Sequence variation of MC1R gene in Iraqi native Ducks and its association with feathers colour trait

Salah Mahdi Alsudany

Department of Animal Production, College of Agriculture, University of Basrah , Basrah, Iraq

salah.mohsen@uobasrah.edu.iq

Abstract

The *MC1R* gene is one of the genes responsible for the pigmentation of feathers in birds by controlling the production of eumelanin and pheomelanin. In this study, blood samples were taken from 50 birds from local Iraqi ducks in order to extract part of the sequence of the *MC1R* gene and to know the number of SNP when aligning the sequence with GenBank : LC480444.1 nucleotide sequence, and it was found through this study that there are three SNP, which were all non-synonymous (G>A 461 , A>C659 , A>G749), which resulted in an amino acid change where the amino acid valine was replaced to methionine and methionine to leucine and threonine to alanine. Although there were three SNP, two of them did not change the function of the protein because the amino acids resulting from the exchange of nucleotides (G>A 461 , A>C659) were also non-polar (neutral) and hydrophobic, while A>G749 the polar amino acid was replaced Neutral hydrophilic to non-polar neutral hydrophobic, so we may notice a change in the shape of the protein as a result of the change of nucleotides (A>G749) and thus a change may occur in the function of the protein , Therefore, it is possible to rely on single nucleotide polymorphisms (SNP) of the (MC1R) gene as genetic markers in distinguishing local Iraqi ducks from other breeds.

Keywords: Duck , Iraqi native breeds, Polymorphism , Sequence variation, *MC1R* gene , SNP

Introduction

The *MC1R* gene plays an important role in the process of regulating the formation, transport, and deposition of melanin in animals and is important for determining the color of skin, feathers, and hair (1). Feather color variation in birds is determined by the relationship between two SNP in The melanocortin 1 receptor (*MC1R*) gene (2). A sequence variation polymorphism in *MC1R* is associated with plumage coloration in several bird species including domestic chicken, Gallus Gallus (3). Chicken plumage color is controlled by several genes and the E locus is one of the basic genes associated with a color and has various alleles, including E*E, extended black; E*R, Perchin; E*WH, dominant wheat; E*N, wild type; E*B, brown; E*BC, Buttercup; and E*Y, recessive wheat (4) and (5). The E locus encodes the melanocortin 1 receptor (*MC1R*), a seven-transmembrane G protein-coupled receptor found primarily in melanocytes and affecting the production and relative distribution of pigments (eumelanin and pheomelanin) (6). Studies have found that there is a relationship between the variation in the genotypes of the *MC1R* gene with the colors of birds, as well as the association of this gene with growth or infection with some diseases (7) and (8). Ducks' feather color is important because determines carcass quality and influences consumer acceptance (2). The *MC1R* gene is located on chromosome number 11 and consists of 1129 base pairs, only one exon which encodes 314 amino acids (3). Concerning, *MC1R* is mainly involved in melanogenesis. Therefore, its genetic diversity was investigated to demonstrate its effect on hair and skin color in

humans and coat color in animals. It has been reported that human skin pigmentation (eg skin, hair, and eye) is controlled by about 120 genes and that *MC1R* has a key role in this process (9). Over the past two decades, the study and knowledge of polymorphisms in the *MC1R* gene and its role in plumage color have increased. Largely, because this gene provides genetic identification useful for identifying subspecies/subspecies in mammals and birds (10). A large number of studies have been conducted examining the potential relationship between variations of the *MC1R* gene and hair/skin/feather color in a diverse population. from unrelated species (10), (11), (12), (13), (14), (15), (16) and (17). Furthermore, polymorphisms of *MC1R* have been associated with feather pigments eumelanin, pheomelanin, and albino (18). Eight mutations were found Causative non-synonymous mutations associated with eumelanin and pheomelanin pigments (14)and (3). It has been suggested that six non-synonymous mutations (Met71Thr, Glu92Lys, Ala126Ile, Thr143Ala, Cys213Arg and His215Pro) were related to eumelanin and pheomelanin pigmentation, where These mutations have been associated with chicken plumage colors (8), (14) and (3). The genetic variation of 741 bp of the *MC1R* gene in geese with five plumage color patterns was investigated and five synonymous polymorphisms were identified by (19). Interestingly, the majority of these studies, particularly in birds, have made efforts to associate single nucleotide polymorphisms (SNP), and corresponding amino acid changes, with observed polymorphisms (12), (19), (20), (16) and (21). With regard to the *MC1R* gene, it is one of the basic genes in chicken coat color, so the aim of this study was to find out the SNP of the *MC1R* gene in local Iraqi ducks and their association with feather color trait.

Materials and working methods

the birds

Fifty blood samples were collected from the veins of duck feet of Iraqi domestic duck breeds. All samples used in this study were obtained from local markets and people (Basra, Iraq) and stored at low temperatures for DNA extraction. The approval of this study was obtained by the Scientific Committee of the Department of Animal Production at the University of Basrah.

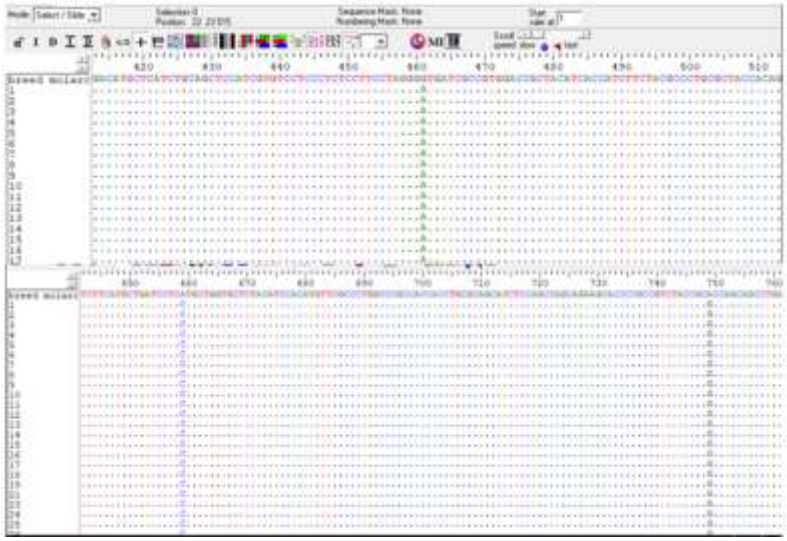
DNA extraction and amplification

DNA was extracted from blood samples using a kit and then samples were stored at -20 °C until used for PCR amplification. Based on the sequence of the *MC1R* gene in ducks found in Genbank. Through the Primer3 program, appropriate primers were designed to amplify a segment of the *MC1R* gene, which is as follows F:5'-ATCCGCCACATGGACAACAT-3) and (R:5'-GACCACCGAGTTGCAGATGA-3).

PCR was performed in a 30 µL reaction mixture, containing 15 µL of PCR Master mix, 1 µL of each primer, 2 µL of DNA and 11 µl of nuclease-free water.PCR temperature profiles consisted of an initial denaturation at 95 °C for 5 minutes followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 63 °C for 30 seconds, and extension at 72 °C for 45 sec and final extension at 72 °C for 5 min. After obtaining the desired gene sequence, the duck nucleotide sequence in the MC1R gene was aligned with GenBank: LC480444.1 nucleotide sequence using the ClustalW program available in the Bioedit program, then the amino acid sequence alignment was made with NP_001297734.1 amino acid sequence, and a phylogenetic tree was drawn. The Mega11 program and also the I-TASSER program was used to design the three-dimensional (3D) structure of the *MC1R* protein.

Results and discussion

In the beginning, a total of 50 samples from local ducks were sequenced and compared with the reference sequence of LC480444.1 in the GenBank through the use of the Bioedit program, through which it was found that there were three SNP in the coding regions of part of the *MC1R* gene sequence, and this was also observed. A single single-nucleotide polymorphism (SNP) at 293 bp was non-synonymous (G>C) (2). Also showed the presence of 4 SNP g.18838722 G>C, g.18838624 T>C, g.18838694 G>A, and g.18838624 C>T) in the MC1R gene (1). Three polymorphisms were also detected. SNP for the *MC1R*, TYR, and *ASIP* gene in chicken (398 T>A, 637T>C, and 920 G>C) in (22). The SNP we obtained in this study were (G>A 461,659A>C, A>G749), which can be seen in Figure (1).



Figure(1) compares the nucleotide sequence of the *MC1R* gene in the native duck of Iraq to the NCBI reference sequence LC480444.1

As the polymorphisms that appeared in this study were not synonymous, which resulted in a change in amino acids, where the amino acid valine was replaced by the amino acid methionine, the amino acid methionine was changed to the amino acid leucine, and the amino acid threonine was changed to the amino acid alanine, as shown in Figure (2). And table (1). Also, three non-synonymous SNPs of the MC1R exon 1 gene (c.52G>A, c.376G>A, and c.409G>A) were found in Tsaya white ducks (23). Eight non-synonymous mutations were also found in Chicken (14)and (3).

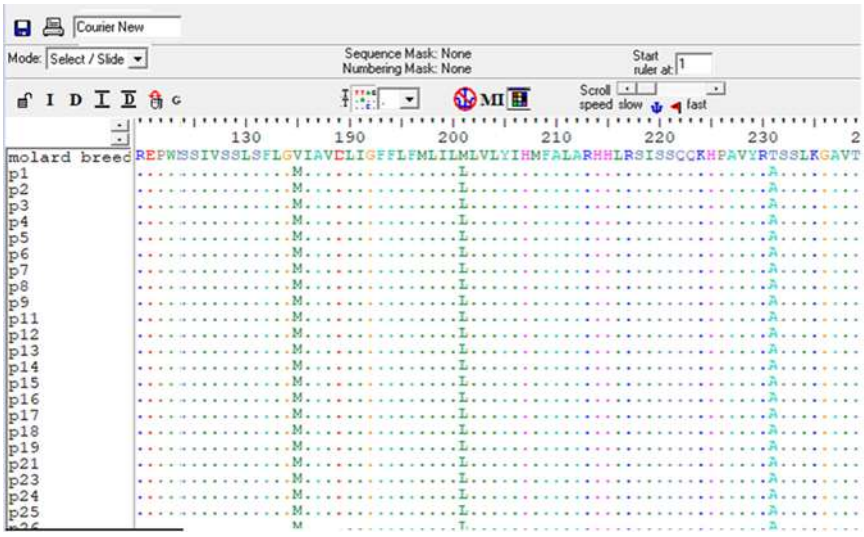


Figure (2) compares the Amino acid sequence of the *MC1R* gene in the native duck of Iraq to the NCBI reference sequence NP_001297734.1

Table (1) represents the location and type of snp and amino acid change

The position of SNP	Nucleotide substitution	The type of SNP	Changed amino acid
461	G>A	nonsynonymous	Valine converted into a Methionine
659	A>C	nonsynonymous	Methionine converted into a Leucine
749	A>G	nonsynonymous	Threonine converted into an Alanine

Table (2) representing the change of polarity and Hydropathy Index of an amino acid

Nucleotide substitution SNP	Changed amino acid	polar type	Hydropathy Index
G>A	Valine converted into a Methionine	non polar-non polar	hydrophobicity-hydrophobicity
A>C	Methionine converted into a Leucine	non polar-non polar	hydrophobicity-hydrophobicity
A>G	Threonine converted into an Alanine	Polar- non polar	hydrophilicity – hydrophobicity

We note from Table (2) that the physical and chemical variables of the protein resulting from the replacement of amino acids as a result of polymorphism in the local ducks did not result in the site (G>A 461,659A>C) indicating the type of polarity, type of charge and hydrotreatment despite the change of amino acids valine to the amino acid methionine and

the change of the amino acid methionine to the amino acid leucine and thus did not change the amino acid sites of the resulting protein, while at site (A>G749) the polar neutral hydrophilic amino acid threonine changed to the non-polar hydrophilic alanine Water, which causes a change in the amino acid location of the resulting protein. These results indicate that this histidine site change can affect *MC1R* protein function, suggesting that these polymorphisms can be used as molecular markers for chicken plumage color (10). According to previous studies, changing the polarity of amino acids can affect the alteration of the protein function of the *MC1R* gene and eventually lead to differences in plumage coloration. There was a significant association between 4 SNP and plumage color in Taihang chickens (1). It was suggested that six non-synonymous SNP (Met71Thr, Glu92Lys, Ala126Ile, Thr143Ala, Cys213Arg, and His215Pro) were related to eumelanin and pheomelanin pigmentation, as these SNP were associated with chicken plumage colors (8), (14), (3)and (2) The identification of genetic markers associated with plumage color has increased dramatically in the recent period because these markers can provide useful information to identify subspecies, and thus can be used as effective molecular markers to identify the original Iraqi subspecies. By using the I-TASSER program to design the three-dimensional (3D) structure of the *MC1R* protein, we notice in Figure (3) There is a difference in the local duck protein with that of Mullard ducks when compared with it. Figure (4) shows the phylogenetic tree that was drawn through the Mega11 program, in which a great similarity appears in the *MC1R* gene between the local Iraqi ducks and the mallard duck, while the least similarity appeared with the guinea fowl .While it was found that the Chinese duck is the closest to the local Iraqi duck compared to the English duck, which is farthest from the local Iraqi duck in the phylogenetic tree (1).

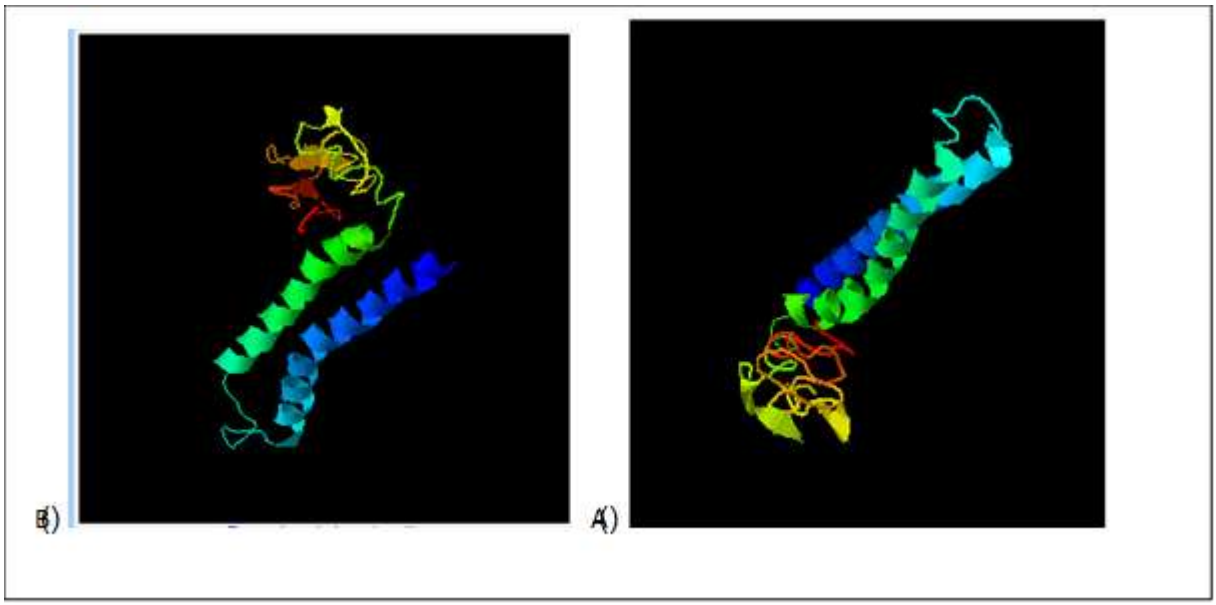


Figure (3) represents a 3D of the local duck part of the protein (A) and the Mueller duck part of the protein (B)

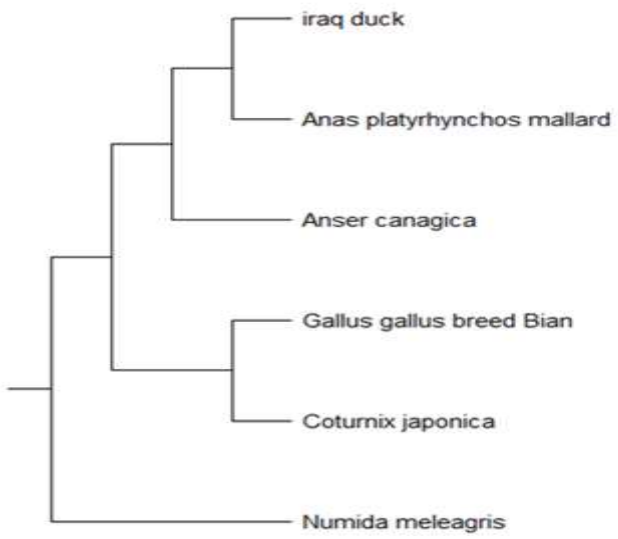


Figure (4) Using the ClustalW and MEGA 11 methods of sequence alignment, the phylogeny tree of the *MC1R* gene sequences was depicted.

Conclusion

It could be concluded that the presence of three SNPs in a segment of the *MC1R* gene (G>A 461, 659A>C, A>G749) can be relied upon as genetic markers in distinguishing the local Iraqi ducks from the rest of the other breeds.

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