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Detection of Melanocortin 1 Receptor (MC1R) polymorphism and its relation to plumage colors in local turkey

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Abstract. Identification of genetic markers in relation to phenotypic differences is important for local turkey breed identification, Melanocortin 1 Receptor (MC1R) is a transmembrane G-protein-coupled receptor, which has a fundamental role in the regulation of black (eumelanin) and red (pheomelanin) pigment synthesis. In an attempt to detect and analyze the MC1R gene, also to find the relatedness among three most common colors of turkey (White, Red, and Black) in Sulaimani city of Iraq. A total of 75 turkeys (25 White, 25 Red and 25 Black birds) (6 ± 1 month old) blood samples (3 ml/bird) were collected into 5 ml vacutainer tubes containing the EDTA for DNA extractions, the quantity and quality of DNA were determined by Nanodrop spectrophotometer. The forward (5'-GCTTTGTAGGTGCTGCAGTTGTG-3') and reverse primer (5'-CCATCCATCCTCCTGTCTGT-3') were used to PCR amplify a 1050 bp fragment of the MC1R gene. The results showed that we have successfully detected and amplified the MC1R gene in Sulaimani local Turkeys. The black color Turkeys showed two bands of PCR amplification, that is mean for showing the black color phenotype the birds need the two MC1R gene express themselves on the two locus on the chromosome, while each of red and white phenotype needs only one MC1R gene to show these two phenotypes. In accordance with the dendrogram of the current study, the result revealed that genetic variation and relatedness among turkey of the three feather color of local turkey of distance 15%. The cluster divided the local turkey in to three main groups; the first group includes all birds of red color, the second group was mainly of black birds. On the other hand, the third group was mixture between the three colors. In conclusion, based on the results obtained in this study, there are significant associations between plumage colors and genetic variants of the MC1R gene in local turkey. However, further studies are essential to confirm this conclusion.

1. Introduction

Turkey (*Meleagris gallopavo*, MGA) is an important avian species and is the second largest contributor to the world's poultry meat production [1]. Identification of genetic markers in relation to phenotypic differences is important for local turkey breed identification. Enormous progress with MC1R gene polymorphisms has been achieved in relation to coat color variations in vertebrate animals [2; 3; 4]. In turkey, the MC1R gene plays a key role in the regulation of eumelanin (black/brown) and pheomelanin (red/yellow) feather pigmentation [2; 5]. Turkey MC1R gene is located on chromosome 11 and encodes a 314 amino acid protein in only one exon [6; 4]. At least



five loci were characterized as responsible for the great color variation in this species [7]. MC1R is known as a key regulator of melanin synthesis and has been associated to melanism in several vertebrate species [8; 9].

Molecular characterization of the MC1R gene can be useful in breeding programs aiming at maintaining or developing different color varieties in species such as turkeys, in which the different and exquisite plumages have ornamental value. Turkeys bred in backyards for ornamental or family consumption purposes usually have predominantly dark plumage, while turkeys raised for the poultry industry have white plumage. White turkeys have the recessive allele *c* in homozygosity in an epistatic locus for color, and therefore may have any allele combination in the bronze locus [7; 10]. Even though white turkeys may serve as a reservoir of MC1R variation to be used in turkey breeding programs, DNA sequence diversity of this locus has never been studied in commercial varieties, likewise, turkey breeders from Iraq especially Sulaimani city use an informal, though traditional, classification of turkey color patterns based on general plumage features, and, therefore, it is unknown whether this classification is useful for predicting genotypes or alleles present different colors locus. In this study, we performed the first characterization of the MC1R locus in local turkeys from Sulaimani city. In addition, we characterized the MC1R variation found in colored turkeys raised in private fields, test the predictive potential of the informal phenotypic classification made by breeders. Understanding the genetic variation for different colors may assist small producers in developing breeding strategies focused in producing feathers with decorative commercial value. To our best knowledge, this study is the first attempt to detect and analyse the MC1R gene in local turkey in Sulaimani region, also to find the relatedness among three most common colors of turkey in this region.

2. Materials and Methods

This study was conducted on three local turkey colors (Figure 1), white, black and red. A total of 75 turkeys (25 White, 25 Red and 25 Black birds) (6 ± 1 month) blood samples (3 ml/bird) were collected, the sampling procedure involved a small puncture in the foot of the birds, and was carried out after informed consent given by the birds' owners, into 5 ml vacutainer tubes containing the EDTA for DNA extractions (DNA was extracted from the blood sample using QIAamp® DNA Blood Mini Kit, QIAGEN GmbH Qiagenstr.1 40724 Hilden Germany) All laboratory work was done in the biotechnology laboratory at the department of animal science, College of Agricultural Engineering Sciences / Sulaimani University. The quantity and quality of DNA were determined by Nanodrop spectrophotometer and 1% agarose gel electrophoresis (Figure 2), respectively but for PCR products we used 1.5% agarose gel to determine the bands.

2.1. PCR amplification and DNA purification

Primers were designed from National Center for Biotechnology Information (NCBI) reference data (GenBank accession no. D78272) using Primer3 program (v. 0.4.0). The forward (5'-GCTTTGTAGGTGCTGCAGTTGTG-3') and reverse primer (5'-CCATCCATCCTC CTGTCTGT-3') were used to PCR amplify a 1,050 bp fragment in the MC1R gene.

Amplifications were performed using a thermal cycler (MJ RESEARCH-PTC-200 Gradient Peltier Thermal Cycler® 60- Well) with the final reaction volume of 20 µL. Two µL (60 ng) sample DNA was added to each tube to make the final volume (20 µL). Each reaction contained: 11 µL of Red Master Mix (AMPLIQON A/S Stenhuggervej 22-Germany), 25 Units/mL Taq polymerase, each dNTPs is 200 µM and MgCl₂ was 1.5 mM), 2 µL of primer (197.13 µM–599.26 µM), 2 µL (60 ng) of DNA template and 5 µL of DNase free water. Many protocols were used but only one protocol gave clear bands, programmed for 35 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 1.5 min. An initial denaturation step of 5 min at 95 °C and a final extension step of 7 min at 72 °C were included in the first and last cycles, respectively. The PCR amplification products were run in a 1.5% agarose gel (Staining with Ethidium bromide in Tris-

borate EDTA buffer) and visualized under UV transillumination. The control reactions were set up without genomic DNA to avoid any DNA contamination.

2.2. Genotypic and statistical analysis

The MC1R bands were scored as present (1) or absent (0) in each pattern. All genetic parameters in present study were calculated by using Genepop software, version, 3.3.

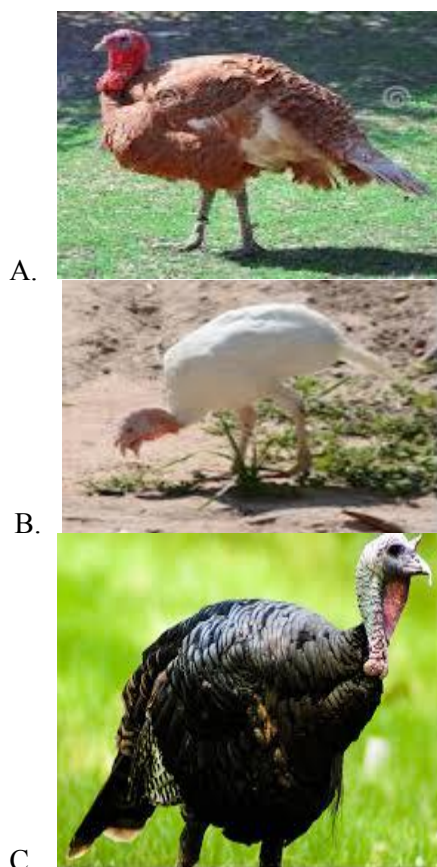


Figure 1. shows A. Red. B. White. C. Black plumage color of local turkey

3. Results and Discussions

The aims of the current study were to detect and investigate the Melanocortin 1 Receptor (MC1R) gene in three different colors (red, white and black) of local turkey in Sulaimani city of Iraq. To our best knowledge, this study is the first to report the detection of MC1R gene in local turkey in Sulaimani region, after collecting the blood samples and then DNA extraction, 3 pooled samples were made from each color. As illustrated in Figure (2), the gel electrophoresis of genomic DNA from three different colors of local turkey, which were later used for the identification of MC1R gene polymorphisms by PCR protocols.

We have analysed 75 domestic turkeys, being 25 white, 25 red and 25 black birds (Figure 1). A fragment of the MC1R gene was amplified with primers: the forward (5'-GCTTTGTAGGTGCTGCAGTTGTG-3') and reverse primer (5'-CCATCCATCCTC CTGTCTGT-3') were used to amplify a PCR product of a 1,050 bp fragment in the MC1R gene. Figure (3) shows the result of PCR amplification, as it is illustrated that black color turkeys have two bands of PCR amplification, that is mean for showing the black color phenotype the birds need the two MC1R gene express themselves on the two locus on the chromosome, while each of red and white phenotype needs only one MC1R gene to show these two phenotypes.

There are three genes code for enzymes in the melanin synthesis pathway. The first of these is the tyrosinase gene (TYR), which encodes a key enzyme in melanin synthesis and is required for the production of both pheomelanin (paler yellow/reddish-brown melanin) and eumelanin (darker black/brown) [11]. This gene has been isolated in chickens [12], and known mutations result in albinism [13] or white plumage [14]. The other two are the tyrosinase-related protein-1 (TYRP1) and the tyrosinase-related protein-2 (also known as DOPA chrome tautomerase, DCT). These are both required for the production of eumelanin but not pheomelanin and have both been identified in chickens [15; 16]. Although mutations with effects on pigmentation in birds have yet to be found, in mice a loss-of-function mutation at the TYRP1 locus produces a brown coat [17], and a mutation at the DCT locus, which reduces but does not abolish enzyme activity, produces a “slaty” phenotype [18].

The other pigmentation gene included here codes for the melanocortin-1-receptor (MC1R). This is a 7-transmembrane G-protein-coupled receptor that sits in the membrane of the melanin producing cell (melanocyte) [19]. Activation of this receptor stimulates melanogenesis and switches it from synthesis of pheomelanin to synthesis of eumelanin. MC1R has been identified in chickens [6] and several mutations with effects of feather color have been identified in chickens [20; 4; 21] and other bird species [22; 23].



Figure 2. Genomic DNA, lane 1-5; White, lane 6-10; Red, and lane 11-15 Black plumage color.

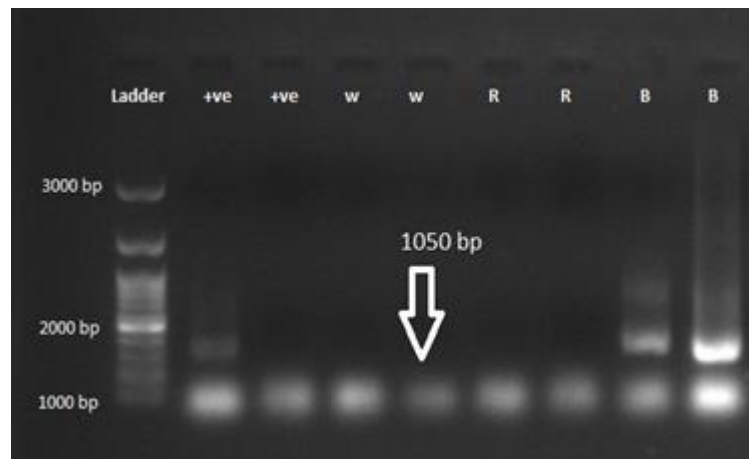


Figure 3. Gel-electrophoresis of MC1R gene in three different plumage colors of Sulaimani local turkey.

In accordance with the dendrogram of the current study (Figure 4), the result revealed that genetic variation and relatedness among turkey of the three feather color of local Turkey of distance 15%. The cluster divided the local turkey in to three main groups; the first group includes all birds of red color. The second group was mainly of black birds. On the other hand, the third group was mixture between the three colors. Low levels of genetic variation and high levels of genetic relatedness were found within each color, while A allele frequencies were high in the white feathered breed. In the case of 427A>G SNP, high frequencies of the GG genotype were observed only in red (63.3%) and yellow (66.7%) feathered breeds, while the black and white feathered chicken breeds displayed predominant AA genotypes. Therefore, only the A allele was observed in KB, O and W breeds,

while red and yellow feathered breeds contained the G allele with high frequencies. Previously, these SNPs have been associated in plumage colors in Chinese chickens [24]. These results reveal that the genotypes taken in the study are genetically diverse. The genetic distance identified in this research can be employed in the crossing programs. This study shows the MC1R gene polymorphisms and their relations to plumage color variation in local Turkey breeds. To maintain the valuable local Turkey breeds, an appropriate conservation breeding program will ultimately be needed. Based on the results presented here, molecular markers in MC1R gene provide some directions toward better breeding system related to color selection.

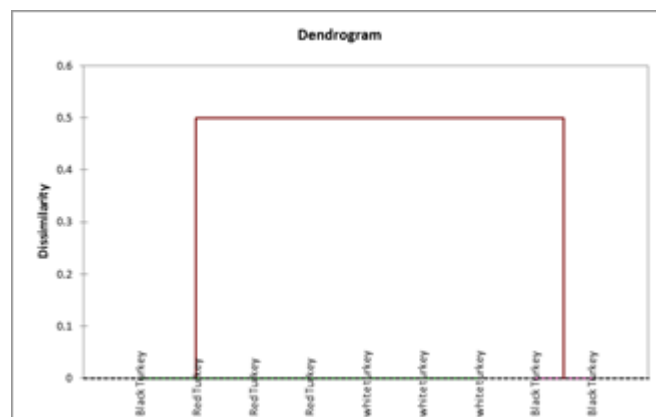


Figure 4. Dendrogram for local turkey with three different plumage colors using MC1R gene polymorphisms.

4. Conclusions

Based on the results obtained in this study, we concluded that there are significant associations between plumage colors and genetic variants of the MC1R gene in local Turkey. However, further studies are essential to confirm this conclusion.

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