

## Polymorphism in melanocortin 1 receptor gene and its association with body weight in composite rabbit population

<sup>1</sup>Benneth, H. N., <sup>1</sup>Agaviezor, B. O.\* and <sup>2</sup>Orheruata, A. M.

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, University of Port Harcourt, Nigeria.

<sup>2</sup>Department of Animal Science, Faculty of Agriculture, University of Benin, Nigeria.

\*Corresponding author (Email:brilliant.agaviezor@uniport.edu.ng; Phone: +2348030697250)

### ABSTRACT

This study was undertaken to identify and analyse the polymorphism in Melanocortin 1 receptor (MC1R) gene and to determine the relationship of this polymorphism with body weight of composite rabbits. 2 – 3 ml of blood of forty five (45) randomly selected rabbits from a population of seventy five (75) rabbits was drawn aseptically through the ear vein into EDTA tubes and transported to the laboratory for DNA extraction. Polymerase Chain Reaction (PCR) was performed and the products were cleaned up using a ZR DNA sequencing cleanup kit and sequenced using a 3130xl genetic analyser. Sequences were aligned and Single Nucleotide Polymorphism (SNPs) detected using Mega 6.0. DNA polymorphism and genetic distances were determined by DNAsp version 5 and the association between body weight and MC1R was done using Genstat v12. Pairwise comparison of 868.00 sites revealed nucleotide diversity of 0.00070 between the coat colour variants. Two polymorphic sites were observed at g.138 (A>G) and at g.527 (T>C) and genetic distance of 0.000 - 0.004 between the coat colour variants. Phylogenic analysis showed 2 clusters. Brown with White patched coat coloured rabbit had consistently higher body weight value and a non-synonymous polymorphism which by implication changed the amino acid coding for the gene and as such, suspected to be responsible for higher value observed in the Brown with White patched coat coloured individuals. Therefore, breeding programmes should focus on rabbits with Brown with White patched coat colours if the breeding objective is targeted at improving body weight.

**Keywords:** Composite rabbit, performance trait, MC1R gene, SNP

### INTRODUCTION

Rabbit production holds great potential especially in developing countries where its huge genetic resources have not been fully exploited (Lukefahr, 2000). Two types of rabbit predominate in the developing world: the non standard breeds and the exotic breeds. Nigeria rabbit industry as obtainable in some other developing countries is characterized by a small scale production, comprised predominantly of a composite population described by Lukefahr (2000) as non standard breeds with apparently small body size and low productivity compared to

their exotic counterparts. Rabbit adaptation to varying environmental factors expresses the likelihood of a genetic variation present in their genome and such variations are capable of causing genetic differentiations amongst populations or breeds (Houdebine and Fan, 2009). Their immense phenotypic diversity resulting from these genetic differentiations however, reflects in their wide variety of uses both commercially and in laboratories (Fan and Watanbe, 2003; Houdebine and Fan, 2009; Rogel-Gaillard *et al.*, 2009).

To effectively conserve and improve rabbits, will require information on their genetic variability (Rahimi *et al.*, 2005). The variability in rabbit breeds are defined using gene frequencies and this is possible with genes identifiable through their visible effects (Coloration and hair structure). Most research undertaken on livestock breeds in Africa are based on morphological evidences which are rather subjective and imprecise (Mwambene *et al.*, 2012). These criteria obviously provide insufficient information for the purpose of prioritization of improvement programmes. Therefore, there is the need for complimentary analysis using genetically based markers which will measure diversity at the molecular level so as to provide valuable information that can be used for selection and conservation decisions (FAO, 2007). Information derived from molecular characterization using molecular markers after amplifying genomic DNA provides an important toolkit for detecting genetic variation (Holsinger *et al.*, 2002). They are more detailed and precise, based mainly on polymorphic and highly informative markers responsible for thoroughly inspecting genetic diversity at the molecular level (Mukesh *et al.*, 2004).

The molecular determination of the individuals' genotype at the DNA levels which involves mapping genes and gene polymorphisms on a variety of traits equipped a researcher and/or animal breeder with information which is useful to select livestock species based on their genetic blueprint (Naqvi, 2007). Therefore, it is expected that a complete knowledge and understanding of the molecular basis of coat colour variants using polymorphisms associated with colour variants at the genomic level could form some sort of genetic marker for selection purposes especially with relation to certain production traits (Body weight). The present study aimed at identifying Single Nucleotide Polymorphism in Melanocortin 1 Receptor

(MC1R) gene and to assess its association with body weight in composite rabbit population.

## MATERIALS AND METHODS

A total of 45 rabbits from the composite rabbit population of 75 rabbits were used for the study. The coat colours of the rabbits were 10 black, 10 brown, 10 white, 5 black with white patches, 5 black with brown patches, and 5 white with brown patches. The rabbits were kept in battery cages and were fed growers mash containing 16% crude protein in the morning while they were fed with grasses and forages in the evening. Weekly body weight of the rabbits was taken using Camry Dial Spring Balance. Blood sample of 2-3ml was drawn aseptically from each animal through the ear vein into a vacutainer tube containing Ethylene Diamine Tetraacetic Acid (EDTA) as anti coagulant to ensure that the samples were properly preserved for analysis. Genomic DNA was isolated at the Animal Science Laboratory, University of Port Harcourt, using a Zymobead™ Genetic DNA Kit (Irvine, CA, USA) following the manufacturers' protocol. The amplification of DNA was carried out using PCR in a PTC-100 Thermal Cycler with forward and reverse primer (5'ATCTGCTGCCTGGCCTTG3'), (5'CAGATGAGCACGTCGATGA3'), respectively using a touchdown procedure as described by Agaviezor *et al.* (2018).

The presence and concentration of the DNA isolated as well as PCR products were checked using 1.5% gel electrophoresis. 10µl of PCR product was loaded in the agarose gel pre-stained with ethidium bromide and electrophoresis was carried out at room temperature for 1 hour at 100 volts using a Biorad Power Pac™ electrophoresis apparatus (Biorad, Hercules, CA, USA). The resulting amplified bands were visualized with UV light, photographed and were scored using GENE Mate Quanti-

Marker 100bp DNA ladder (Bioexpress, UT, USA). PCR products for sequencing were cleaned up using ZR DNA Sequencing Clean-up Kit, thereafter, purified sequences were sent to the Bioscience Centre of the International Institute for Tropical Agriculture (IITA) where sequencing was done using a 3130xl Genetic Analyzer from Applied Biosystems. Sequences were aligned and Single Nucleotide Polymorphism (SNPs) detected using Mega 6.0. DNA polymorphism and genetic distances were determined by DNAsp version 5 and the association between body weight and MC1R was done using Genstat v12. Data collection on body weight started at weaning till the animals were 5 months old. The weight of individual rabbit in grams per coat colour was taken on a monthly interval for 18 weeks from March

through July 2016. The data obtained were subjected to analysis of variance using GenStat.v12 (VSN International, 2011) to determine the mean post weaning weight of the composite rabbits per month per colour and per SNP using the model below.

$$Y_{ij} = \mu + s_i + e_{ij}$$

Where  $Y_{ij}$  is the individual rabbit observed;  $\mu$  is the populations mean;  $s_i$  is the effect of SNP on body weight and  $e_{ij}$  is the experimental error.

## RESULTS

Sequence information on MC1R locus showed a nucleotide diversity of 0.00070 within samples sequenced. On Analysis of the 868.00 individual sites (column by column), the number of polymorphic sites revealed was 2, whereas the average number of differences was 0.611 (Table 1).

**Table 1:** Results of Pairwise Comparisons and at Individual Sites (column by column)

Parameters	Values
<b>Analysis in Pairwise Comparisons</b>	
Number of pairwise comparisons	36
Average number of sites analyzed	868.00
Nucleotide diversity, (Pi)	0.00070
<b>Analysis at Individual Sites (column by column)</b>	
Number of polymorphic sites, (S)	2
Nucleotide diversity, (Pi)	0.611
Theta-W, per sequence	0.73587
Theta-W, per site	0.00085

The results of the genetic distance between rabbits of different coat colour are presented in Table 2. There was no detectable genetic distances between the rabbits based on their colour combinations except for 0.004 that was observed between white versus Black,

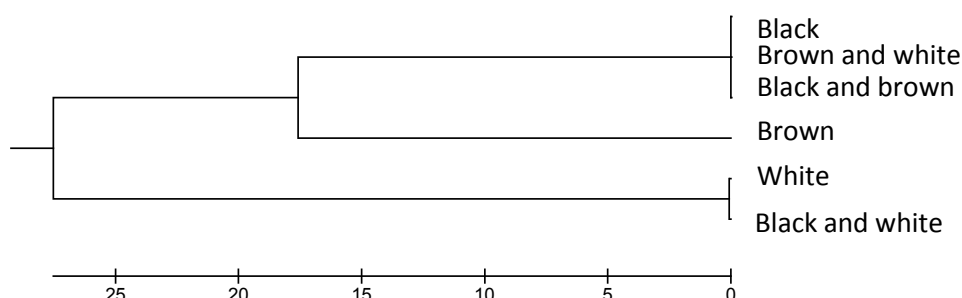
White versus Brown and white, Brown versus Black, Brown versus Brown and white, Black versus Black and brown, Black versus Black and white. Black and brown versus Brown and white and Black and white versus Brown and white.

**Table 2:** Genetic distance between colour variants in rabbits

	White	Brown	Black	Black and brown	Black and white
Brown	0.000				
Black	0.004	0.004			
Black and brown	0.000	0.000	0.004		
Black and white	0.000	0.000	0.004	0.000	
Brown and white	0.004	0.004	0.000	0.004	0.004

Two (2) clusters can be inferred from the dendrogram showing genetic clusters between the coat colours as depicted in Figure 1. Cluster 1 is made up of sole White coat colour and White with patches of Black (combination of Black and white) coat

colour. The other cluster bifurcated forming a sub cluster where one sub cluster is characterized with coat colours sole Black, Black with Brown patches and Brown with White patches while the second sub cluster is characteristically sole Brown coat colour.

**Fig. 1.** Dendrogram showing the genetic cluster between colour variants of rabbits

The results of the mutations were exonic. The first (g.138 A>G) was observed among all the coat colours considered in this study.

The other change was non-synonymous (g.527 T>C) and was observed only in coat colour “Black” and “Brown and white”.

1. White	CCCATCCCCA	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
2. Brown	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
3. Black	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
4. Blk and Brn	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
5. Blk and wht	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
6. Blk and Brn	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
7. Blk and Brn	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
8. Brn and Wht	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT
9. Blk and Brn	CCCATCCCCG	TCTTCCTCAGGGGCTGGTGACCA	TACGGCCGTCCTGCTCTGCCTCAT

**Figure 2: Mutations in MC1R sequences of composite rabbits.**

Key: Blk – Black, Brn – Brown, Wht – White,

The mean body weight across the coat colours under study increased systematically and constantly per month from March through July as shown in Table 3. Black and white colour combination had the highest initial body weight in March which was significantly different from others ( $P < 0.05$ ).

There was a marked increase in the body weight of brown colour rabbits from April through July. The highest body weight was significantly different from others ( $P < 0.05$ ), except in the month of July where it was not significantly different from white coloured rabbits.

**Table 3: Effect of Coat colour per monthly body weight of rabbits under study**

Month	Brown	Brown & White	White	Black	Black & White
March	0.330 <sup>a</sup> ± 0.020	0.376 <sup>ab</sup> ± 0.016	0.338 <sup>ab</sup> ± 0.020	0.347 <sup>a</sup> ± 0.023	0.414 <sup>a</sup> ± 0.018
April	0.592 <sup>ab</sup> ± 0.020	0.656 <sup>b</sup> ± 0.016	0.599 <sup>a</sup> ± 0.020	0.592 <sup>ab</sup> ± 0.023	0.642 <sup>ab</sup> ± 0.018
May	0.854 <sup>ab</sup> ± 0.020	0.951 <sup>d</sup> ± 0.016	0.793 <sup>a</sup> ± 0.020	0.888 <sup>bc</sup> ± 0.023	0.844 <sup>b</sup> ± 0.018
June	1.164 <sup>c</sup> ± 0.020	1.234 <sup>c</sup> ± 0.016	1.014 <sup>a</sup> ± 0.020	1.089 <sup>ab</sup> ± 0.023	1.116 <sup>bc</sup> ± 0.018
July	1.432 <sup>bc</sup> ± 0.028	1.496 <sup>c</sup> ± 0.023	1.318 <sup>a</sup> ± 0.028	1.311 <sup>a</sup> ± 0.033	1.364 <sup>ab</sup> ± 0.025

<sup>abc</sup> Means on the same row with common superscript are not significantly different ( $p > 0.05$ )

## DISCUSSION

Based on the results obtained, low nucleotide diversity of 0.0007 was observed in the target locus between the colour variants. This observation may have stemmed from the uncontrolled breeding practices in the study population. It is important to note that the low polymorphism (i.e. the two SNP detected) could be as a result of the few rabbits used in this study or as a result of mixed breeding. According to Chantry-Darmon *et al.* (2006), low variation can be attributed to the use of inbred rabbit strains for building the reference families. Checkmating these practices can avail the opportunity for rabbit improvement through applying breeding practices that will increase genetic divergence and hence improve vigour as well as meat rabbit production. The low

genetic distance observed did not differ much from 0.009 recorded by Mamuris *et al.* (2002), in wild Greek hare population.

Also, the phylogenetic tree showed an interesting pattern of variance and/or relatedness in the rabbit population indicating that certain colour variants were more related than others at that target gene locus and hence should pose as an indicator to checkmate inbreeding between flocks based on their coat colours. Clustering of this nature can be seen in the work of Galal *et al.* (2013), where the Baladi black breed clustered with the Gabali breed being more genetically related than the New Zealand White breed. This relatedness, according to the authors, could be attributed to geographical location/ origin and or breed history. The first change (g.138 A>G) did

not lead to a change in the amino acid coded for Proline, hence it is a synonymous polymorphism. This means that they do not cause any obvious functional or regulatory role, but can be used as DNA markers in linkage and association studies as reported by Fontanesi *et al.* (2012), for MYO5A gene responsible for the dilute coat pigmentation in rabbits. The (g.527 T >C) SNP detected in the rabbit population in the study caused a change in Amino acid from Isoleucine to Threonine and this was the reason for implicating it in the regulatory role leading to a higher body weight recorded especially for the polymorphic coat colour brown and white. This mutation can be suspected to be connected to a functional or regulatory role in rabbit performance as observed by Dodds *et al.* (1996) who reported that in most SNPs, at least of two from every three involved substituting of Cytocine (C) for Thymine (T) as shown in Figure 2.

Reports gathered from thermo-physiological data on related studies shows that coat colour in rabbits directly or indirectly affects animal performance (production, carcass, fitness and or functional) traits. This is evident in the work of Adelodun (2015), where he opined that light pigmentation performed better than its dark counterparts since they will reflect solar radiation more and absorb less of it into the body.

## REFERENCES

- Adelodun, O. 2015. Thermo physiological traits of Californian, New Zealand white, Havanna black and Palomino brown rabbits raised in Humid Tropics. *Journal of Biology, Agriculture and Healthcare* 5(3), 204 - 210.
- Agaviezor, B.O., Ajayi, F.O. and Nwachukwu, C.C. 2018. Association between chicken growth hormone gene and growth performance in chickens raised in South-South Nigeria, *African Journal of Agriculture, Technology and Environment* 7(2), 188 – 194
- Chantry-Darmon, C., Urien, C., de Rochambeau, H., Allain, D., Pena, B., Hayes, H., Grohs, C., Cribiu, E.P., Deretz-Picoulet, S., Larzul, C., Save, J.C., Neau, A., Chardon, P. and Rogel-Gaillard, C. 2006. A first-generation microsatellite-based integrated genetic and cytogenetic map for the European rabbit (*Oryctolagus cuniculus*) and localization of angora and albino. *Animal Genetics*, 37,335-341.
- Dodds, K. G., Tate, M. L., McEwans, J. C. and Crawford, A. M. 1996. Exclusion probabilities for pedigree testing farm animals, *Theor. Appl. Genet.* 92, 966 - 975.
- Fan, J. L. and Watanabe, T. 2003. Transgenic rabbits as therapeutic protein bioreactors and human disease models. *Pharmacol Therapeut.* 99, 261–282.
- FAO, 2007. The state of the world's Animal Genetic Resources for food and agriculture, edited by Barbara Rischkowsky and Dafydd Pilling. Rome.
- Fontanesi, L., Scotti, E., Dall'olio, S., Oulmouden, A. and Russo, V. 2012. Identification and analysis of single nucleotide polymorphisms in the Myosin Va (*Myo5a*) gene and its exclusion as the causative gene of the *dilute* coat colour locus in rabbit. *World Rabbit Sci.*, 20, 35 – 41.
- Galal, O. A., Rehan, M. and Abd El-Karim, R. E. 2013..Analysis of genetic diversity within and among four rabbit genotypes using biochemical and molecular genetic markers. *Afr. J. Biotechnol.*, 12 (20), 2830-2839.
- Holsinger, K. E., Lewis, P. O. and Dey, D. K. 2002. A bayesian approach to inferring population structure from

- dominant markers. *Mol. Ecol.*, 11, 1157 – 1164.
- Houdebine, L. M. and Fan J. 2009. Rabbit Biotechnology: rabbit genomics, transgenesis, cloning and models. Springer, pp. 65–75 New York.
- Lukefahr, S. D. (2000). National rabbit project of Ghana: A genetic case study. ICAR technical series 3.pp 307 -317.
- Mamuris, Z., Sfougaris, A. I., Stamatis, C. and Suchentrunk, F. 2002. Assessment of genetic structure of Greek brown hare (*Lepuseuropaeus*) populations based on variation in random amplified polymorphic DNA (RAPD). *Biochem. Genet.*, 40, 323-338.
- Miller, P. 1997. Tools for Population Genetic Analyses (TFPGA) version 1.3 A Windows® program for the analysis of allozyme and molecular population genetic data Copyright © Mark.
- Mukesh, M., Sodhi, M., Bhatia, S. and Mishra, B. 2004. Genetic diversity of Indian native cattle breeds as analysed with 20 microsatellites. *J Anim. Breed Genet*, 121, 416–424.
- Mwambene, P. L., Katula, A. M., Cheyambuga, S. W. and Mwakilembe, P. A. A. 2012. Fipa cattle in the southwest highlands of Tanzania: socio economic roles, traditional management practices and production constraints. *Anim. Genet. Resour* 10, 1017-2078.
- Naqvi, A. N. 2007. Application of Molecular Genetic Technologies in Livestock Production: Potentials for Developing Countries. *Advances in Biological Research* 1 (3-4), 72-84.
- Rahimi, G., Khanahmadi, A., Nejati-Javareni, A. and Smailkhanian, S. 2005. Evaluation of Genetic variability in a breeder flock of native chicken based on randomly amplified polymorphic DNA markers. *Iranian Journal of Biotechnology*, 3 (4), 231 - 234.
- Rogel-Gaillard, C., Ferrand, N. and Hayes, H. (2009). Genome mapping and genomics in domestic animals. Rabbit. Springer Heidelberg. pp. 165–230
- VSN International (2011). GenStat for Windows 12th Edition. VSN International, Hamel Hempstead, UK. Web page: GenStat.co.uk