serine and 2-threonine phosphorylation sites and 12-ubiquitination sites. The evolutionary relationship study found Japanese quail to be in a sister branch close to DBL and chicken. Motifs detected in the avian species revealed the gene to be highly conserved. The secondary structure consisted of 16-helices, 3-sheets and 14-strands. The homology modelling was 87.25% sequence identity with human MC-HSP90-alpha. The study elucidates the components and characteristics of HSP90AA1 in DBL in response to heat stress.

Keywords: HSP90AA1; dominant brown layers; motifs; protein structure; phylogeny; phosphorylation.

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INTRODUCTION

Heat shock proteins (HSPs) are primarily known for the protection of cells from the deleterious effect of stress (Timperio et al., 2008; Kalmar and Greensmith, 2009). The expression of most HSPs is increased if cells are exposed to elevated temperature or other stressful conditions, although some HSPs are expressed even in non-stressful conditions (Itoh et al., 2005). Through protein-protein interactions, HSPs also regulate fundamental cellular processes such as protein turnover, mitochondrial and endoplasmic reticulum trafficking, cell cycle progression, and steroid signalling (Beato and Klug, 2000; Taipale et al., 2010).Heat shock proteins are named according to their molecular weight, such as HSP60 for 60-kiloDalton, HSP70 for 70-kiloDalton, HSP90 for 90-kiloDalton, HSP100 for 100-kiloDalton, and so on (Schlesinger, 1990). The HSP90 which is commonly known to be ubiquitous and highly conserved protein is comprised up

to 2% of total cell proteins under non-stressed conditions and up to 4–6% when stress arises (Picard, 2002; Whitesell and Lindquist, 2005; Stravopodis et al., 2007; Taipale et al., 2010; Finka and Goloubinoff, 2013). A study by Chen et al. (2005) proposed a new nomenclature system for the HSP90 gene family. They divided the gene family into five subfamilies namely; HSP90A, HSP90B, HSP90C, TRAP (tumour necrosis factor receptor-associated protein) and HTPG (high-temperature protein G).

HSP90 protein has been reported to have two cytosolic isoforms in eukaryotes: HSP90-α (HSP90AA1 gene) and HSP90-β (HSP90AB1 gene). HSP90AA1 is stress- or heat-induced and it is tissues-specific (Sreedhar et al., 2004), while HSP90AB1 is constitutive and is highly associated with the early development of the embryo and several cellular pathways (Csermely et al., 1998). HSP90AA1 also plays important role in basic cellular events like assisting unfolded proteins, giving the cell the needed aid in the repair of damaged proteins (Wegele et al., 2004; Walter and Buchner, 2004).

Poultry production in hot climates has negative effects that come with it, thereby, decreasing production efficiency. An increase in temperatures and incidence of heat waves has been reported to cause stress in poultry, resulting in reduced productivity, anorexia, heat stress and mortality (Turnpenny et al., 2001). In layer birds, heat stress has also been reported to cause a reduction in total egg production, egg mass and decreased shell strength (Mashaly et al., 2004).

The expression of the HSP90AA1 gene was first characterized in transformed mouse cells, where it was reported to increase in expression following heat shock compared to normal conditions (Ullrich et al., 1989). A study by Wang et al. (2015) indicated that HSP90AA1, among other HSP genes, was induced during acute heat stress, as a response by the testes of broiler-type chicken. It was also documented that mRNA expression including those of the HSP90AA1 gene, was up-regulated in heat-stressed layer-type roosters (Wang et al., 2013). These results have indicated that HSP90AA1 in chicken is activated in response to heat stress and an extended study of the HSP90AA1 gene in chicken would provide insight into its characteristics and importance.

Computational analysis has been very helpful in the characterization of protein structure and function (Edwards and Cottage, 2003). The study aimed to perform a computational analysis of the biochemical and structural features and phylogenetic analysis on part of intron 7 and exon 8 of HSP90AA1 gene and some selected avian species using bioinformatic tools and web servers. This would provide insight into the complex nature and characteristics of the HSP90AA1 gene in the laying chicken strain in comparison to other avian species. The study would also provide information on the degree of similarities and relatedness of the HSP90AA1 in a hybrid layer to some selected avian species.

MATERIALS AND METHODS

A consensus sequence of HSP90AA1 gene (728 bp) gotten from Dominant brown layer chicken in our previous study (Irivboje et al., 2020) was used for the bioinformatic analyses of this research.

Subcellular localization

Prediction of subcellular localization of the nucleotide sequence was carried out by CELLO v.2.5 program (http://cello.life.nctu.edu.tw/) (Yu et al., 2006).

Retrieval of other HSP90AA1 sequences of high percent identity, translation and alignment of the sequences

HSP90AA1 nucleotide sequences of other avian sequences were searched from the National Centre for Biotechnology Information (NCBI) database using BLAST algorithm (www.ncbi.nlm.nih.gov/Blast.cgi) (Zhang et al., 2000) optimized for highly similar sequences. The consensus sequence of Dominant brown HSP90AA1 was used for the search. Translation of all the nucleotide sequences was performed using an online server available at https://web.expasy.org/translate/. Multiple sequence alignments of the deduced HSP90AA1 amino sequences were performed using ClustalX 2.1 (Thompson et al., 1997).

Protein sequence analysis

The translated amino acid sequences were used for the remaining bioinformatic analysis as follows. The physiochemical properties (molecular weight, theoretical isoelectric point, instability index, aliphatic index, and grand average of hydropathicity) of Dominant brown chicken HSP90AA1 protein were predicted using the ProtParam online tool (http://web.expasy.org/protparam/) (Gasteiger et al., 2005). Transmembrane sequence analysis was performed using the system SOSUI accessed through http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.html (Hirokawa et al., 1998). The motifs scan was executed using the Scanprosite program available online at http://www.prosite.expasy.org/scanprosite/. It contains a large collection of biologically useful and equally annotated signatures for detection of short motifs or larger domains (Castro el al.,

2006). Phosphorylation profile analysis was done with the program, Netphos-3.1 server (http://www.cbs.dtu.dk/services/NetPhos/) (Blom et al., 1999). Prediction of Ubiquitination sites with Bayesian Discriminant Method (BDM-PUB) program (http://bdmpub.biocuckoo.org/) was used to predict ubiquitination sites (Li et al., 2009). The evolutionary relationship tree was constructed using the Neighbour-Joining statistical method and the reliability were evaluated with bootstrap analysis performed 1000 times to obtain support values for the branches using the MEGA 6 program (Tamura et al., 2013). The web server MEME (http://meme-suite.org/tools/meme) (Bailey and Elkan, 1994) was used to search for motifs in HSP90AA1 gene in Dominant brown layer and the retrieved avian species. Number of motifs to find was set to 15, optimum motif width was set to ≥ 6 and ≤ 50 and optimum number of sites for each motif was set at ≥ 2 and ≤ 600.

Structural analysis

The protein secondary structure was predicted using a pictorial database, PDBsum software (http://www.ebi.ac.uk/pdbsum) (Laskowski et al., 2018). Protein modelling and the prediction of its tertiary structure was done with the SWISS-model available at https://swissmodel.expasy.org//SWISS-MODEL.html (Waterhouse et al., 2018) and viewed with SWISS-PDB Viewer (Guex et al., 2009). The model was verified with Ramachandran plot calculations (Ramachandran et al., 1963; Morris et al., 1992) computed with PROCHECK program (Laskowski et al., 1993; Rullmann, 1996).

RESULTS AND DISCUSSIONS

Gene information

The consensus sequence of the HSP90AA1 of Dominant brown layer chicken was aligned with the Reference Sequence of Gallus gallus (Chicken) available at an online database (https://www.ensembl.org) to provide detailed information about the sequence. The coding sequence (CDS) of the HSP90AA1 in Dominant brown was confirmed to be located in exon 8 at 5:49,680,063 – 49,679,795.

Subcellular localization

The prediction of the subcellular localization of the HSP90AA1 gene of Dominant brown is represented in Table 1. The CELLO prediction showed that the Cytoplasmic region had the highest value with a reliability score of 1.937. Indicating HSP90AA1 to be dominantly located in the cytoplasm while also presented in other parts of the cell in varying quantities. Cellular localization analysis predicted HSP90AA1 protein of Dominant brown to be localized in the cytoplasm, suggesting it is its major site. HSP90A proteins had been reported to be largely cytosolic by Csermely et al. (1998), have ATPase activity, and play a key role in the folding of cell regulatory proteins and the re-folding of stress-denatured polypeptides (Obermann et al., 1998).

Table 1. Subcellular localization of the *HSP90AA1* gene of Dominant brown layer chicken

SVM	Localization	Reliability
Amino Acid Comp.	Cytoplasmic	0.362
N-peptide Comp.	Cytoplasmic	0.514
Partitioned seq. Comp.	Cytoplasmic	0.429
Physico-chemical Comp.	Chloroplast	0.369
Neighbouring seq. Comp.	Cytoplasmic	0.364
CELLO Prediction:	Cytoplasmic	1.937*
	Nuclear	0.934
	Chloroplast	0.712
	Extracellular	0.703
	Mitochondrial	0.461
	Plasma Membrane	0.080
	ER	0.043
	Peroxisomal	0.031
	Vacuole	0.029
	Golgi	0.029
	Cytoskeletal	0.023
	Lysosomal	0.018

* - SVM (Support Vector Machine)

Retrieved sequences of other avian species

The retrieved avian species with high identity to Dominant brown layers' HSP90AA1 nucleotide sequence is depicted in Table 2. The table showed Chicken (reference sequence), Japanese quail and Common pheasant to be having the highest percentage identity ranging from 97.00 to 100%. Golden eagle and North-Island Brown Kiwi had the least identity with 90.63% and 88.73% respectively.

The nucleotide sequence of the HSP90AA1 gene of Dominant brown showed a per cent identity range between 93.75 and 100% to twenty-six selected avian species. This confirms the gene to be highly conserved in the Dominant brown layer chicken. 100% identity was observed with *Gallus gallus* (NM_001109785.1), which is the reference sequence in the database. This is closely followed by the Japanese quail in a sister branch, the followed by Common pheasant, Helmeted guinea fowl and Turkey.

Table 2. Retrieved Sequences showing their accession number and percent identity

S/N	Species	Common name	Accession number	Percentage Identity to Dominant Brown (%)
1	*Coturnix japonica*	Japanese quail	AB517674.1	97.00
2	*Gallus gallus*	Chicken	NM_001109785.1	100.00
3	*Aquila chrysaetos*	Golden eagle	LR606182.1	90.63
4	*Phasianus colchicus*	Common pheasant	XM_031592984.1	97.06
5	*Numida meleagris*	Helmeted Guinea-fowl	XM_021401219.1	96.69
6	*Meleagris gallopavo*	Turkey	XM_010711927.3	96.32
7	*Apteryx australis mantelli*	North-Island Brown Kiwi	LK064790.1	88.73
8	*Anser cygnoides domesticus*	Swan goose	XM_013198244.1	94.85
9	*Pelecanus crispus*	Dalmatian pelican	XM_009483244.1	94.85
10	*Calypte anna*	Anna's humming bird	XM_030452390.1	94.49
12	*Opisthocomus hoazin*	Hoatzin (Stink bird)	XM_009936837.1	94.49
13	*Aptenodytes forsteri*	Emperor penguin	XM_009280847.1	94.49
14	*Aythya fuligula*	Tufted Duck	XM_032187887.1	94.12
15	*Athene cunicularia*	Burrowing Owl	XM_026849311.1	94.12
16	*Anas platyrhynchos*	Mallard (Wild duck)	XM_027458158.1	94.12
17	*Apteryx rowi*	Okarito Brown Kiwi	XM_026079728.1	94.12
18	*Fulmarus glacialis*	Northern fulmar	XM_009582499.1	94.12
19	*Pygoscelis adeliae*	Adelie penguin	XM_009327997.1	94.12
20	*Tyto alba*	Barn-Owl	XM_009974161.2	93.75
21	*Calidris pugnax*	Ruff	XM_014957488.1	93.75
22	*Pterocles gutturalis*	Yellow-throated sandgrouse	XM_010083045.1	93.75
23	*Leptosomus discolor*	Cuckoo roller	XM_009955579.1	93.75
24	*Apaloderma vittatum*	Bar-tailed trogon	XM_009869211.1	93.75
25	*Egretta garzetta*	Little egret	XM_009636860.1	93.75
26	*Phalacrocorax carbo*	Great cormorant	XM_009510560.1	93.75

Physico-chemical characteristics

The physical and chemical characteristics analysed revealed that Dominant brown HSP90AA1 gene had a molecular weight of 24681.19 Da. The predicted isoelectric point was 8.51. The instability index of the protein was computed to be 27.60, which classified the protein as stable. A protein is classified as stable or unstable by the measure of its instability index (Giroux et al., 1996; Rani et al., 2013). Aliphatic index was found to be 30.29 while Grand average of hydropathicity (GRAVY) was recorded to be -0.024. The Atomic composition formula was given as follows: C1124H1812N284O312S12.

Post-translational modification sites

Transmembranes sequences were not detected in the protein sequence of the HSP90AA1 gene of Dominant brown layers but the transmembrane sequence analysis also revealed that it has a negative hydrophobicity value of -0.05720, confirming it to be a soluble protein. The post-translational modification site analysis of HSP90AA1 in Dominant brown layers is shown in Table 3. Four motifs were detected and included; one MYRISTYL (N-myristylation site), two PKC_PHOSPHO_SITE (Protein kinase C phosphorylation site) and one TYR_PHOSPHO_SITE_2 (Tyrosine kinase phosphorylation site 2). The same motifs and more were also discovered by Chen et al., (2006) in the HSP90 family of genes.

Table 3. Post-translational modification sites (Motifs scan)

Pattern	Predicted feature	Phosphorylation site	Position	Motif sequence
MYRISTYL	-	-	8 - 13	GSvqCS
PKC_PHOSPHO_SITE	MOD_RES	Phosphoserine	34 - 36	SlK
PKC_PHOSPHO_SITE	MOD_RES	Phosphothreonine	54 - 56	TlK
TYR_PHOSPHO_SITE_2	-	-	150 - 157	KhglEviY

MYRISTYL - N-myristoylation, PKC_PHOSPHO_SITE - Protein Kinase C phosphorylation site, TYR_PHOSPHO_SITE_2 - Tyrosine kinase phosphorylation site 2, MOD_RES (Modified residue)

Phosphorylation sites

Table 4 shows the phosphorylation sites predicted in Dominant brown HSP90AA1 gene. The phosphorylation site predictor program (NetPhos) has the tendency to over predict, therefore stringency was increased and only those motifs with NetPhos score of 0.8 or higher were selected (Blom et al., 1999). Phosphorylation sites detected in the HSP90AA1 gene of Dominant brown layers were only on Serine and Threonine while there was no phosphorylation on tyrosine residues. Researches have shown that phosphorylation sites like other important post-translational modification sites have major effects on the structure and function of a protein (Zhao and Liu 2003). The position of the predicted sites, their context and score values (which ranges from 0.802 to 0.963) are all listed in the table.

Table 4. Prediction of phosphorylation sites

Position	Context	Score	Prediction	Kinase
34	IALA**S**LKLR	0.874	S	PKC
53	KLRV**S**TLKQ	0.963	S	Unspecified
54	LRVS**T**LKQL	0.941	T	Unspecified
87	SHQV**S**KITS	0.918	S	Unspecified
135	PAGE**T**KDQV	0.820	T	Unspecified
180	KTLV**S**VTKE	0.802	S	PKC

S refers to phosphorylation on serine residue, *T* refers to phosphorylation on threonine residue (scores above the threshold of 0.800 are assigned as '*S*' or '*T*')

Protein ubiquitination Site

The residues in HSP90AA1 protein of Dominant brown that undergo ubiquitination are listed in Table 5. Some of the processes that depend on ubiquitination include receptor and lysosomal trafficking, transcription factor activity among others (Radivojac et al., 2010). Identifying ubiquitination sites is important in understanding the molecular mechanism of the ubiquitin system and regulatory roles of ubiquitination (Li et al., 2009). In the analysis, twelve (12) ubiquitination sites were predicted.

Table 5. Protein ubiquitination sites prediction

Peptide	Position	Score	Threshold
SAMLLFFKHCCSFET	20	0.50	0.3
FIALASLKLRENELK	36	2.14	0.3
KLRENELKAGFLPKL	43	0.42	0.3
LKAGFLPKLRVSTLK	49	0.80	0.3
KLRVSTLKQLDFCFN	56	0.70	0.3
TNTLVGLKSHQVSKI	82	1.08	0.3
YVTVLRLKQNFMLPA	125	0.52	0.3
MLPAGETKDQVANSA	136	1.60	0.3
EDEEEKKKQEEKKAK	197	0.71	0.3
EKKKQEEKKAKFENL	201	2.05	0.3
KKKQEEKKAKFENLC	202	2.36	0.3
MKDILEKKVEKVISV	219	0.35	0.3

Phylogenetic analysis

The phylogenetic analyses procedure is used to reconstruct the evolutionary relationship, predict certain features, track gene flow and to determine genetic relatedness (Khan et al., 2014). A dendrogram illustrating the evolutionary relatedness of different avian species including Dominant brown layers is shown in figure 1. As indicated in the dendrogram, Brown dominant layers was found to be on the same clade with Chicken (Reference sequence) confirming the 100% identity they share, while Japanese quail which is closely related to the two is sited on a sister clade. The tree was constructed using the Neighbour joining statistical method and Poisson model available on the Molecular Evolutionary Genetics Analysis version 6.0. (MEGA 6.0). All gaps and missing data were completely deleted while the pattern among lineages were homogeneous.

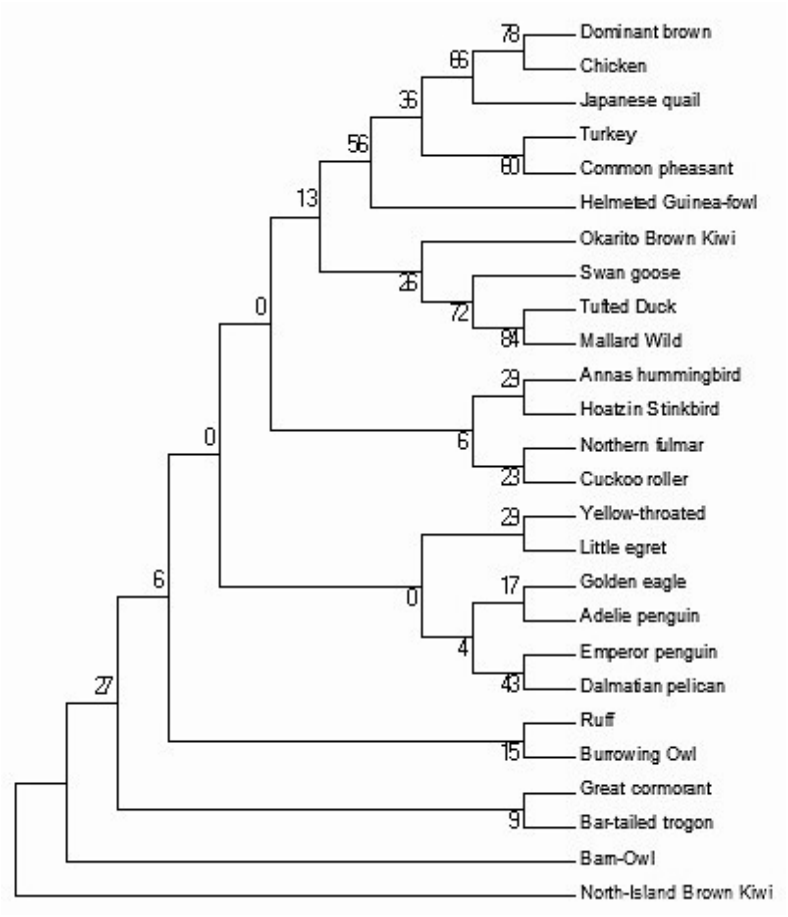

Figure 1. Phylogenetic tree illustrating the relationship between the HSP90AA1 of Brown dominant layers and other avian species.

Motif analysis

Motif analysis using MEME motif detection software revealed the diversification in the conserved motifs. The details of the 15 putative motifs are shown in Figure 2. The figure showed most of the avian species sharing similar motifs with *HSP90AA1* of Dominant brown layers with the exception of Golden eagle and North island brown kiwi, having fifteen and thirteen motifs respectively. The divergence seen in these two species could be a result of evolution over time. Similar motifs across the sequences showed the gene to be highly conserved in them.

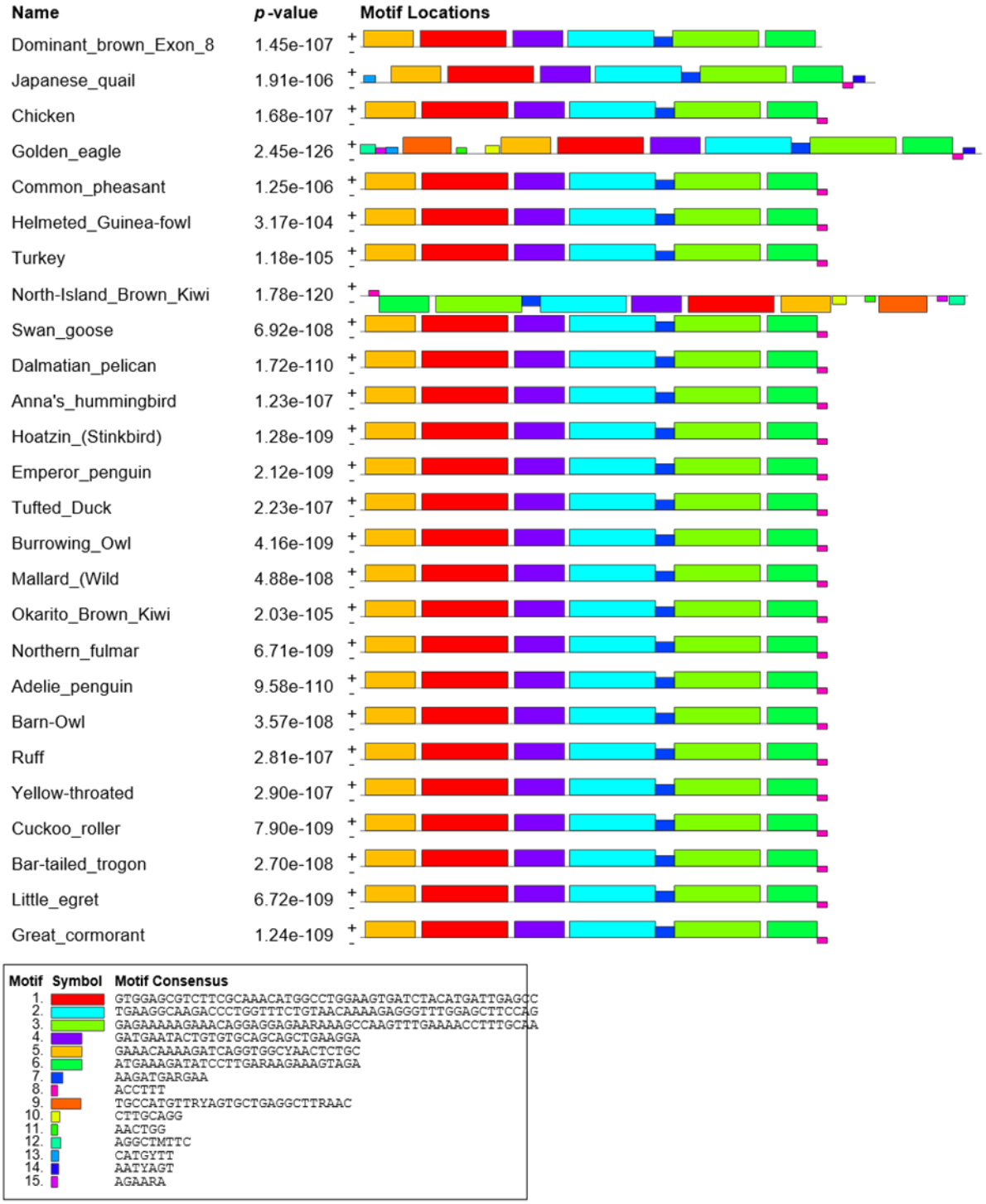

Figure 2. Conserved motifs arrangement in the *HSP90AA1* gene of Brown dominant layer chicken and other avian species

Structural analysis

Protein secondary structure prediction

The secondary structure analysis is shown in figure 3. The secondary structure of the *HSP90AA1* protein of Dominant brown was dominated majorly by Alpha helices. The protein consisted of 3 sheets, 1 beta alpha beta unit, 5 beta hairpins, 3 psi loops, 2 beta bulges, 14 strands, 16 helices, 18 helix-helix interacts, 35 beta turns and 3 gamma turns. The summary of the structure as seen in Table 6, depicted alpha-helix to be the main known component of the protein.

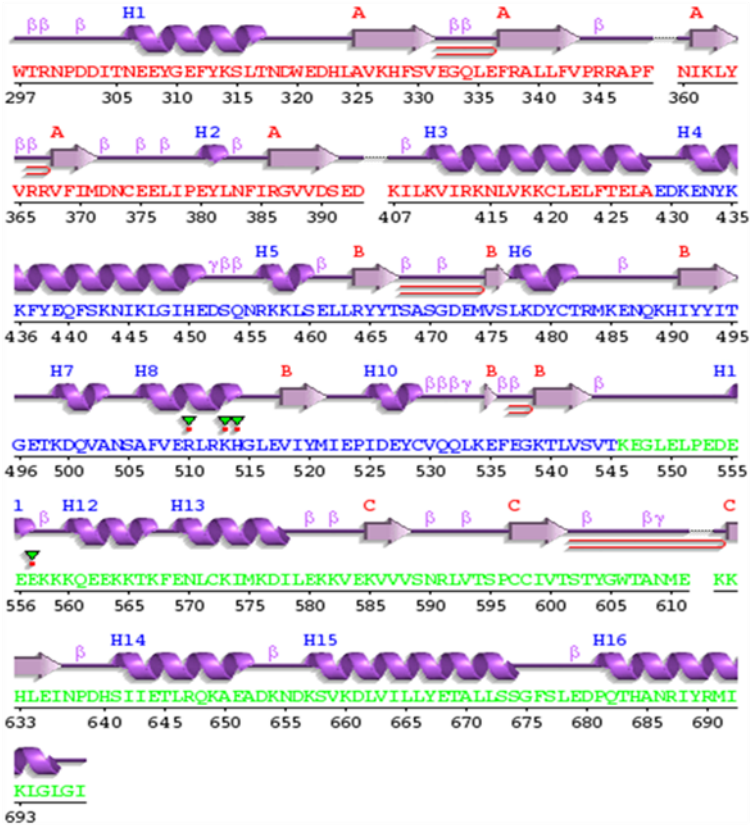

Figure 3. Secondary structure analysis of *HSP90AA1* protein of Dominant brown layer.

Key:

Secondary structure: Helix Strand — Helices labelled H1, H2, ... and strands by their sheets A, B, ...

Motifs: beta turn; gamma turn; beta hairpin

Residue contacts: to ligand

PDB SITE records: AC1 AC2 AC3 AC4 AC5 AC6

Table 6. Secondary structure summary

Strand	Alpha helix	3-10 helix	Other	Total residues
65 (18.1%)	143 (39.7%)	8 (2.2%)	144 (40.0%)	360

Tertiary structure prediction

The tertiary structure or homology modelling as displayed in figure 4 and the validation of the structure was computed with the Ramachandran plot calculations as displayed in figure 5. Protein tertiary structure is important in knowing the interaction, function and localization of the protein. In homology modelling, a suitable template for the structure was selected was based on the X-ray structure of Heat shock protein HSP 90-alpha Crystal Structure of Human MC-HSP90 in the P21 space group (PDB ID: 3q6n) (Lee et al., 2011). The target sequence was searched by BLAST (Camacho et al., 2009) against the primary amino acid sequence available in the SWISS-MODEL template library with a sequence identity of 87.25%. The protein model of the *HSP90AA1* protein of Dominant brown layer validated with Ramachandran plot revealed 99.1% of the residues were in both favoured and allowed regions.

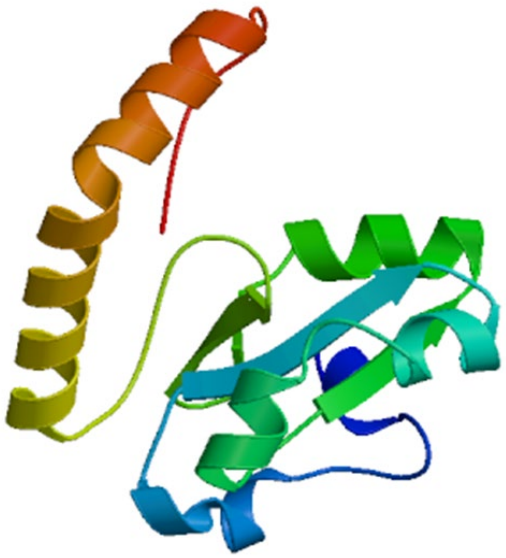

Figure 4. 3D structure of *HSP90AA1* protein in Dominant brown layer chicken with 87.25% sequence identity.

CONCLUSIONS

The *HSP90AA1* gene of Dominant brown layers is important in this study as the layer chicken strain is a very common brown plumage layer bird for table egg production in Nigeria. The study – functional and structural analysis elucidates its components and characteristics in response to heat stress. It shares a perfect identity with Chicken (Reference sequence) and is closely related to Japanese quail, Turkey and Common pheasant. The *HSP90AA1* protein was revealed to be highly conserved in Dominant brown layers and the other avian species as they share similar motifs. Dominant brown layers are a popular hybrid layers' strain that has over the years been used for breeding and table egg production in Nigeria and generally in the tropics. The detected features may have contributed to the adaptability of Dominant black layers to the climatic conditions of the tropics. Further study is required to reveal the level of tolerance and expression of the *HSP90AA1* gene in Dominant brown layers in response to heat stress.

Author Contributions: P Y.I., C.I. and A.F. Conceived and designed the experiment; Y.I. Carried out the research, Performed the analyses, Wrote the paper; Y.I. and O.I. Collected data; C.I. and A.F. Supervised the research; O.I. Proof read the paper.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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