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Original Article

Identification of *Mx* gene nucleotide dimorphism (G/A) as genetic marker for antiviral activity in Egyptian chickens

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ABSTRACT

Egyptian chickens, representing 2 breeds and 7 strains, were genotyped using the PCR-RFLP and sequencing techniques for detection of a non-synonymous dimorphism (G/A) in exon 14 of chicken Myxovirus resistance (*Mx*) gene. This dimorphic position is responsible for altering *Mx* protein's antiviral activity. Polymerase Chain reactions were performed using Egyptian chickens DNA and specific primer set to amplify *Mx* DNA fragments of 299 or 301 bp, containing the dimorphic position. Amplicons were cut with restriction enzyme *Hpy*81. Genotype and allele frequencies for the resistant allele A and sensitive allele G were calculated in all the tested chickens. Results of PCR-RFLP were confirmed by sequencing. The three genotypes AA, AG, GG at the target nucleotide position in *Mx* gene were represented in all the studied Egyptian chicken breeds and strains except Baladi strain which showed only one genotype AA. The average allele frequency of the resistant A allele in the tested birds (0.67) was higher than the sensitive G allele average frequency in the same birds (0.33). Appling PCR-RFLP technique in the breeding program can be used to select chickens carrying the A allele with high frequencies. This will help in improving poultry breeding in Egypt by producing infectious disease-resistant chickens.

1. Introduction

Mx proteins are Dynamin-like guanosine triphosphate metabolizing enzymes (GTPases). They are the products of interferon-stimulated *Mx* genes which exist in almost all vertebrates ranging from fish to humans [1–3]. *Mx* proteins are known to be involved in inhibiting the multiplication of several RNA viruses, including Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Bunyaviridae and Togaviridae as well as some DNA viruses, including Hepadnaviridae [4–10]. *Mx* proteins are members of the large GTPases family. These GTPases share an N-terminal GTPase domain, a middle domain (MD), and a C-terminal GTPase effector domain (GED). In the primary structure of the *Mx* protein, the GTPase domain is followed by the middle domain (MD) and the C-terminal GTPase effector domain (GED). The MD and GED are very important for the conformation and activity of the *Mx* proteins. The MD is important for oligomerization and virus target recognition [11]. On the other hand, the GED functions as an intramolecular GTP-activating domain: the C-terminal leucine zipper motif (65–70 amino acids) in the GED folds back to join the N-terminal GTP-binding domain, forming the enzymatically active center of *Mx* proteins [12]. Some vertebrates have up to three *Mx* gene copies in the same organism. For Example, mice and humans carry more than one *Mx* gene [13].

Chickens have a single *Mx* gene (*Mx*1) that is induced by type I interferon [14]. The *Mx* gene is located on chicken chromosome 1 in approximately 20767 bp fragment and consists of 14 exons. The *Mx* mRNA or cDNA molecule length is 2545 bp with a 2115 bp coding region that codes for 705 amino acids protein [15,16]. The chicken *Mx* protein has been reported to confer antiviral activity against the influenza viruses from the Orthomyxoviridae family and the recombinant vesicular stomatitis virus (VSV) from family Rhabdoviridae [17–21]. It has been also reported that *Mx* protein exhibited antiviral activity against the Newcastle Disease virus

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(NDV) from the Paramyxoviridae family [22,23]. A non-synonymous dimorphism G/A in exon 14 of the chicken *Mx* gene (this position corresponds to nucleotide number 2032 in *Mx* cDNA reference sequence accession number: Z23168) results in the presence of serine (Ser) or asparagine (Asn) at amino acid (aa) 631, located in the C-terminal GTPase effector domain of the *Mx* protein. The substitution of Ser with Asn and vice versa alters the *Mx* protein's antiviral activity [19].

Several *in vitro* studies have indicated that the presence of Asn at aa 631 (Asn631allele), which results from homozygous AA genotype, had higher antiviral activity than the presence of Ser631 allele, from GG individuals, against VSV and NDV in chickens [19,20,22,24]. Sasaki et al. [24] also found that replacing Ser with Asn at 631 aa altered intracellular localization of the *Mx* protein. Moreover, Yin et al. [22] reported that the AA individuals from Beijing-You and White Leghorn chicken breeds had higher *Mx* expression levels than GG individuals. The higher antiviral activity of the AA genotype against the ND virus was confirmed *in vivo* study by Pagala et al. [23]. They confirmed that AA and AG genotypes were resistant against NDV and showed better production than GG genotype in Tolaki chickens.

Several studies have also presented conflicting results regarding whether or not the substitution of Ser with Asn in the *Mx* protein at the aa 631(S631N) is associated with resistance to avian influenza virus infection in chickens [25]. In an *in vitro* study by Ko et al. [19] demonstrated that S631N substitution in the *Mx* protein confers more resistance to the highly pathogenic avian influenza (HPAI) H5N1 subtype in chickens. In an *in vivo* study, Ewald et al. [26] reported that chickens homozygous for Asn631 allele were significantly more resistant to disease based on early mortality, morbidity, or virus shedding than Ser631 homozygotes when infected with HPAI H5N2 subtype. Wang et al. [27] also reported that chickens with AA genotype had high gene expression and a non-significant tendency for lower virus titer, when infected with influenza virus H5N3 subtype, than GG individuals. Two other groups have failed to support the anti-influenza activity of chicken *Mx* Asn631 against influenza virus subtypes H1N1, H4N6, H5N1, H5N3 and H7N1 *in vivo* and *in vitro* systems [28,29].

Despite the previously mentioned conflicting results regarding the anti-influenza activity of chicken *Mx* Asn631, the latter could be important for chicken response to other viruses. Therefore, it is possible to breed infectious disease-resistant chickens that carry the A allele at the above-mentioned nucleotide position, which could help to prevent the spread of viral infections.

Egyptian chickens are grouped into around 15 breeds and strains. Egyptian chickens have a small body and a dual purpose for meat and egg production. They have some useful genetic attributes such as adaptability to local environment, resistance to some diseases, possessing a good nicking ability and lower clutch [30]. Genetic improvement programs for poultry breeding in Egypt will be of a great economic importance. Therefore the aim of the present study was to identify the Egyptian chickens that carry the *Mx* gene resistant allele with the high frequencies using the PCR-RFLP genotyping and sequencing techniques. The obtained data could help in improving poultry breeding in Egypt by producing infectious disease-resistant chickens.

2. Materials and methods

2.1. Chicken samples

The study was performed on 246 Egyptian chickens, representing 2 breeds Dandarawi and Fayoumi in addition to 7 strains: El-Salam, Golden Montazah, Dokki-4, White egg commercial, Red egg; commercial, Gemmizah and Baladi.

2.2. Blood sampling

The blood samples were collected from brachial vein in the chicken wing area in sterile tubes containing 0.5 ml EDTA, transferred to the lab and kept frozen until DNA extraction. Details of the collected blood samples are presented in Table 1.

2.3. DNA isolation and quantification

DNA was extracted from whole blood using commercial Kit (GeneJET Whole Blood Genomic DNA Purification Mini Kit, K0781) according to the manufacture instructions. DNA concentration was measured using UV spectrophotometer (Shimadzu UV 2401) at 260 nm wavelength.

2.4. PCR amplification and visualization

PCR was performed using the primer set designed by Sironi et al. [31] for the amplification of a DNA fragment (299 bp or 301 bp) from the *Mx* gene that contains the target dimorphic nucleotide position G/A. The primers sequences were: forward (F) 5′-GCACTGTCACCTCTTAATAGA-3′ and reverse (R) 5′-GTATTGG TAGGCTTTGTTGA-3′. The F primer anneals to the last intron of the *Mx* gene, and the R primer anneals to the last exon of the gene. PCR was carried out in a total volume of 25 μL, with 50 ng genomic DNA, 10 pmol of each primer, 2.5 μL 10X buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, and 1 U from Dream *Taq* (Thermo Scientific). The reaction was accomplished in TM Thermal Cycler (MJ Research PTC-100 thermocycler, USA). The thermal cycling was as follows: initial denaturation step at 95 °C for 4 min followed by 35 cycles

Table 1
The collected blood samples from Egyptian chickens.

Chickens	Collection site	No. of blood samples		
		Male	Female	Total
Dandarawi breed	Fayoum Poultry Station "El Fayoum"	8	30	38
Fayoumi breed	Fayoum Poultry Station "El Fayoum"	10	28	38
El-Salam strain	Fayoum Poultry Station "El Fayoum"	6	30	36
Golden Montazah strain	Fayoum Poultry Station "El Fayoum"	14	18	32
Dokki-4 strain	Animal Production Research Center "Sakha, Kafr El Sheikh"	–	–	20
White egg commercial strain	El Noubaria Farm "El Behera"	0	17	17
Red egg; commercial strain	El Noubaria Farm "El Behera"	0	19	19
Gemmizah strain	Collected from 3 different farms:(1) Gemmizah Poultry Station "El Gharbia",(2) Tkamoly farm, El Azab village "El Fayoum"(3) Sabahia Farm "Alexandria"	1	24	25
Baladi strain	El Noubaria Farm "El Behera"	11	10	21

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