Association of chicken MC1R gene polymorphism with coat colour trait in Iraqi native chicken

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Abstract: MC1R gene is mainly involved in melanogenesis and has crucial key in hair and skin colour in humans and coat colour in animals. The aim of this study was to investigate the genetic diversity of the partial coding regions of the MC1R gene and its association with coat colour in Iraqi native chicken. Blood samples from 95 Iraqi native chickens were collected to extract DNA and the 681-bp fragment of the MC1R gene was amplified and DNA sequencing method was adopted for single nucleotide polymorphism (SNP) discovering and genotyping. Sequence analysis showed that there were four SNPs in chicken MC1R gene, two of them were synonymous (D78272:g.1094A>G and D78272:g.1292C>T) and others were non- synonymous (D78272:g.915A>G and D78272:g.1095C>T). The allele frequency of these mutations is 0.92/0.08, 0.95/0.05, 0.94/0.06 and 0.96/0.04 for D78272:g.915A>G, D78272:g.1094A>G, D78272:g.1095C>T, and D78272:g.1292C>T mutations, respectively. The association analysis showed that no significant differences between different genotypes and coat colour ($p > 0.05$). However, our finding identified four novel SNPs for MC1R gene in Iraq indigenous chicken and it is useful for considering theirs association with other biophysical and biochemical indexes.
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Introduction

In chickens, the wide variety of plumage colors has been created by the selection of natural and human. Recently, identification of genetic markers associated with plumage color has increased considerably because these markers could provide useful information for the identification of breeds. The plumage color of chickens controls by several genes. The E locus is one the fundamental genes associated with color and it has different alleles, including: E*E, extended black; E*R, birchen; E*WH, dominant wheaten; E*N, wild type; E*B, brown; E*BC, buttercup; and E*Y, recessive wheaten(Crittenden et al., 1996; Smyth Jr, 1990). The linkage analysis showed that the E locus is located on chromosome 1 (Carefoot, 1993; SMYTH Jr and de LEON, 1992). It has been reported that the E locus is equivalent to the Melanocortin 1 Receptor (MC1R) gene. This gene is one the gene the receptors of melanocortins families and this family has five members (MC1R – MC5R) (Switonski et al., 2013). The encoded receptors bind four ligands: α-, β- and γ-melanocyte-stimulating hormone (α-, β-, γ-MSH) and the adrenocorticotropic hormone (ACTH). MC1R and MC2R bind to α-MSH and ACTH, respectively (Switonski et al., 2013). These five members expressed in the different tissue, as MC1R is mainly expressed in melanocytes, MC2R in the adrenal cortex, MC3R and MC4R in the nervous system, and MC5R in brain, muscles, lung and kidney(Yang, 2011). Among them, MC1R and MC4R were extensively studied in domestic mammals and the association between the polymorphism of them with coat colour variability, fat tissue deposition and feed conversion ratio(Switonski et al., 2013). As regards, MC1R is mainly involved in melanogenesis. So, the genetic diversity of it was investigated for founding its effect on hair and skin colour in human and coat colour in animals. It has been reported that human cutaneous pigmentation (e.g. skin, hair and eye) is controlled by about 120 genes and MC1R has a role key in this process(Dessinioti et al., 2011). It has found 16 causative polymorphisms in seven species (pig 5, dog 3, sheep 2, cattle arctic fox 2, horse 1 and red fox 1) for MC1R gene (Switonski et al., 2013). And also, 22 polymorphic sites were observed in coat colour variation in canids (10 in the dog, 8 in the red fox, 3 in the arctic fox and 1 in the Chinese raccoon dog (Nowacka – Woszuk et al., 2013). In addition, a study was investigated the association of the plumage colors with MC1R and TYR genes in Korean chickens (Heo et al., 2011). Furthermore, the polymorphism of MC1R linked with the eumelanin, pheomelanin and albino plumage pigmentations. For plumage colors, eight causative nonsynonymous mutations were found in association with eumelanin and pheomelanin pigments (Kerje et al., 2003; Ling et al., 2003). It has

suggested that six nonsynonymous mutations (Met71Thr, Glu92Lys, Ala126Ile, Thr143Ala, Cys213Arg and His215Pro) were related to eumelanin and pheomelanin pigmentation, as, these mutations were associated in chicken plumage colors (Guo et al., 2010; Kerje et al., 2003; Ling et al., 2003). Also, the genetic diversity of MC1R gene associated with melanic polymorphisms in vertebrate species (Hoekstra, 2006). These results show that MC1R gene has a crucial key in the coat colour of chicken. Iraqi native chicken has different colors and as regards, MC1R gene is one of the fundamental genes in the coat colour of chicken, so the objective of the present study was to investigate the genetic diversity of the partial coding regions and its association with coat colour in Iraqi native chicken.

Materials and methods

Animals

Blood samples were collected from the external jugular vein of chickens belonging to Iraqi native chicken breeds. All samples used in this study were obtained from Abu Ghraib's research station (Baghdad, Iraq) and stored at -20 °C for DNA extraction. This study was approved by the Institutional Animal Care and Use Committee of Tehran University.

DNA extraction and amplification

Genomic DNA was extracted from whole blood samples using salting out method (Miller et al., 1988) and stored at -20 °C until being used for PCR amplification. Based on the chicken *MC1R* gene sequence present in the Genbank with accession number of D78272.1, one pair of primers (F:5'-TGTCATCGACATGCTCATCTGC -3' and R:5'-CATCCACCCATCTGTTTGTCCATC -3') was designed to amplify a 681-bp -bp fragment of the coding region of chicken *MC1R* gene using polymerase chain reaction (PCR). The 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. The PCR temperature profiles consisted of an initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 63 °C for 30 s, extension at 72 °C for 45 s and a final extension at 72 °C for 5 min. Electrophoresis of PCR products was performed in 1% (w/v) agarose gel in parallel with 100 bp DNA marker, in 1x TAE buffer at a fixed voltage of 90 V for 30 min. After ethidium bromide staining, the products were visualized by ultraviolet transillumination.

Sequencing and Analysis

All PCR products were subjected to sequence analysis using ABI 3730 XL DNA Analyzer (BioNeer, Daejeon, South Korea). The chicken *MC1R* nucleotide sequences were aligned using ClustalW program available in Bioedit software and then, SNPs were determined and each animal was genotyped for SNPs. Genotypic, allelic frequencies and Hardy–Weinberg equilibriums were estimated with use of GenAlEx 6.41 software.

The analysis of associations of the animal genotypes with coat colour were done in the Genmod of SAS 9.1 (Institute, 1985).

Results and Discussion

A total of 95 individuals were initially sequenced and compared to NCBI reference sequence D78272.1 by using Bioedit software. The results revealed four polymorphisms in the partial coding regions of the MC1R gene in Iraqi native chicken and these mutations are D78272:g.1094A>G, D78272:g.1292C>T, D78272:g.915A>G and D78272:g.1095C>T (figure 1).

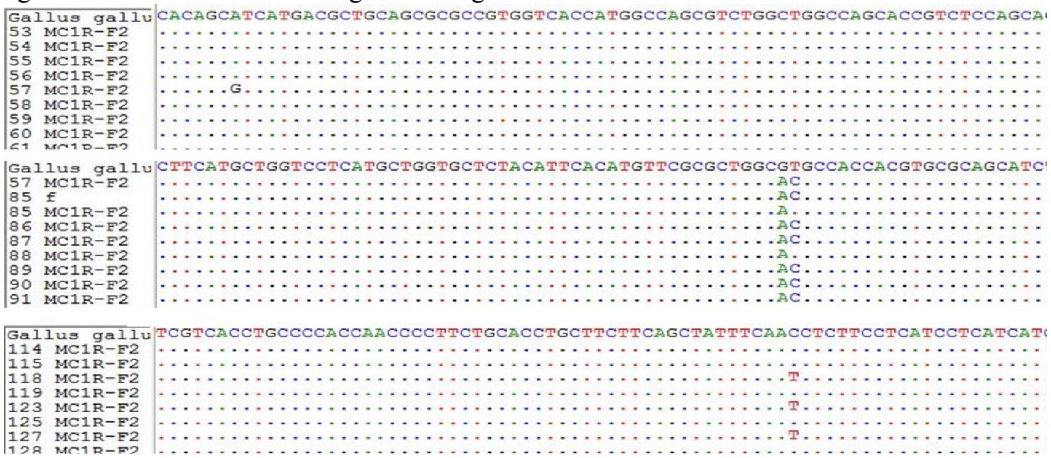

Fig. 1. Comparative alignment of conceptualized nucleotide sequence of MC1R gene in Iraqi native chicken with NCBI reference sequence D78272.1.

Also, the allele frequency and the Chi square ($\chi 2$) test of Hardy-Weinberg equilibrium (HWE) of these mutations were showed table 1 in analyzed population. These results revealed that the whole of these SNPs have low genetic diversity within MC1R gene (table 1). The allele frequency of these mutations is 0.92/0.08, 0.95/0.05, 0.94/0.06 and 0.96/0.04 for D78272:g.915A>G, D78272:g.1094A>G, D78272:g.1095C>T, and D78272:g.1292C>T mutations, respectively (table 1). In addition, one of mutations was in HWE ($p<0.01$) and others were not in HWE ($p>0.05$) (Table 1).

Table 1 Allele frequencies of the MC1R gene and the test Hardy–Weinberg for level of significance of the deviation within population.

The position of SNP				Allele					value (HWE)
	A	G	A	G	C	T	C	T	
D78272:g.915A>G	0.92	0.08							p<0.05
D78272:g.1094A>G			0.95	0.05					p<0.05
D78272:g.1095C>T					0.94	0.06			p<0.05
D78272:g.1292C>T							0.96	0.04	p<0.05

In domestic animal species, coat colour is an important characteristic of breeds. So, the genetic markers have been extensively for finding the association between host genetic and coat colour. It has been reported that some associated polymorphisms with different phenotypes (ranging from the dominant extended black to the recessive yellow) have been identified in the chicken *MC1R* gene (Kerje et al., 2003; Takeuchi et al., 1996). It has been reported that one of the SNPs of MC1R gene (69T>C) has different genotype ferquency in different breeds; TT genotype is prevalent in black Korean native chicken (56.7%) and black silky (80%), while the CC genotype is prevalent in yellow Korean native chicken (80%), red Korean native chicken (66.7%), and white Leghorn (100%) chicken breeds(Hoque et al., 2013). In cattle, the ED and E+ are associated with black coat colour and a combination of red or reddish brown/black coat colours, respectively(Klungland et al., 1995).

In lines with different plumage color, 8 polymorphic sites are associated with 4 haplotypes in the coding region of MC1R (Tixier-Boichard et al., 2006). It has been observed that the expression of MC1R was significantly different at 56 d of age in a cross between chickens of white and black color but it was not different in other days. Several mutations of the MC1R gene linked with the E locus and they may be associated with feather pigmentation. Furthermore, Okimoto et al. (1999) and Ellett and Okimoto (2000) reported that there was a close association between MC1R polymorphism and the E locus(Dávila et al., 2014; Okimoto et al., 1999). According to our sequence analysis, two out of four SNPs detected in Iraqi chicken MC1R were nonsynonymous (Table 2).

Table 2 The effect of nucleotide substitutions on amino acid of chicken MC1R protein.

Nucleotide substitution	Position	Changed amino acid	The type of SNP
A/G	D78272:g.915A>G	Isoleucine converted into a valine	nonsynonymous
A/G	D78272:g.1094A>G	Alanine	synonymous
C/T	D78272:g.1095C>T	Cysteine converted into an arginine	nonsynonymous
C/T	D78272:g.1292C>T	Asparagine	synonymous

They include D78272:g.915A>G and D78272:g.1095C>T in which, an isoleucine to valine substitution and a cysteine to arginine substitution was detected, respectively (Table 2). The two other SNPs (D78272:g.1094A>G and D78272:g.1292C>T) were synonymous. So, these results imply that these markers affect the function of MC1R protein, suggesting that these polymorphisms can be used as molecular markers for coat colour in chicken. The association analysis showed that no significant differences between different genotypes and coat colour ($p> 0.05$). One of the reasons for lack of association between genotypes and coat colour is low diversity for observed SNPs in this population and another is small size population. It has been suggested that six nonsynonymous SNPs (p.M71T, p.E92K,

p.A126I, p.T143A, p.C213R, and p.H215P) are high significant associated with plumage colors in chicken. Hoque et al., 2013 reported that two non-synonym SNPs in chicken (Val1 26 Ile and Ala 143 Thr). Dávila et al., 2014 found 11 SNPs for MC1R gene in Spanish breeds of chickens, two of them were synonymous (C69T and C834T) and others were nonsynonymous (T212C, G274A, G376A, T398AC, G409A, A427G, C637T, A644C, and G646A), these synonymous corresponding to amino acid changes Met72Thr, Glu92Lys, Val126Ile, Leu133GlnPro, Ala137Thr, Thr143Ala, Arg213Cys, His215Pro, and Val216Ile). Takeuchi et al. (1996a, b) determined a non-synonymous SNP (Glu92Lys) in MC1R that leads an active receptor to produce eumelanin. Furthermore, it has been reported that amino acid polarity could affect the signal transduction of the MC1R gene and ultimately lead to color variations.

Conclusion

It can be assumed that the partial coding regions of the MC1R gene in Iraq indigenous chicken breed has low genetic diversity and four mutations in MC1R gene identified by DNA sequencing. Two of them were synonymous (D78272:g.1094A>G and D78272:g.1292C>T) and others were non-synonymous (D78272:g.915A>G and D78272:g.1095C>T). The allele frequency of these mutations is 0.92/0.08, 0.95/0.05, 0.94/0.06 and 0.96/0.04 for D78272:g.915A>G, D78272:g.1094A>G, D78272:g.1095C>T, and D78272:g.1292C>T mutations, respectively. The association analysis showed that no significant differences between different genotypes and coat colour. However, our finding identified four novel SNPs for MC1R gene in Iraq indigenous chicken and it is useful for considering theirs association with other biophysical and biochemical indexes.

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