Research Square

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Differential growth, Cardiovascular risk, and Polymorphism in Targeted Regions of Ryanodine Receptor 2 (RYR2) gene in Three Breeds of Chickens.

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Abstract

Sudden death syndrome (SDS) is a stress-related genetic disease in broilers with no diagnostic clinical or necropsy finding. SDS is often preceded by elevated markers of cardiovascular risks (Obesity, Blood glucose, Lipidemia and Inflammation) in broilers. Mutation of Ryanodine Receptor 2 (RYR2) gene, which controls rapid release of ca^{2+} from the sarcoplasmic reticulum (SR) into the Cystol during muscle contraction has been associated with sudden death syndrome in broilers, but there are no reports on the genetic association between RYR2 mutation and cardiovascular risk markers in broiler. This study aims to investigate the role of RYR2 mutation in the development of SDS using the linkage association approach. The study compares exotic broiler chicken (Ross 308, Arbor Acres) with the less susceptible indigenous Noiler chicken breed as a contrast to identifying genetic markers linked to SDS susceptibility.

Key findings revealed a Single Nucleotide Polymorphism (SNP) near a functional RYR2 region at chicken genome location 3:36968262. The Noiler and Arbor Acres breeds carried an A (Adenosine) allele, while Ross 308 carried a G (Guanine) allele, with the G allele associated with increased cardiovascular risk and stress. Moreover, Arbor Acres and Ross 308 breeds exhibited higher stress levels and cardiovascular risk than the Noiler breed using certain metrics of measurement. These results underscore the critical role of RYR2 genetic differences in growth and cardiovascular traits in poultry, these findings suggest that genetic differences in the RYR2 gene play a crucial role in growth and cardiovascular traits in poultry, offering valuable insights for breeding programs.

Implications

This research provides critical insights into the genetic basis of growth and cardiovascular health in poultry, emphasizing its implications for sustainable breeding in tropical and subtropical regions. By identifying a Single Nucleotide Polymorphism (SNP) in the Ryanodine Receptor 2 (RYR2) gene associated with sudden death, the study highlights genetic markers that can guide breeding programs to enhance productivity and resilience in poultry. These findings hold economic importance by promoting healthier, stress-resilient chicken breeds, reducing losses in poultry production. Environmentally, the results support breeding strategies that minimize stress-induced mortality, improving resource efficiency. Socially, this research contributes to food security in tropical regions.

Introduction

Broiler chickens play a crucial role in addressing Nigeria's protein needs (Olorunwa, 2018). Broiler production provides employment, income, and an affordable protein source within 6–10 weeks (Olorunwa, 2018). Broilers are favored in Nigeria for their high feed conversion ratio, rapid growth, and profitability. With over 200 million chickens raised commercially (FAO, 2008; Oloso et al., 2020), broiler production is significant to the economy. However, the fast growth rate of broilers comes with challenges, including sudden death syndrome (SDS), where healthy birds suddenly die, often due to cardiovascular complications (Sosnowka-Czajka & Skomorucha, 2022). Years of selection for rapid growth and feed efficiency have increased the cardiovascular system's workload, predisposing broilers to metabolic disorders like right ventricular failure, cardiac arrhythmias, and sudden death (Julian, 2005). SDS accounts for a 3–6% mortality rate, with further studies indicating up to 17–35% of fast-growing birds suffer from cardiac arrhythmias, a key cause of sudden death (Olkowski et al., 2005, 2008, 2010).

Slow-growing chicken breeds are less prone to SDS, as they have slower metabolic rates and reduced cardiovascular stress (Olkowski & Classen, 1998). One such breed is the Noiler chicken, a Nigerian crossbreed between exotic broilers and pullets. Though smaller and slower growing, Noilers are hardy and less susceptible to sudden death, making them a preferred breed for many (Animashahun et al., 2022).

Genetic selection has been linked to SDS in broilers, with rapid growth and feed efficiency also increasing cardiovascular risks (Ajayi, 2010). Traditional animal improvement methods, such as black box selection, are less efficient than molecular approaches like marker-assisted selection (Meuwissen et al., 2001). One promising approach is candidate gene studies, which investigate known genetic variations associated with traits or diseases. For SDS, studies have identified polymorphisms in genes like calsequestrin 2 and ryanodine receptor 2 (RYR2), which are linked to cardiac disorders and sudden death (Basaki et al., 2019;

Peng et al., 2016). This research will focus on RYR2's role in cardiovascular risk in broilers and Noiler chickens and will also explore SDS by examining cardiovascular stress and risk factors, including obesity, lipidemia, inflammation, and blood glucose.

Materials And Method

Experimental site

The experiment was conducted at the University of Ilorin Teaching and Research Farm, Ilorin, Kwara State, Nigeria (Latitude: 8°27'09.5"N, Longitude: 4°39'44.5"E). Molecular biology analyses were performed at three locations:

- University of Ilorin Central Research Laboratories (DNA extraction).
- University of Ilorin Teaching Hospital, Molecular Diagnostic and Research Laboratories (PCR amplification).
- Inqaba Biotechnology Limited, South Africa (DNA sequencing).

Experimental Birds

A total of 150-day-old chicks from three breeds were used: 50 Noiler (from Amo Sieberer Hatchery, Ibadan), 50 Abor Acres (from Valentine Hatchery, Ilorin), and 50 Ross 308 (from Agrited Hatchery, Lagos).

Management

Upon arrival, the chicks received electrolytes (Vitranor by Jubaili) and were wing-tagged for identification. The birds were divided into three compartments based on breed and fed ad-libitum with commercial feeds. For the first 4 weeks, they were fed Ultima broiler starter (23% protein), and for the remaining 2 weeks, they were fed Ultima broiler finisher (18% protein). Vaccinations against infectious bursal disease and Newcastle disease were administered weekly.

Housing

The birds were housed in a deep-litter system for the entire experiment.

Data Collection

Weekly data were collected on growth parameters: body length, wing length, thigh length, drumstick length, shank length, keel length, and body girth. Body weight was measured using a top-loading scale. Body Mass Index (BMI) was also calculated as a metric of evaluating cardiovascular risk, providing an indicator of susceptibility to sudden death related to obesity. Growth rates were determined using Maciejowski and Ziêba's formula (1982).

- BODY LENGTH: The distance between the top of the head and pygostyle (Oludoyi & Toye, 2012)
- WING LENGTH: Distance between the tip of the phalanges and the coracoids-humerus joint (Oludoyi & Toye, 2012).
- THIGH LENGTH: The distance between the beginning of the fibula to the hock joint.
- DRUMSTICK LENGTH: The length of the Femur bone (Oludoyi & Toye, 2012).
- SHANK LENGTH: The distance between the beginning of the hock joint to the last ring before the tarsal or meta-tarsal digit (Oludoyi & Toye, 2012).
- KEEL LENGTH: The distance from the V-joint to the end of the sternum (Oludoyi & Toye, 2012).
- BODY GIRTH: This was taken from the point of depression to the breast round the body back to the point of depression (Oludoyi & Toye, 2012).
- BODY WEIGTH: Body weight was measured weekly using a top loading 20kg scale with sensitivity of 10g (Oludoyi & Toye, 2012).

- BODY MASS INDEX (BMI): The body mass index was derived by dividing body weight expressed in gram units by the square of body
- length expressed in cm units (Oludoyi & Toye, 2012).
- GROWTH RATE: For body weight (g), and eachof the linear measures of body growth recorded (cm), Growth rate/velocity/potential for 1, 3 and 6 successive week periods were determined by use of an adapted version of the formula of Maciejowski and Ziêba (1982),

I = [(T2 T1) / (T2 + T1)/2] x 100 …… Eq. 1

Where:

I = Growth rate per unit period (%).

T1 = Average initial reading for the stated period (g or cm)

T2 = Average final reading for the stated period (g or cm)

- These measurements were taken with the use of a tailor rule and recorded in centimeter (cm) except for body weight which was recorded in Grams (g), following the procedures of (Oludoyi & Toye, 2012).

Primer Design

The RYR2 gene (ENSGALT00010021632.1) on chromosome 3, with five variants in Gallus gallus domesticus, was the target gene in this experiment. This gene, associated with sudden death syndrome in broilers, has 104 exons (Basaki, 2019). Genomic organization and gene sequences were obtained from ENSEMBL (www.ensembl.org). Forward and reverse primers were designed to amplify exons 23, 40, and 44 (Table 1), identified as mutational hotspots across species (Alvarado *et al.*, 2019). Using Primer3web Version 4.0.0, primers were anchored 50–100 base pairs from each exon's flanking regions. These primers were synthesized by Inqaba Biotechnical Industries, South Africa.

Table 1
Summary Of Results from Forward and Reverse Primer Sequencing Of Ryr2 Gene And Exon Pcr Products From Ross 308, Arbor Acre And Noiler Chicken.

Primer Name	Primer sequence (5' – 3')	Primer length (bases)	Actual primer temperature (°c)	Product size (bp)	Exon position (nt)	Sequencing result		
						R3	AA	NO
RYR2_XN23-F	GACAACTGTGGAAGCCTGTT	20	51.78	253	37,010,713 –	S	S	S
RYR2_XN23-R	TCCCATTAGCACACTGAAATTGA	23	51.71		37,010,894	S	S	S
RYR2_XN40-F	TCTGCAGACAAAGTAGAAAGCA	22	51.11	344	36,968,268 –	S	S	S
RYR2_XN40-R	TTGGGGTGCACAGATCTTTC	20	51.78		36,968,758	S	S	S
RYR2_XN44-F	GTGCAAGATGATTATAAATTGAGTGGT	27	53.66	464	36,959,346 –	NS	NS	NS
RYR2_XN44-R	TCAAAGTTCAATCATCCAGAGCA	23	51.71		36,959,916	NS	NS	NS

*Ryr2- Ryanodine Receptor 2; Xn- Exon; F- Forward; R- Reverse; Ta- Annealing Temperature; Bp- Base Pair; Nt- Nucleotide; S- Sequenced; Ns- Not Sequenced; R3- Ross 308 Chicken; Aa- Arbor Acre Chicken; No- Noiler Chicken. *Exon Position And Location Relating To Nt- 100 (3'utr) Primary Transcript (Ryr2- Ensgalt00010021632.1)

Blood Collection and DNA Extraction

Blood samples were collected for hematological analysis, serum biochemistry, and genotyping.

1ml of blood was collected via the brachial vein of each bird using sterilized disposable 2ml syringe and needle into EDTA (Ethylene-di-amine-tetra-acetic acid) bottles to prevent coagulation. DNA was then extracted from blood samples using the procedure described by Udeze et al. (2020). DNA yield and purity were accessed by the use of Nano-drop spectrophotometry analysis.

Target Fragment Amplification by PCR

Fifteen DNA templates from three chicken breeds (five replicates per breed) were amplified using polymerase chain reaction (PCR) following standard protocol. The PCR process was conducted at the Molecular Diagnostic and Research Laboratory, University of Ilorin, Nigeria, using a MyGene™ Series Peltier Thermal Cycler. The NEB PCR cocktail included 2X Master mix with buffer, chicken DNA template, primers, and deionized water, totaling 25µl. The thermal cycle used are: denaturation at 95°C, annealing at 55°C, 58°C, or 62°C, and extension at 72°C. PCR products were resolved on a 2% agarose gel with ethidium bromide, revealing a single clear band.

Sequencing

The band of the five replicates of each breed's PCR products was purified and sequenced through a commercial service (Inqaba Biotec Company, South Africa; using LIZ500 (ThermoScientific), ABI 3500XL Genetic Analyzer for Applied Biosystems, POP7™). Sequences results (electrophoretogram) supplied by Inqaba were viewed using Finch TV v1.4.0.

Multiple Sequence Alignment

The sequence of each successfully sequenced Exon from each of the examined breeds was aligned (MultAlin; Corpet, 1996) against the known sequence of the corresponding chicken gene (RYR2; ENSGALT00010021632.1) to confirm that the intended fragment of the target gene has been correctly amplified and sequenced.

Phylogenetic Analysis

Phylogenetic analysis was carried out using MultAlin (Corpet, 1996) and phylogenyfr (Dereeper et al., 2008) to determine the evolutionary relationship amongst orthologs.

Search for Regulatory Sequences (MOTIFS)

Tfsitescan (http://www.ifti.org/cgi-bin/ifti/Tfsitescan.pl) online Motif searching software was used to scan for known gene regulatory sequences within the polymorphic intronic flanks of Exons DNA.

Search for Endonuclease Restriction Site

NEBcutter (https://nc3.neb.com/NEBcutter/) online endonuclease cleaving site software was used to scan for known gene restriction site sequences within the overlapping intronic SNPs.

SNP Association with Trait Related to Cardiovascular Risks and Physiological Stress.

Data collected was analyzed by observing the effect of SNP on traits of cardiovascular risks and physiological stress by using SPSS version 27.

Results

All three exons from each of the 15 DNA templates were successfully amplified. However, clear bands were observed in only 66.7%, 66%, and 60% of the template DNA for exon 40, 23, and 44, respectively (Fig. 1&2). The sharpest bands from the five replicates of each breed were selected for sequencing. Despite attempts, sequencing exon 44 did not yield successful results in any of the reactions.

Nucleotide Sequence Polymorphism in RYR2

RYR2 gene Exon 23

Multiple sequence alignment of RYR2 exon 23 from Ross 308, Arbor acres and Noiler breeds (including flanking intron DNA) against corresponding region of corresponding chicken gene (RYR2; ENSGALT00010021632.1) RYR2 revealed no sequence polymorphism (deletion, insertion, inversion or substitution) in the coding and non-coding portion (Fig. 3)

RYR2 gene Exon 40

Multiple sequence alignment of RYR2 exon 40 from Ross 308, Arbor acres and Noiler breeds (including flanking intron DNA) against corresponding region of corresponding chicken gene (RYR2; ENSGALT00010021632.1) RYR2 revealed no sequence polymorphism (deletion, insertion, inversion or substitution) in the coding portion. However, a purine (Adenine A) to purine (Guanine G), Inversion single nucleotide polymorphism (SNP) located at base 3:36968262 of the primary transcript (numbering from nt-100 in the 3'UTR following Exon 40) was observed. This polymorphism falls in the 3' non-coding portion of the amplified exon 40 fragment; the primary transcript (ENSGALT00010021632.1) together with the Arbor Acre and Noiler breed carries the 36968262 Guanine (G) allele and the Ross 308 carrying the Adenine (A) allele (Fig. 4). (See Figs. 5,6 and 7 for the chromogram of a segment of sequence at the 3' flanking intron DNA sequence (within intron 40–41) of RYR2 Exon 40 of Arbor Acre, Noiler and Ross 308 chicken respectively.)

Phylogenetic Analysis

RYR2 Gene Exon 23

The phylogenetic analysis of RYR2 Exon 23 (intron 23) for Noiler, Arbor Acre, and Ross 308, compared to the red jungle fowl and other avian orthologs, revealed multiple conserved regions. These include conserved sequences within the exon and the 5' and 3' flanking intronic regions, such as an 8-base stretch (GTACCTTC) near the 3' splice donor site (GT) corresponding to position 37010779 – 37010771 of the primary transcript and a 7-base stretch (TCTCTTT) at the 5' splice acceptor site (AG) corresponding to position 37010898 – 37010891 of the primary transcript. Within the exon, sequences like (ATTGTTTTGCCTCC, CATGAACTTTGGGTTATGAATAAGATT) were conserved (see Fig. 8). However, conservation decreased when non-avian species were included, except for the dinucleotide GA within the exon (See Figs. 8 & 9).

RYR2 Gene Exon 40

The phylogenetic analysis of RYR2 Exon 40 (intron 40) for Noiler, Arbor Acre, and Ross 308, compared to the red jungle fowl and other avian orthologs against the primary transcript (ENSGALT00010021632.1), revealed multiple conserved regions within the exon and flanking intronic regions. Conserved sequences include a 5-base stretch (TTC*AG*) at the 5' splice acceptor site except in duck corresponding to position 36968349 of the primary transcript and a 3-base stretch (T*GT*) at the 3' splice donor site corresponding to position 36968494 of the primary transcript, along with the canonical splice dinucleotides AG and GT. Within the exon, conserved sequences such as TTGA, TGG, ATGG, and GGAA were observed from positions 36,968,350 – 36,968,493 in the chicken gene (See Figs. 10,11 & 12)

Regulatory Sequence (MOTIFS)

RYR2 Gene Exon 23

The search for regulatory signatures in the intron sequences flanking RYR2 Exon 23 in Ross 308, Arbor Acre, and Noiler chickens revealed no regulatory elements. No polymorphisms were found in flanking introns of exon 23, corresponding to positions 37,010,785–37,010,889 in the broiler reference genome ENSGALT00000020816. Thus, the analysis did not identify any regulatory signatures in these regions for the three chicken breeds.

RYR2 Gene Exon 40

A sequential search for regulatory signatures in the polymorphic intron DNA sequence 3' flanking RYR2 Exon 40 of Ross 308, Arbor Acre, and Noiler chickens revealed no regulatory elements. Ross 308 carries an alternate allele (Adenosine; A) while Arbor Acre and Noiler carry Guanine (G) at positions 36,968,350–36,968,493 of the broiler reference (ENSGALT00010021632.1). Despite this, no regulatory signature was found at the polymorphic site, next to this site is an important motif, EF II (AAGCAACATTA), a transcription factor known as Elongation Factor 2, vital for protein synthesis and essential for cellular growth and development. The EF II motif, though critical, has not been reported in the RYR2 gene.

Search for Endonuclease Restriction Site

A search for endonuclease restriction sites in the polymorphic intron DNA sequence 3' flanking RYR2 Exon 40 in Ross 308, Arbor Acre, and Noiler chickens revealed no restriction signature. Ross 308 carries an alternate allele (Adenosine; A), while Arbor Acre and Noiler carry Guanine (G) at positions 36,968,350–36,968,493 of the broiler reference (ENSGALT00010021632.1). Despite being a highly endonuclease-restrictive region, PCR by Restriction Fragment Length Polymorphism (RFLP) is unsuitable for determining allele inheritance at this SNP. An alternative approach, such as genotyping by tetra-arms PCR, should be used instead.

SNP Association with Trait Related to Cardiovascular Risks and Physiological Stress.

Bird genotypes were classified at each SNP locus, with Ross 308 broilers carrying the Adenosine (A) allele and Noiler and Arbor Acre chickens carrying the Guanine (G) allele. This classification aimed to assess genotype effects on cardiovascular risks, including obesity (BMI), blood glucose, inflammation, lipidemia, and stress levels, measured by the Heterophil/lymphocyte ratio.

Effect of Genotype on Obesity

At week 1, there was no significant BMI difference between genotypes (ROSS 308 = Arbor Acre & Noiler). From weeks 2 to 6, BMI was significantly different ($p < 0.05$) between genotypes, with Ross 308 having higher BMI than Arbor Acre and Noiler (See Table 2).

Table 2
Effect of genotype on body weight growth rate from week 1 to 6. BMI: Body Mass Index (g/cm^2), means with different superscript within row are significantly different.

TRAITS	GENOTYPE SNP (rs734634989)	
	A (ROSS 308)	G (Arbor Acre & Noiler)
BMI_wk1 (g/cm^2)	9.8 ± 0.29(49)[a]	9.1 ± 0.18(99)[a]
BMI_wk2 (g/cm^2)	23.5 ± 0.83(49)[a]	17.2 ± 0.52(99)[b]
BMI_wk3 (g/cm^2)	38.3 ± 1.52(49)[a]	26.4 ± 0.96(99)[b]
BMI_wk4 (g/cm^2)	54.1 ± 2.0(49)[a]	32.6 ± 1.26(99)[b]
BMI_wk5 (g/cm^2)	69.5 ± 2.65(49)[a]	41.7 ± 1.69(99)[b]
BMI_wk6 (g/cm^2)	82.5 ± 3.34(40)[a]	49.5 ± 2.11(97)[b]

Effect of Genotype on Blood Glucose.

There was no significant difference in glucose levels between the genotypes A (ROSS 308) and G (Arbor Acre & Noiler) (See Table 3).

Table 3
Effect of breed on body weight growth rate from week 1 to 6.

BLOOD PROFILE	GENOTYPE SNP (rs734634989)	
	A (ROSS 308)	G (Arbor Acre & Noiler)
TC (mg/dl)	21.7 ± 0.95(10)[a]	21.6 ± 0.67(10)[a]
LDL (mg/dl)	13.4 ± 1.3(10)[a]	11.3 ± 0.88(10)[a]
HDL (mg/dl)	6.5 ± 0.52(10)[a]	8.4 ± 0.36(10)[b]
GLUCOSE (mg/dl)	59.2 ± 2.7(10)[a]	62.1 ± 1.9(10)[a]
HB (g/dL)	7.7 ± 0.32(10)[a]	8.4 ± 0.22(10)[a]
RBC ($x10^6$/mcl)	3.8 ± 0.18(10)[a]	4.02 ± 0.13(10)[a]
MCV (fL)	60.9 ± 0.54(10)[a]	61.3 ± 0.38(10)[a]
MCH (pg)	20.3 ± 0.18(10)[a]	20.4 ± 0.13(10)[a]
MCHC (g/dL)	33.2 ± 0.2(10)[a]	33.2 ± 0.14(10)[a]
WBC ($x10^3$/mcl)	2.6 ± 0.24(10)[a]	2.7 ± 0.17(10)[a]
LYM (%)	71.6 ± 4.1(10)[a]	65.8 ± 2.9(10)[a]
HETERO	28.7 ± 4.0(10)[a]	38.7 ± 2.8(10)[a]
H/L RATIO	0.41 ± 0.66(10)[a]	0.57 ± 0.47(10)[a]
TRYGLY (mg/dl)	3.9 ± 0.9(10)[a]	3.95 ± 0.06(10)[a]

TC: Total cholesterol, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, HB: Hemoglobin, RBC: Red Blood Count, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Concentration, WBC: White Blood Count, LYM: Lymphocytes, HETERO: Heterophils, TRYGLY: Triglycerides, H/L RATIO: Heterophils/Lymphocytes Ratio. means with different superscript within row are significantly different.

Effect of Genotype on Inflammation (White Blood Count, Heterophil, Lymphocyte).

There were no significant differences in white blood cell counts, heterophil percentages, or lymphocyte percentages between genotypes A (ROSS 308) and G (Arbor Acre & Noiler) (See Table 3).

Effect of Genotype on Lipidemia (Tc, Ldl, Hdl).

There were no significant differences in total cholesterol (TC) or LDL levels between genotypes A (ROSS 308) and G (Arbor Acre & Noiler), as shown in Table 4.9.2. However, HDL levels were significantly different ($p < 0.05$), with genotype A having lower levels than genotype G (See Table 3).

Effect of Genotype on Physiological Stress (Heterophil/Lymphocyte Ratio)

Genotype does not significantly ($p < 0.05$) affect Heterophil/Lymphocyte ratio. However, with the p value approaching 0.05 may point to a weak but significant difference detectable only in experiments with high power (higher replication per group and lower environmental noise). Facilitating a stronger distinction between group variance from within group variance (See Table 3).

Author's Point of View

The absence of polymorphisms in RYR2 Exon 23 for Noiler, Ross 308, and Arbor Acre chickens suggests this exon is not responsible for cardiovascular risk and stress differences. However, intronic polymorphisms identified could serve as molecular markers, creating breed-specific identities for future research on distinct variants.

Evidence from 167 chicken genotypes across diverse geographical regions, including China, Indonesia, Korea, and Tibet, supports the existence of the SNP. Studies indicate this SNP is conserved across various populations, suggesting its evolutionary significance or selective pressure related to environmental adaptation. Notably, the exon 40 sequence of Arbor Acre and Ross 308 in the RYR2 gene has not been previously described, highlighting an intronic SNP that could be useful for selective breeding.

Using the SNP haplotypes identified outside RYR2 Exon 40, the three breeds—Arbor Acre Broiler, Ross 308 Broiler, and Noiler chicken—can be distinguished at the molecular level regarding cardiovascular risk and physiological stress. This study underscores the importance of comparative sequencing of candidate genes for identifying breed-specific haplotypes, paving the way for gene association studies. However, the SNP haplotype does not fully distinguish effects on cardiovascular risk and stress, except for HDL levels. Genotype A is associated with about 10% lower HDL than Genotype G, indicating that birds inheriting allele A may experience higher stress due to reduced HDL. Although this SNP likely does not directly cause HDL differences, it may be in linkage disequilibrium with a causal variant nearby. Further sequencing studies are needed to identify the actual causal variant within this gene.

Conclusion

The unsampled RYR2 Exon 44 should be sequenced and analyzed by future researchers. Further studies are needed to identify additional mutations to address the gaps in this research. Additionally, other candidate genes linked to cardiovascular risks and stress in different chicken breeds should be amplified and sequenced in Noiler and indigenous birds to enhance local chicken populations. The haplotypes identified outside RYR2 Exon 40 could serve as molecular markers for distinguishing Ross 308 broiler chickens. Future investigations should also explore how variations in RYR2 Exon 40 haplotypes contribute to differences in cardiovascular risks and stress levels.

Declarations

Ethical Approval

All experimental procedures involving animals were conducted in compliance with the university of Ilorin department of animal production ethical committee guidelines and approved by the Animal Ethics Committee of the department.

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Author Contributions

Kolawole Olayiwola Faisal: Conceived and designed the study, performed the experiments, analyzed the data, and wrote the manuscript.

Prof. Toye, A. A: Supervised the research and offered guidance.

Dr. Sulaiman, M.K: Provided expertise in the molecular techniques used, supervised the laboratory aspect of the project, and helped revise the manuscript.

Declaration of Interest

The author declares no conflict of interest regarding the publication of this study.

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Figures

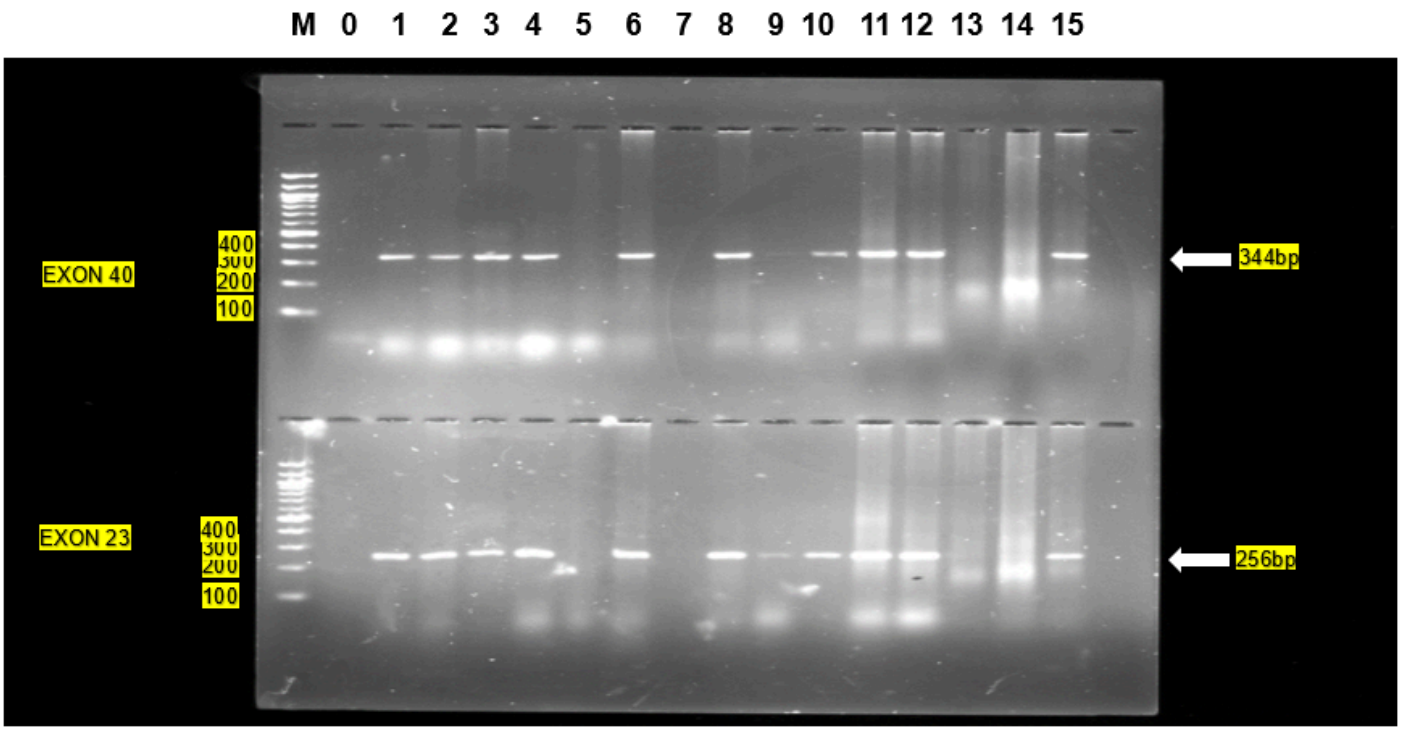


Figure 1

Gel image of electrophoretically resolved RYR2 Exon 40 and 23 PCR amplified products. Ladder shown (lane M) and fragment size to the right.

Lane M= 100 base pair DNA ladder; Lanes 1,2,3,4 and 5 = Exon 40 and 23 of Ross 308 respectively; Lanes 6,7,8,9 and 10 = Exon 40 and 23 of Arbor Acres respectively; Lanes 11,12,13,14 and 15 = Exon 40 and 23 of Noiler respectively; bp = base pair.

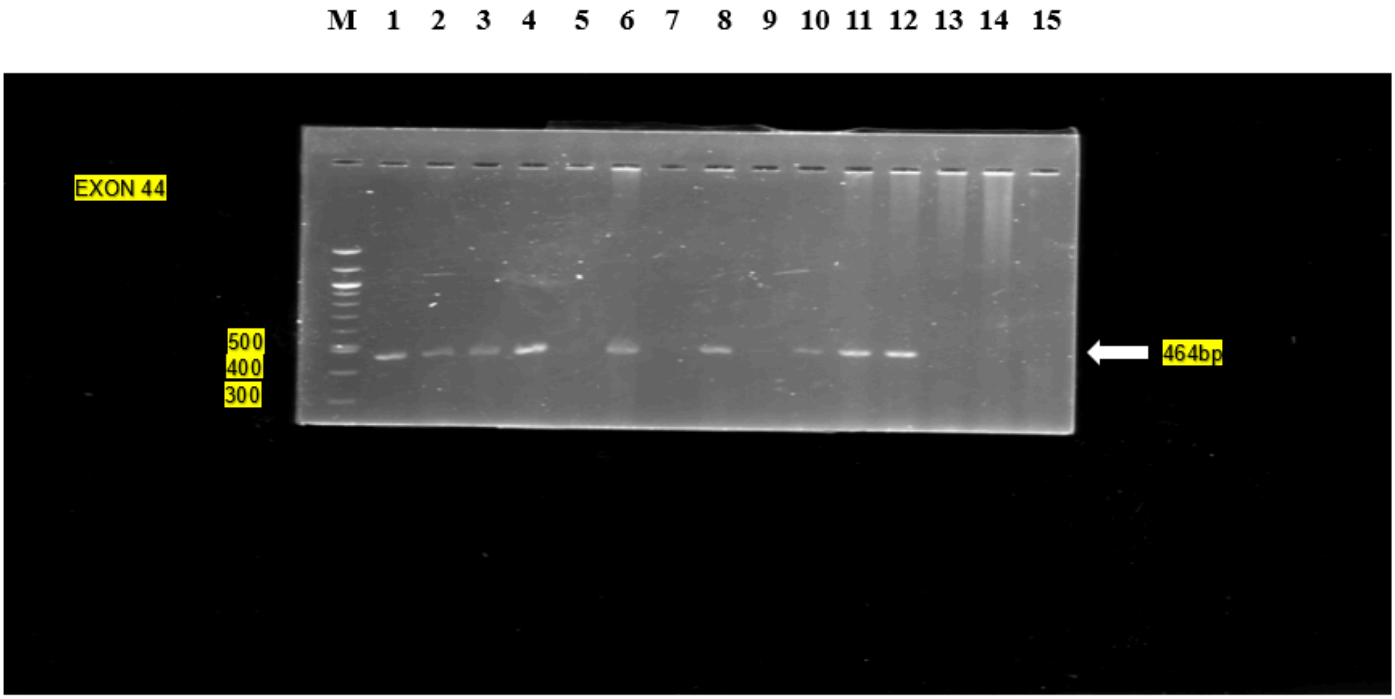


Figure 2

Gel image of electrophoretically resolved RYR2 Exon 44 PCR amplified products. Ladder shown (lane M) and fragment size to the right.

Lane M= 100 base pair DNA ladder; Lanes 1,2,3,4 and 5 = Exon 44 of Ross 308; Lanes 6,7,8,9 and 10 = Exon 44 of Arbor Acres; Lanes 11,12,13,14 and 15 = Exon 44 of Noiler; bp = base pair.

Electrophoresis setup: 2% agarose, 145 volts for 45 minutes.

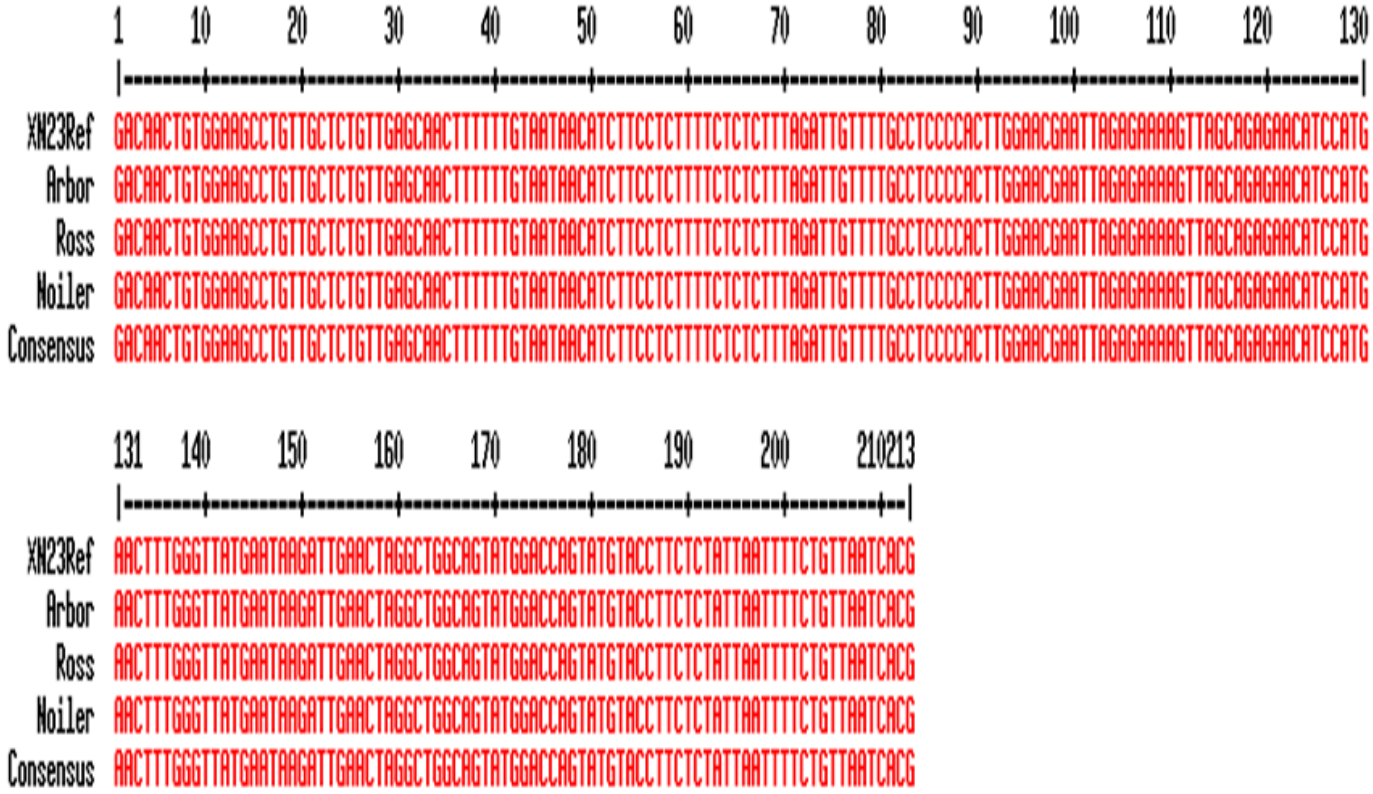


Figure 3

Multiple sequence alignment of RYR2 Exon 23 and flanking intron DNA sequence for Ross 308 broiler (Reverse: Ross 308), Arbor acre broiler (Forward: Arbor) and Noiler chicken (Reverse: noiler) amplified in this study against RYR2 Exon 23 and flanking intron DNA sequence for broiler reference (XN26Ref) (ENSGALT00010021632.1) with no polymorphic base arrowed.

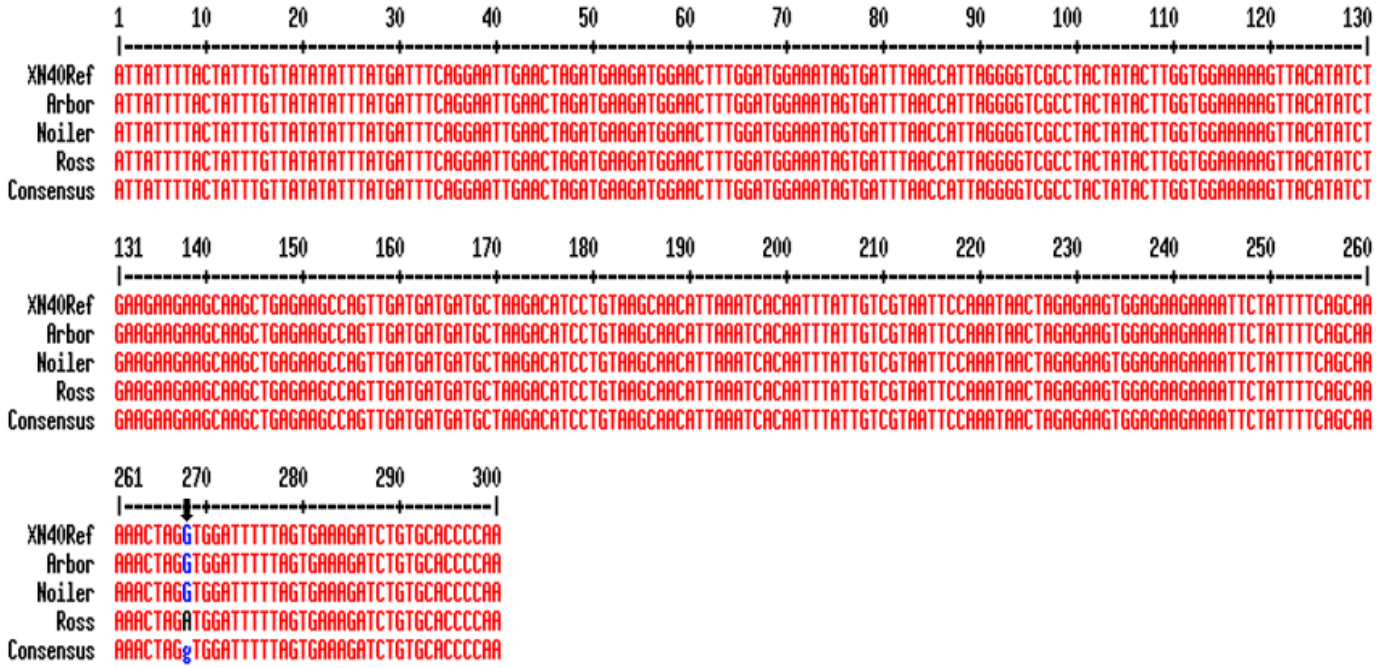


Figure 4

Multiple sequence alignment of RYR2 Exon 40 and flanking intron DNA sequence for Ross 308 broiler (Reverse and Forward: Ross 308), Arbor acre broiler (Forward and Reverse: Arbor) and Noiler chicken (Reverse: noiler) amplified in this study against RYR2 Exon 40 and flanking intron DNA sequence for broiler reference (XN40Ref) (ENSGALT00010021632.1) with polymorphic base arrowed.

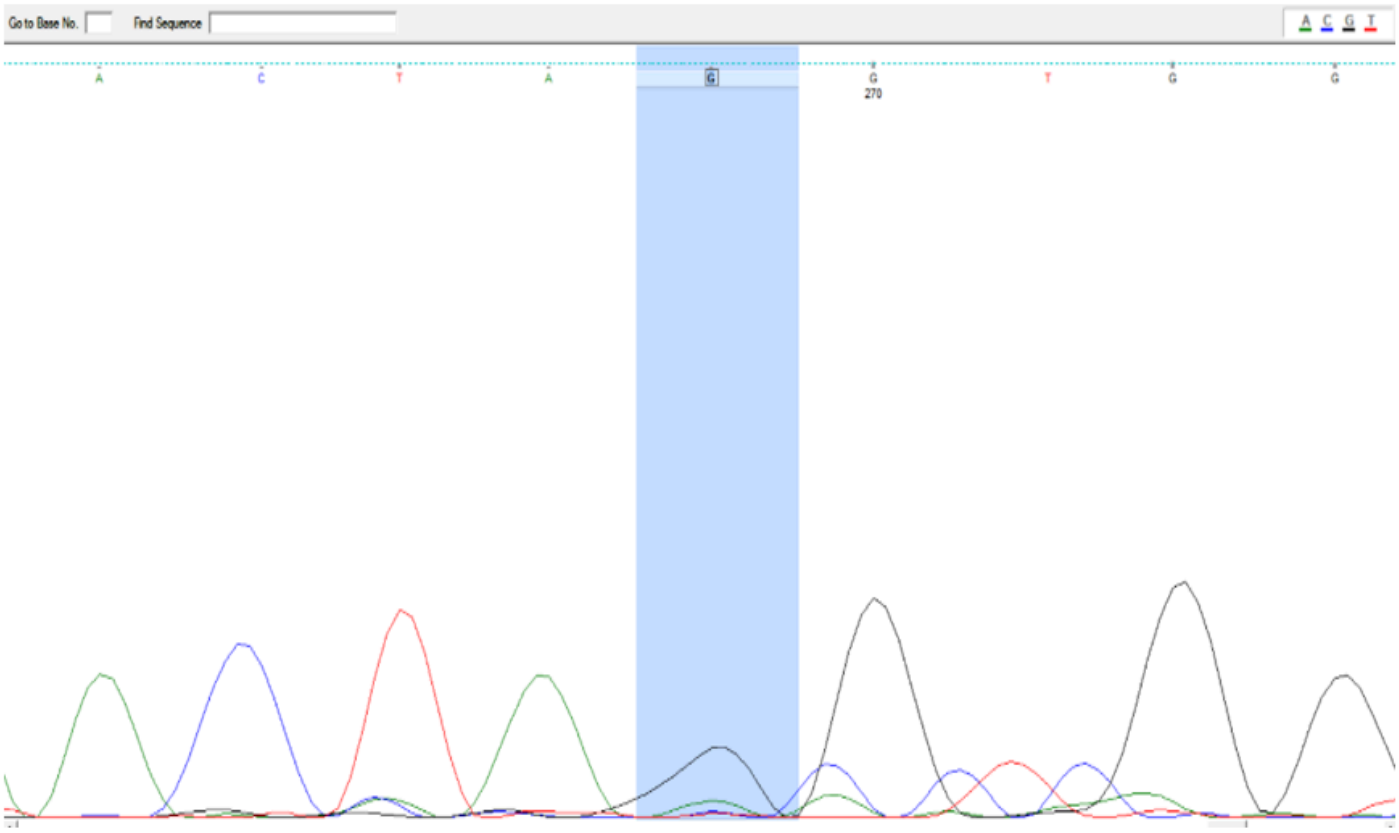


Figure 5

Chromatogram of a segment of sequence at the 3' flanking intron DNA sequence (within intron 40-41) of RYR2 Exon 40 of Arbor Acre broiler showing the G insertion region corresponding to the bases 36968444insA of the broiler reference (ENSGALT00010021632.1) primary transcript.

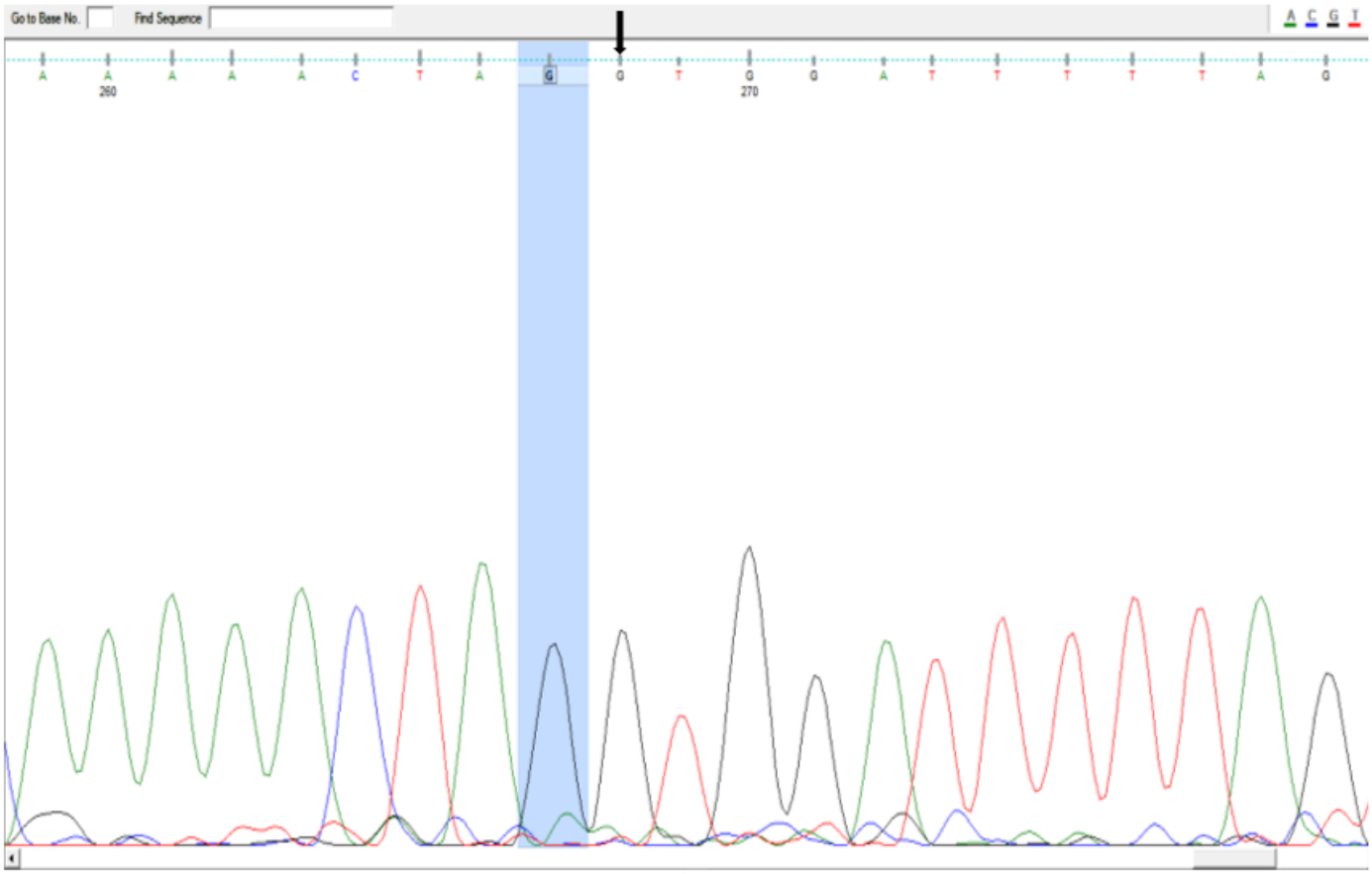


Figure 6

Chromatogram of a segment of sequence at the 3' flanking intron DNA sequence (within intron 40-41) of RYR2 Exon 40 of Noiler chicken showing the G insertion region corresponding to the bases 36968444insA of the broiler reference (ENSGALT00010021632.1) primary transcript.

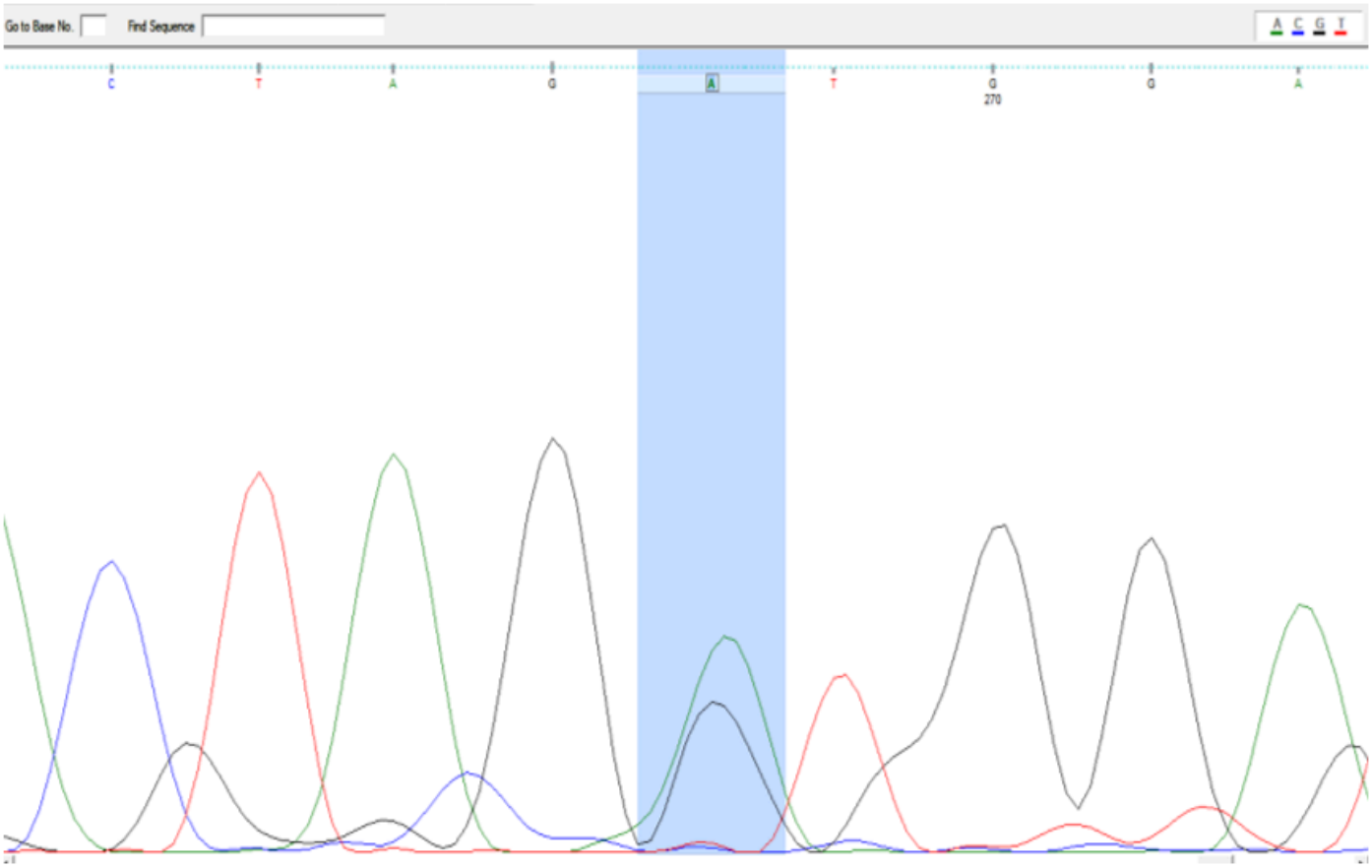


Figure 7

Chromatogram of a segment of sequence at the 3' flanking intron DNA sequence (within intron 40-41) of RYR2 Exon 40 of Ross 308 broiler chicken showing the A substitution region corresponding to the bases 36968444insA of the broiler reference (ENSGALT00010021632.1) primary transcript.

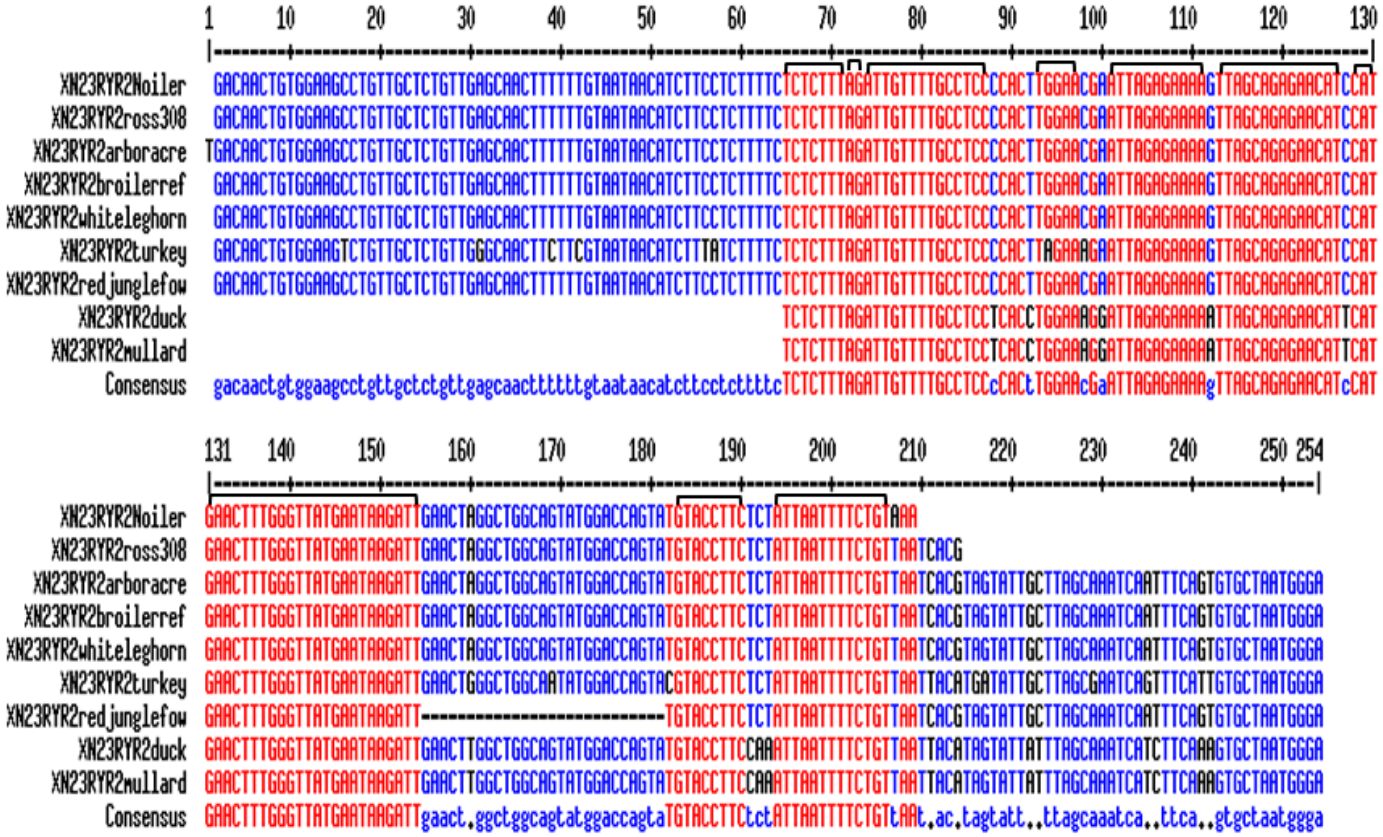


Figure 8

Phylogenetic analysis alignment result showing conserved regions within RYR2 intron and Exon 23 (i.e. 100 bases before and after Exon) amongst arbor acre broiler, Ross 308 broiler and noiler chicken against red jungle fowl and other avian orthologs.

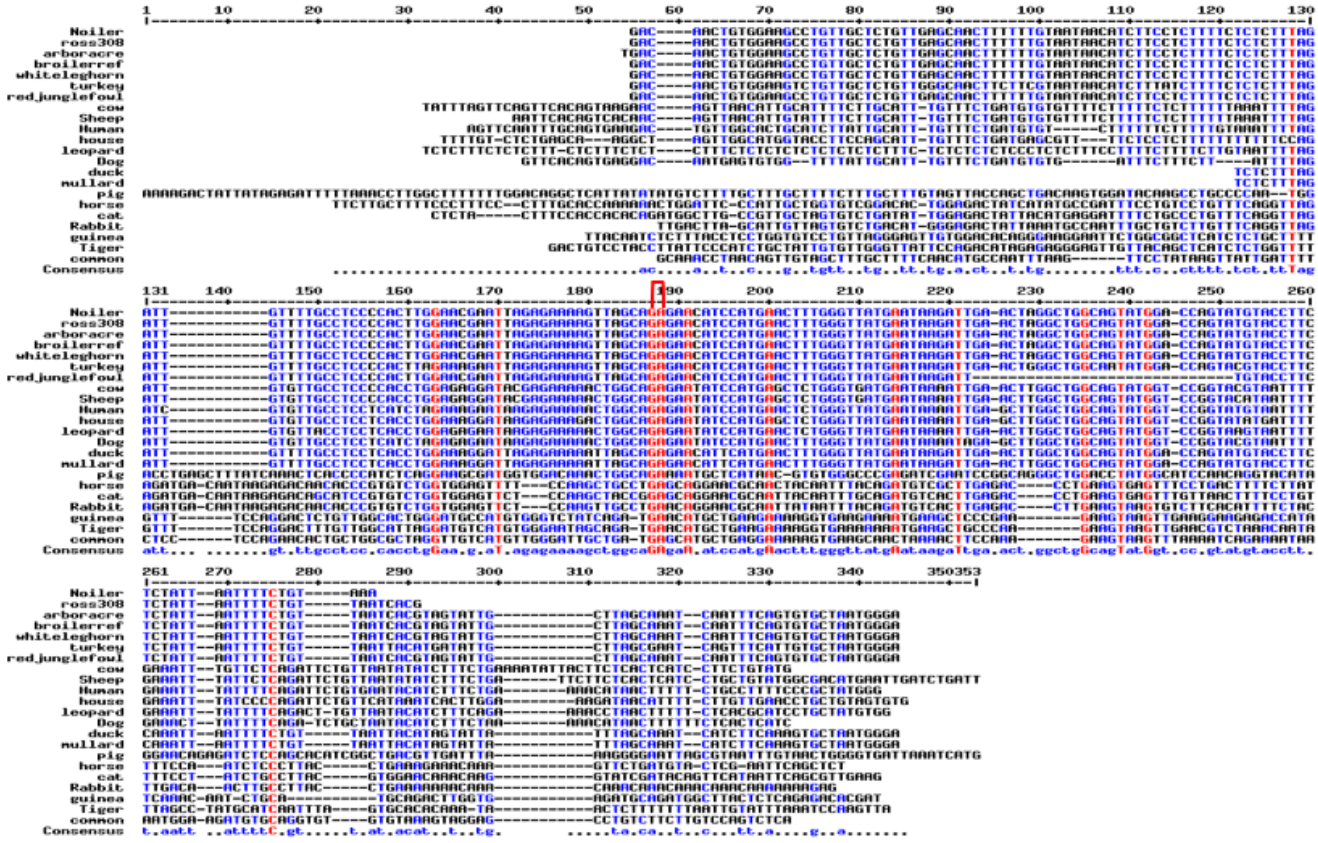


Figure 9

Phylogenetic analysis alignment result showing conserved regions within RYR2 intron and Exon 23 (i.e. 100 bases before and after Exon) amongst arbor acre broiler, Ross 308 broiler and noiler chicken against red jungle fowl and other orthologs spanning Aves, Eutherian and non-eutherian mammals, Pisces, and other vertebrates.

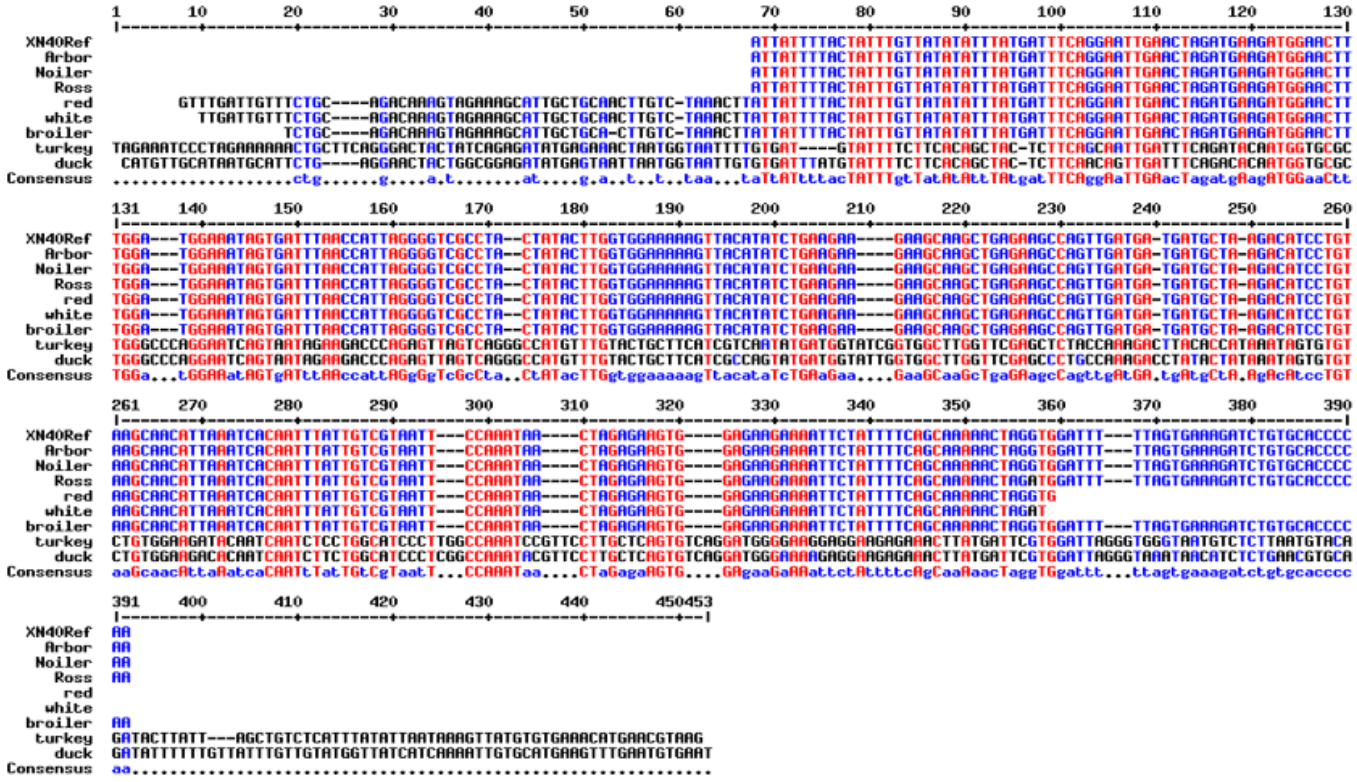


Figure 10

Phylogenetic analysis alignment result showing highly conserved region within RYR2 exon 40 flanking intron DNA (100bp before and after the exon) for arbor acre broiler, Ross 308 broiler and noiler chicken against red jungle fowl and other avian orthologs.

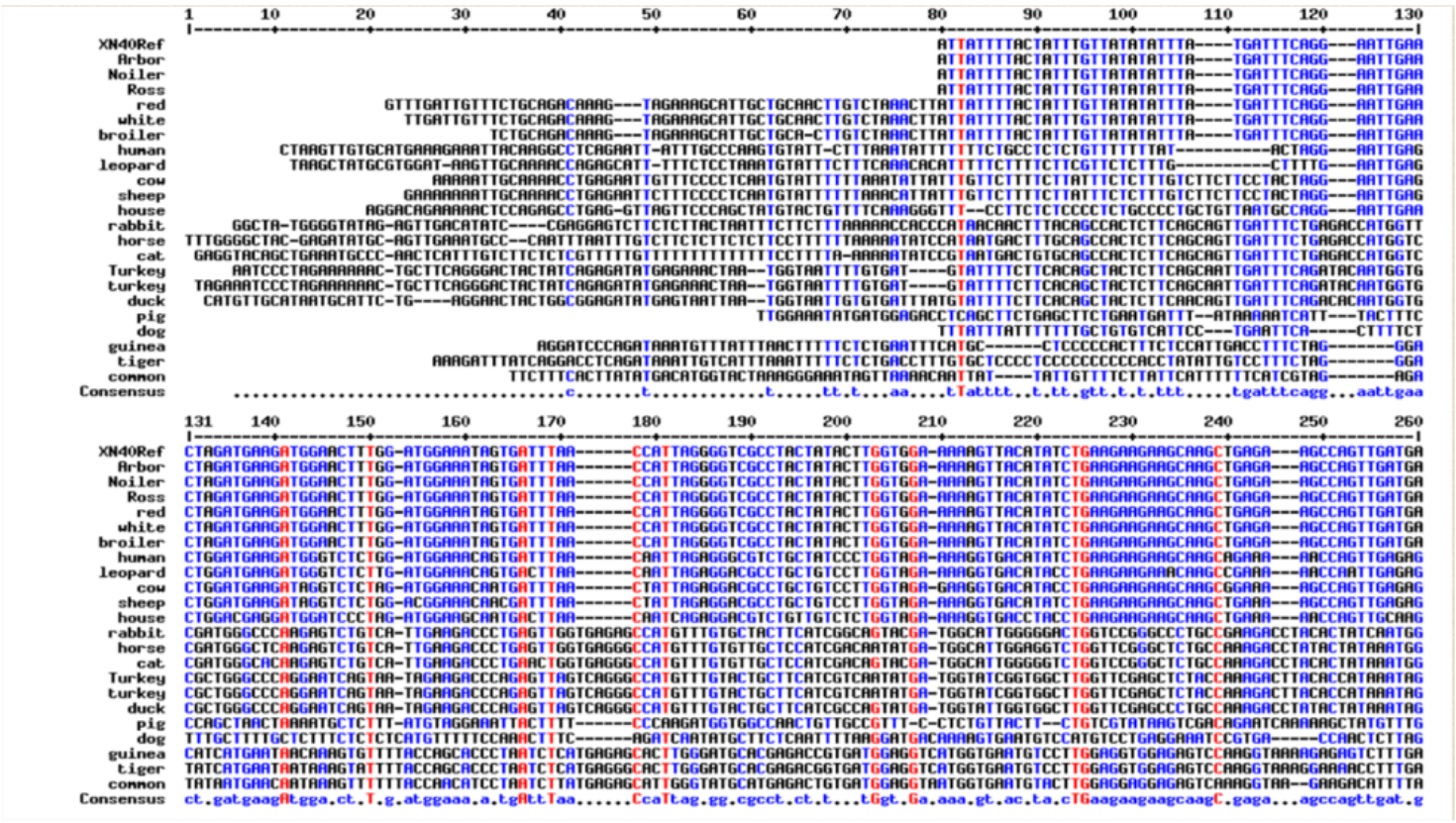


Figure 11

Phylogenetic analysis alignment result showing limited conserved region within RYR2 exon 40 flanking intron DNA (100bp before and after the exon) for arbor acre broiler, Ross 308 broiler and noiler chicken and orthologs spanning Aves, Eutherian and non-eutherian mammals, Pisces, and other vertebrates.



Figure 12

(Continuation) Phylogenetic analysis alignment result showing limited conserved region within RYR2 exon 40 flanking intron DNA (100bp before and after the exon) for arbor acre broiler, Ross 308 broiler and noiler chicken and orthologs spanning Aves, Eutherian and non-eutherian mammals, Pisces, and other vertebrates.

Supplementary Files

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- 26APrimer2H1022copy.ab1
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- Ryr2XN261ROSSRPrimer1B0306copy.ab1
- Ryr2XN261ROSSRPrimer2C0309copy.ab1
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- RYR2exon23bottom40top.tif
- RYR2exon44.tif
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