AJBS Asian Journal of Biological Sciences

OPEN ACCESS

Mutational and Evolutionary Analyses of MC1R Gene in Exotic and Nigerian Indigenous Turkeys with Different Colored Feathers

[1]David Oluwafemi Oguntade, [1]Ekinadose Orose, [1]Ruth Noseregbe, [2]Babatunde Moses Ilori, [2]Samuel Olutunde Durosaro and [2]Dick Obasoyo

[1]Department of Animal and Food Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria

[2]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

ABSTRACT

Background and Objective: Understanding the genetic basis for breed variations is an important step toward achieving breed improvement. Investigations were conducted on the MC1R gene mutations and evolutionary assessments in Nigerian local and exotic turkeys with various colored feathers. **Materials and Methods:** The experiment, which lasted for 16 weeks, included 60 poults in total, 15 of which were each of three genotypes of indigenous Nigerian turkeys (white, black and lavender in color) and exotic turkeys. Quick-DNA Mini-prep Plus extraction kits were used to extract genomic DNA and product guidelines were followed to the letter. Using NCBI primer BLAST, a set of primers was developed to amplify exon 1 of the MC1R gene in Turkey. **Results:** In both Nigerian local and exotic turkeys, nine SNPs were found in exon 1 of the MC1R gene. The major allele frequency was in the range of 0.57 to 0.86. Seven haplotypes in all were found, with five belonging to the exotic, three to the Nigerian local white, one to the Nigerian local black and two to the lavender genotypes. In all genotypes, Tajima's D values were negative and non-significant ($p>0.05$), however, Fu's Fs values were negative in exotic turkey. According to the phylogenetic study, Nigerian local black and lavender turkeys have a closer relationship, whereas Nigerian local white turkeys are more closely related to exotics. **Conclusion:** Nigerian indigenous turkeys can be classified into distinct genotypes according to their plumage colours using the SNPs and haplotypes found in exon 1 of the MC1R gene.

KEYWORDS

MC1R gene, Nigerian local black turkey, Nigerian local white turkey, lavender, exotic turkey

INTRODUCTION

Plumage colour plays an important role in the evolution of birds and therefore is a useful genetic marker in breed identification and population characterizations[1-2]. The plumage color of birds is mainly related to the pigment distribution or proportion of eumelanin and pheomelanin[3]. Since multiple genes might influence the same feature, the precise genetic mechanism underlying the formation of a certain type of color is still unclear[4]. While some genes have a direct impact on color, others function as regulators and modifiers to influence the pigment's regional and zonal distribution as well as its distribution inside individual feathers such as banding, spotting, edging and other patterns[5].

ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2024.505.513

Received: 25 Mar. 2024
Accepted: 15 May, 2024
Published: 31 Dec. 2024

The pigments responsible for variations in feather colouration in birds include flavins, melanins, porphyrins, psittacofulvins, pterins, purines and turacin[6] and they are majorly expressed in feathers and naked parts such as bills and legs. One of the key genes that regulate the biosynthesis of melanins is melanocortin 1 receptor (MC1R) gene[7]. This gene is responsible for the synthesis of eumelanin and pheomelanin pigments[8], which are the two main types of melanins. Eumelanin gives rise to dark black, brown, or grey colorations while pheomelanin gives rise to lighter yellowish to reddish colorations[9].

The melanocortin 1 receptor (MC1R) is encoded by the solid black color locus in birds; mutations in this locus alter receptor activation and result in distinct variants of the E-locus[10].

A broad evolutionary distribution of the MC1R gene is demonstrated by the presence of banded hairs and/or pale ventral coloring in a variety of mammals, including several monotremes and marsupials[11]. Long noted by Jackson *et al.*[12], loss-of-function of MC1R produces yellow pigment (pheomelanin), while gain-of-function of MC1R appears to produce black pigment (eumelanin). In certain research projects by Feelay and Munyard[13] and Feeley *et al.*[14], 2 linked missense mutations (positions 82 A/G and 901 C/T) were identified in the coding region of the MC1R gene in animals lacking black pigmentation.

Nigeria indigenous turkeys have been described as being hardy, tolerant to most infectious diseases in the tropics, can survive on low nutrient feed resources and are best adapted to prevailing tropical climatic conditions[15]. They are phenotypically classified along plumage colours into pure black; white and lavender[15] with high genetic variability. The exotic turkey on the other hand has been selected and improved for relatively higher body weight and genetic characterizations. The MC1R gene has been proposed to play a vital role in coat colour genesis in mammals but their roles remain ambiguous in exotic and Nigerian indigenous turkeys.

MATERIALS AND METHODS

Description of experimental site: This project work was conducted at the Poultry Unit of the Teaching and Research Farm of the Faculty of Agriculture, Ambrose Alli University, Ekpoma Edo State. The laboratory analysis was carried out at African Biosciences Laboratory, Off Iwo road, Iyana agbala Ibadan Oyo State. The experiment lasted for 16 weeks between April and August, 2023.

Experimental birds: A total of 60 poults comprising 15 each of exotic and the three genotypes of Nigerian indigenous turkeys (white, black and lavender plumage colour) were used for this study. The poults were sourced from a reputable farm in Ibadan, Oyo State Nigeria. The poults were raised on a deep litter pen and brooded for four weeks. They were allowed access to feed and clean water *ad libitum* and subjected to the same management practices throughout the experimental period of 16 weeks.

Blood sample collection: At the end of the experiment (16th week), about 1mL of blood samples were collected with a needle and syringe through the brachial vein of each turkey and transferred into an anti-coagulant bottle (ethylene diamine tetra acetic acid-EDTA) for genomic DNA extraction.

DNA extraction: The genomic DNA was extracted using whole blood collected with Quick-DNA Miniprep Plus extraction kits. The manufacturer's procedures for extraction were strictly followed during the extraction process.

The primer BLAST incorporated in NCBI was used to design a pair of primers that could amplify only one exon (945 bps) of MC1R gene in turkey (Ensembl release 109 Feb, 2023 © EMBL-EBI) for polymerase chain reaction.

Amplification and sequence of amplified DNA: Polymerase Chain Reaction (PCR) was carried out in a micro centrifuge tube using programmable thermocycler (Mastercycler pro by Eppendorf) to amplify the extracted DNA. The amplification reaction was performed in a volume of 20 µL mixture containing 12.8 µL of H_2O MQ, 2.5 µL of 1XPCR reaction buffer, 1 µL dNTP's, 1.5 µL $MgCl_2$, pH 9, 1 µL each of forward and revers primers and 0.2 µL of Taq DNA polymerase. The PCR cycling protocol includes an initial denaturation at 96°C for 15 min followed by 40 cycles of denaturing at 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 70°C for 1 min and 40 sec and final extension at 70°C for 5 min. The reliability of the PCR products was checked using electrophoresis. A volume of 20 µL was used for the sequencing. This was prepared using an ABI 3730 XL Capillary DNA Analyzer (Applied Biosystems, Foster City, USA) with approximately 20 ng of purified PCR product as template DNA, 3.2 Mol of primer, 8 µL of Big Dye Terminator Ready Reaction Mix (a combination of dNTPs, ddNTPs, buffer, enzyme and MgCl2), 8 µL of deionized water, 2 µL of primer and 2 µL of template DNA. The reaction was set up to occur 25 times, with intervals of 10 sec at 960C, 5 sec at 500C and 4 min at 600C. There was a quick thermal ramp to 400C following the last cycle, which was maintained until the sequencing product was purified.

Sequence editing and analysis: Bioedit® software was used to view, manually clean and align the sequences. The edited sequence was blasted (BLASTN)[16] against other sequences in the GenBank to determine the similarity and identity with other turkey sequences of MC1R gene in the database. The edited sequences were further aligned using CLUSTALW for further analyses.

Identification and analyses of single nucleotide polymorphisms: The SNPs present in exon 1 of MC1R gene in Nigerian indigenous turkeys were identified by aligning with the reference exon downloaded from Ensembl database using Clustal W[17]. The SNPs were also confirmed using DnaSP[18].

Evolutionary analysis: The frequency of nucleotides present in exon 1 of MC1R gene in Nigerian indigenous turkeys was determined using MEGA 6 software. Haplotype diversity was generated using HyPhy program incorporated in a program DNASP 4.1.0.8[18].

Heterozygosity of the SNPs was calculated using the formula[19]:

$$\text{Heterozygosity } (H_e) = 1-(p^2+q^2)$$

Polymorphism information content (PIC) of the SNPs was calculated using the formula[20]:

$$PIC = H_e - 2p^2q^2$$

where, p is the major allele frequency, q is the minor allele frequency and H_e is the heterozygosity.

The evolutionary history was carried out using the UPGMA method[21] to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method[22] and are in the units of the number of base substitutions per site. The analysis involved 7 nucleotide sequences including exon 1 of MC1R gene in exotic (hybrid converter), Nigerian local black, Nigerian local white and lavender turkeys and other avian breeds (Red jungle fowl (Accession Number: AB201628), duck (Accession Number: 051783) and pheasant (Accession Number: EF360992)). Evolutionary analyses were conducted in MEGA5[23].

RESULTS

Single nucleotide polymorphisms identified, major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys: The major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 1of MC1R gene in exotic and Nigerian indigenous turkeys are presented in Table 1. The major allele frequency of polymorphisms identified in exon 1 of MC1R gene in exotic turkey ranged from 0.57 to 0.86 with mutation 312A>G having the least major allele frequency. Also, the major allele frequency of SNPs identified in 1 of MC1R genes in Nigerian local white turkey ranged from 0.57 to 0.86 with the least major allele found in mutation 580G<A. In Nigerian local black turkey, the major allele frequency ranged from 0.71 to 0.86 with mutation 580G<A having the least major allele. The least major allele frequency (0.57) was obtained in SNP 580G<A in lavender turkey.

The highest heterozygosity value of 0.49 was obtained in SNPs 312A<G in exotic turkey and 580G<A in lavender turkey. Similarly, the highest polymorphism information content (PIC) of 0.37 was observed for mutations 312A<G in exotic turkey and 580G<A in Nigerian local black turkey.

Table 1: Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys

SNPs	Genotype	Form of SNPs	MA	MAF	(He)	PIC
312A<G	Exotic	Parsimony	G	0.57	0.4902	0.370052
	Local white	Parsimony	G	0.86	0.2408	0.211808
	Local black	Parsimony	G	0.86	0.2408	0.211808
	Lavender	Parsimony	G	0.86	0.2408	0.211808
367G<A	Exotic	Parsimony	G	0.71	0.4118	0.32701
	Local white	Parsimony	A	0.86	0.2408	0.211808
	Local black	Parsimony	A	0.86	0.2408	0.211808
	Lavender	Parsimony	A	0.86	0.2408	0.211808
372G<A	Exotic	Parsimony	G	0.71	0.4118	0.32701
	Local white	Parsimony	A	0.86	0.2408	0.211808
	Local black	Parsimony	A	0.86	0.2408	0.211808
	Lavender	Parsimony	A	0.86	0.2408	0.211808
446G<C	Exotic	Singleton	C	0.86	0.2408	0.211808
	Local white	Singleton	C	0.86	0.2408	0.211808
	Local black	Singleton	C	0.86	0.2408	0.211808
	Lavender	Singleton	C	0.86	0.2408	0.211808
580G<A	Exotic	NP	-	-	-	-
	Local white	Parsimony	A	0.86	0.2408	0.211808
	Local black	Parsimony	G	0.57	0.4902	0.370052
	Lavender	Parsimony	A	0.86	0.2408	0.211808
686C<T	Exotic	Singleton	C	0.86	0.2408	0.211808
	Local white	NP	-	-	-	-
	Local black	NP	-	-	-	-
	Lavender	NP	-	-	-	-
694A<T	Exotic	NP	-	-	-	-
	Local white	Singleton	A	0.86	0.2408	0.211808
	Local black	NP	-	-	-	-
	Lavender	NP	-	-	-	-
715A<G	Exotic	Parsimony	A	0.86	0.2408	0.211808
	Local white	Parsimony	A	0.86	0.2408	0.211808
	Local black	NP	-	-	-	-
	Lavender	NP	-	-	-	-
721T<A	Exotic	Singleton	A	0.86	0.2408	0.211808
	Local white	Singleton	A	0.86	0.2408	0.211808
	Local black	Singleton	A	0.86	0.2408	0.211808
	Lavender	Singleton	A	0.86	0.2408	0.211808

He: Heterozygosity, PIC: Polymorphic information content and NP: Not present

Table 2: Haplotypes present in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys

		Genotype				
Haplotype	Haplotype sequence	Exotic	Local white	Local black	Lavender	Total
1	GGGCGCAGA	1 (0.17%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1
2	AGGCGCAAA	1 (0.17%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1
3	GAACGCAAA	2 (33.33%)	3 (50.00%)	0 (0.00%)	0 (0.00%)	5
4	AGGCGTAAA	1 (0.17%)	0 (00.00%)	0 (0.00%)	0 (00.00%)	1
5	GGGCGCAAA	1 (0.17%)	0 (00.00%)	0 (0.00%)	0 (00.00%)	1
6	GAACACAAA	0 (00.00%)	2 (33.33%)	6 (100.00%)	6 (100.00%)	14
7	GAACGCTGA	0 (00.00%)	1 (0.17%)	0 (00.00%)	1 (16.67%)	1

Table 3: Haplotype diversity of exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys

Indices	Exotic	White	Black	Lavender
Number of haplotypes	5	3	1	2
Haplotypes diversity (Hd)	0.952	0.524	0.286	0.286
Nucleotide diversity (Pi)	0.00370	0.00317	0.00340	0.00238
Average number of nucleotide difference (K)	2.667	2.286	1.714	1.714
Sequence conservation	0.990	0.989	0.992	0.992

Table 4: Test of deviation of exon 1 of MC1R gene from neutrality

Test	Exotic	White	Black	Lavender
Tajima's D	-0.345	-1.576	-0.1524	-1.524
Fu's Fs	-2.522	1.598	2.920	2.920
Fu and Li's D	-0.298	-1.666	-1.6088	-1.6088
Fu and Li's F	-0.311	-1.660	-1.60315	-1.60315
Achaz Y*	-0.287	Na	Na	Na

Haplotypes present in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys: Haplotypes present in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys are shown in Table 2. Seven haplotypes were identified in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys. Haplotype 3 was shared by exotic and Nigerian local white genotypes while haplotype 6 was shared by the three Nigerian indigenous turkeys (local white, local black and lavender turkeys). Haplotypes 1, 2, 4 and 5 were specific to exotic turkeys while haplotype 7 was specific to Nigerian local white turkeys.

Haplotype diversity of exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys: Table 3 shows the haplotype distribution in exon 1 of MC1R gene in exotic and the three genotypes of Nigerian indigenous turkeys.

Seven haplotypes were found, comprising five exotic, three local white Nigerian, one local black Nigerian and two lavender genotypes. The highest haplotype diversity (0.952) was recorded in exotic turkeys. Among the Nigerian indigenous turkeys, the white turkeys had the highest haplotype diversity (0.524) while the black and lavender turkeys had a similar value (0.286) and the least. Also, nucleotide diversity was highest (0.00370) in exotic turkeys, followed by Nigerian local white (0.00317), Nigerian local black (0.00340) and lavender (0.00238) genotypes. The average number of nucleotide differences ranged from 1.714 in Nigerian local black and lavender turkeys to 2.667 in exotic turkeys while the sequence conservation ranged from 0.989 in white turkeys to 0.992 in Nigerian local black and lavender turkeys.

Test of deviation of exon 1 of MC1R gene from neutrality: The test of deviation of exon 1 from neutrality is shown in Table 4. Non-significant ($p>0.10$) and negative values of Tajima's D were estimated for exon 1 of MC1R gene in all the turkey genotypes. Negative Fu's Fs value was observed in exon 1 of MC1R gene in exotic turkeys while positive values were observed in Nigerian local turkeys. The Fu and Li's D and Fu and Li's F were not significant ($p<0.10$) and the values were negative across the genotypes. The Achaz Y* value was negative and not significant ($p>0.10$) in exotic turkeys while no value was obtained in Nigerian indigenous turkeys.

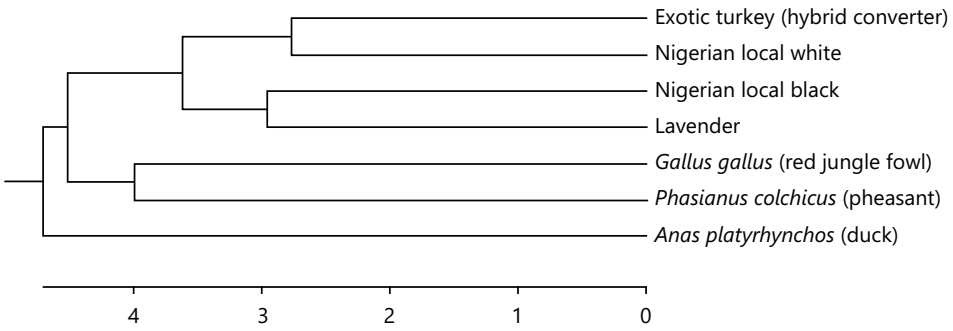

Fig. 1: Phylogenetic relationship between exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys and other avian genotypes

Phylogenetic relationship between exon 1 of MC1R gene in exotic and Nigerian indigenous turkey: Figure 1 shows the phylogenetic relationship between exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys and other avian breeds. Two clades were formed with duck found on a single clade. The second clade gives rise to two sub-clades in which red jungle fowl and pheasant formed sister taxa on the first sub-clade while the second sub-clade split to form Nigerian indigenous white and exotic turkeys as sister taxa and Nigerian local black and lavender turkeys as sister taxa on the same sub-clade.

DISCUSSION

Nine SNPs were identified in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys. The three of the SNPs identified are common to all the genotypes and are parsimonious while SNP 446G<C is also common but singleton in all the genotypes. The presence of parsimonious SNPs suggests that those regions in exon 1 of MC1R gene are polymorphic and have genetic implications on the diversity of exotic and the three genotypes of Nigerian indigenous turkeys. The existence of common SNPs to all the genotypes suggests that such locus may be used as a genetic marker for selection for coat colour in exotic and Nigerian indigenous turkeys. In addition, SNP 580G<A is common and peculiar to Nigerian indigenous turkeys which suggests evolutionary descent among these turkey genotypes which could be employed as one of the genetic bases for phenotypic variations between exotic and Nigerian indigenous turkeys. It was also observed that SNP 715A<G was common to exotic and Nigerian indigenous white turkeys which may also play a biological role in the existence of similar plumage colour between the two turkey populations. The exotic and Nigerian indigenous whites shared white plumage color which is a phenotypic trait.

This study observed the highest heterozygosity value of 0.49 in SNPs 312A<G and 580G<A in exotic lavender turkeys respectively. Also, SNPs 312A<G and 580G<A had the highest polymorphism information content (PIC) in exotic turkey and Nigerian local black turkeys. Generally, the genetic implication of SNPs is the increased distribution of heterozygosity[24,25] of MC1R gene in the exotic and Nigerian indigenous turkey. Therefore, the high heterozygosity recorded especially in exotic and Nigerian local black turkeys implies general fitness and high survivability potential[26] in these turkey populations. Also, since MC1R gene polymorphisms had been associated with colour variations[27], this suggests a possibility for basis of genetic variation in colours among these turkey genotypes. However, further analysis may be required to associate MC1R gene to colour variants in exotic and Nigerian indigenous turkey genotypes.

The polymorphism information content (PIC) ranged from 0.21 to 0.37 for the SNPs identified across the turkey genotypes. Chen *et al.*[28] reported a maximum PIC of 0.38 for single nucleotide polymorphisms which are bi-allelic and 0.37 PIC was obtained for mutations 312A>G in exotic turkey and 580G<C in Nigerian local black turkey. This implies that the markers are informative enough for linkage studies and

any exotic or Nigerian local black turkeys chosen at random at these loci are likely to be heterozygous for that marker when these loci are considered for marker-assisted selection[29]. Various PICs values observed for different SNPs and different genotypes may be influenced by many factors such as sample size, breeding behaviour of species, genetic diversity and sensitivity of genotyping method[30].

Presence of four singleton variable sites out of 721 base pairs was an indication of pathogen/transcription signal pressure effect on the region. A singleton is a rare variant for which genetic variation is carried by a unique chromosome in a sample. Thus, singleton SNPs would enhance fitness in animals, although this was not covered in this study. Therefore, the singleton together with parsimonious SNPs identified in exon 1 of MC1R gene in exotic and Nigerian indigenous turkeys would provide useful information through phylogenic trees, on the origin of plumage colour variations among these turkey populations.

Negative Tajima's D values observed in exon 1 of MC1R gene across all the genotypes show an excess of rare variation which is characterised with population growth[31]. This was an indication of population size expansion which can result from purifying selection, bottleneck, or selective sweep[32]. Negative Tajima's D values may be a result of presence of an excess of rare alleles. Four of the SNPs identified in exon 1 of MC1R gene in all the turkey genotypes were singletons which are rare alleles.

The high haplotype diversity and low nucleotide diversity observed in the current study show that the genetic diversity of the exotic and Nigerian turkey populations is low. It then implies that both exotic and Nigerian indigenous turkey populations have remained stable with an old evolutionary history[33]. Therefore, this suggests rapid expansion after a period of a population bottleneck[33]. Thus, shows a mark of historical and rapid demographic expansion of improved turkey genotypes from a small effective population size[34]. More so, high genetic diversity and low nucleotide diversity of the regions of MC1R gene studied in this experiment might be the result of rich ancestral genetic variation[35].

Since the haplotype and nucleotide diversities were identified majorly in the coding regions of MC1R gene in the four turkey genotypes and the coding regions are subject to natural selection than coding region[36], it, therefore, suggests that most of the variations in feather colour among the studied turkey populations especially the Nigerian indigenous turkeys might be due to natural selection which might have resulted from genetic drift or inbreeding[36]. Several authors also observed high haplotype and low nucleotide diversities in coat colour genes in some livestock species. For instance, in goats, Marín *et al*.[37] observed high haplotype and low nucleotide diversities in coding regions of MC1R gene and coding/noncoding regions of ASIP gene in goats, while Shen *et al*.[38] reported similar results in MC1R gene in Hs chicken.

Phylogenetic analysis revealed that Nigerian local black and lavender turkeys were related based on exon 1 of MC1R gene while Nigerian local white is closely related to exotic turkeys. This close relationship between Nigerian local black and lavender turkeys and Nigerian local white and exotic turkeys imply high comparability and evolution from a most common ancestor. This can be harnessed for selection for genetic improvement of Nigerian indigenous turkeys.

CONCLUSION

This study concluded that exon 1 of MC1R gene of exotic and Nigerian indigenous turkeys is polymorphic and the SNPs and haplotypes identified are informative and could explain the genetic basis for plumage colour variations among these turkey populations. The results obtained can also serve as basis for genetic characterizations of exotic and Nigerian indigenous turkeys into different genetic groups. The knowledge provided can further be used for marker-assisted selection for genetic improvement of Nigerian indigenous turkeys.

SIGNIFICANCE STATEMENT

A great deal of phenotypic variation exists between Nigerian indigenous turkeys and their exotic counterparts. These variations manifest in differences in productivities between these two turkey populations. This study looked at the evolution of Nigerian indigenous and exotic by examining the diversity of MC1R gene in the two populations in order to provide the basis for genetic characterization and improvement of Nigerian indigenous turkeys. Interestingly, the analysis of MC1R gene in both genotypes showed that Nigerian indigenous turkey with white plumage colour was more closely related to exotic turkey than the other two coloured variants (black and lavender). This result is therefore a high-throughput for genetic improvement of Nigerian indigenous white turkey.

REFERENCES

1. Moiseyeva, I.G., M.N. Romanov, A.A. Nikiforov and N.B. Avrutskaya, 2012. Studies in chicken genetics. Commemorating the 120th anniversary of the outstanding soviet geneticist A.S. Serebrovsky (1892-1948). Russ. J. Genet., 48: 869-885.
2. Mitrofanova, O.V., N.V. Dementeva, A.A. Krutikova, O.P. Yurchenko, A.B. Vakhrameev and V.P. Terletskiy, 2017. Association of polymorphic variants in *MSTN*, *PRL*, and *DRD2* genes with intensity of young animal growth in Pushkin breed chickens. Cytol. Genet., 51: 179-184.
3. Prota, G., 1980. Recent advances in the chemistry of melanogenesis in mammals. J. Invest. Dermatol., 75: 122-127.
4. Makarova, A.V., O.V. Mitrofanova, A.B. Vakhrameev and N.V. Dementeva, 2019. Molecular-genetic bases of plumage coloring in chicken. J. Genet. Breed., 23: 343-354.
5. Akumbugu, F.E., G. Dauda and E.A. Anzaku, 2023. Effect of plumage colour genes on body measurements and heat tolerant traits of indigenous chicken. EAS J. Biotechnol. Genet., 5: 15-19.
6. Delhey, K., 2016. The colour of an avifauna: A quantitative analysis of the colour of Australian birds. Sci. Rep., Vol. 5. 10.1038/srep18514.
7. Yang, C.W., J.S. Ran, C.L. Yu, M.H. Qiu and Z.R. Zhang *et al*., 2019. Polymorphism in *MC1R*, *TYR* and *ASIP* genes in different colored feather chickens. 3 Biotech, Vol. 9. 10.1007/s13205-019-1710-z.
8. Ha, T., L. Naysmith, K. Waterston, C. Oh, R. Weller and J.L. Rees, 2003. Defining the quantitative contribution of the melanocortin 1 receptor (MC1R) to variation in pigmentary phenotype. Ann. N. Y. Acad. Sci., 994: 339-347.
9. Galván, I. and F. Solano, 2016. Bird integumentary melanins: Biosynthesis, forms, function and evolution. Int. J. Mol. Sci., Vol. 17. 10.3390/ijms17040520.
10. Dávila, S.G., M.G. Gil, P. Resino-Talaván and J.L. Campo, 2014. Association between polymorphism in the melanocortin 1 receptor gene and *E* locus plumage color phenotype. Poult. Sci., 93: 1089-1096.
11. Wilson, B.D., M.M. Ollmann, L. Kang, M. Stoffel, G.I. Bell and G.S. Barsh, 1995. Structure and function of *ASP*, the human homolog of the mouse *agouti* gene. Hum. Mol. Genet., 4: 223-230.
12. Jackson, P.J., N.R. Douglas, B. Chai, J. Binkley, A. Sidow, G.S. Barsh and G.L. Millhauser, 2006. Structural and molecular evolutionary analysis of agouti and agouti-related proteins. Chem. Biol., 13: 1297-1305.
13. Feeley, N.L. and K.A. Munyard, 2009. Characterisation of the melanocortin-1 receptor gene in alpaca and identification of possible markers associated with phenotypic variations in colour. Anim. Prod. Sci., 49: 675-681.
14. Feeley, N.L., S. Bottomley and K.A. Munyard, 2011. Three novel mutations in *ASIP* associated with black fibre in alpacas (*Vicugna pacos*). J. Agric. Sci., 149: 529-538.
15. Ilori, B.M., S.O. Peters, A. Yakubu, I.G. Imumorin and M.A. Adeleke *et al*., 2011. Physiological adaptation of local, exotic and crossbred turkeys to the hot and humid tropical environment of Nigeria. Acta Agric. Scand. Sect. A: Anim. Sci., 61: 204-209.
16. Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl. Acids Res., 25: 3389-3402.
17. Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.

18. Librado, P. and J. Rozas, 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451-1452.
19. Guo, X. and R.C. Elston, 1999. Linkage information content of polymorphic genetic markers. Hum. Heredity, 49: 112-118.
20. Chen, Z., F. Zhao, Z. He, H. Sun and Q. Xi *et al*., 2024. Expression localization of the *KRT32* gene and its association of genetic variation with wool traits. Curr. Issues Mol. Biol., 46: 2961-2974.
21. Schraiber, J.G. and J.M. Akey, 2015. Methods and models for unravelling human evolutionary history. Nat. Rev. Genet., 16: 727-740.
22. Tamura, K., M. Nei and S. Kumar, 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. U.S.A., 101: 11030-11035.
23. Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol., 28: 2731-2739.
24. Hoekstra, H.E., R.J. Hirschmann, R.A. Bundey, P.A. Insel and J.P. Crossland, 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. Science, 313: 101-104.
25. Kharwar, N.K., K.N. Prasad, M. Rai, V.K. Paliwal and D.R. Modi, 2016. Association of TLR2 and IL-8 polymorphisms and their expression in Guillain-Barré syndrome. Int. J. Pharm. Sci. Res., 7: 3695-3702.
26. Keller, L.F. and D.M. Waller, 2002. Inbreeding effects in wild populations. Trends Ecol. Evol., 17: 230-241.
27. Voisey, J. and A. van Daal, 2002. Agouti: From mouse to man, from skin to fat. Pigm. Cell Res., 15: 10-18.
28. Chen, W., L. Hou, Z. Zhang, X. Pang and Y. Li, 2017. Genetic diversity, population structure, and linkage disequilibrium of a core collection of *Ziziphus jujuba* assessed with genome-wide SNPs developed by genotyping-by-sequencing and SSR markers. Front. Plant Sci., Vol. 8. 10.3389/fpls.2017.00575.
29. Andersson, L., 2001. Genetic dissection of phenotypic diversity in farm animals. Nat. Rev. Genet., 2: 130-138.
30. Singh, N., D.R. Choudhury, A.K. Singh, S. Kumar and K. Srinivasan *et al*., 2013. Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. PLoS ONE, Vol. 8. 10.1371/journal.pone.0084136.
31. Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123: 585-595.
32. Suharyanto and S. Shiraishi, 2011. Nucleotide diversities and genetic relationship in the three Japanese pine species; *Pinus thunbergii*, *Pinus densiflora*, and *Pinus luchuensis*. Diversity, 3: 121-135.
33. Grant, W.A.S. and B.W. Bowen, 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. J. Hered., 89: 415-426.
34. Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Massachusetts, United States, ISBN: 978-0-674-26870-8, Pages: 464.
35. Zhao, Z., N. Yu, Y.X. Fu and W.H. Li, 2006. Nucleotide variation and haplotype diversity in a 10-kb noncoding region in three continental human populations. Genetics, 174: 399-409.
36. Deng, Y., T. Liu, Y. Xie, Y. Wei, Z. Xie, Y. Shi and X. Deng, 2020. High genetic diversity and low differentiation in *Michelia shiluensis*, an endangered magnolia species in South China. Forests, Vol. 11. 10.3390/f11040469.
37. Marín, J.C., R. Rivera, V. Varas, J. Cortés and A. Agapito *et al*., 2018. Genetic variation in coat colour genes *MC1R* and *ASIP* provides insights Into domestication and management of South American Camelids. Front. Genet., Vol. 9. 10.3389/fgene.2018.00487.
38. Shen, X., Y. Wang, C. Cui, X. Zhao and D. Li *et al*., 2019. Detection of Snps in the Melanocortin 1-Receptor (MC1R) and Its Association with Shank Color Trait in Hs Chicken. Braz. J. Poult. Sci., Vol. 21, No. 3 10.1590/1806-9061-2018-0845.