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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

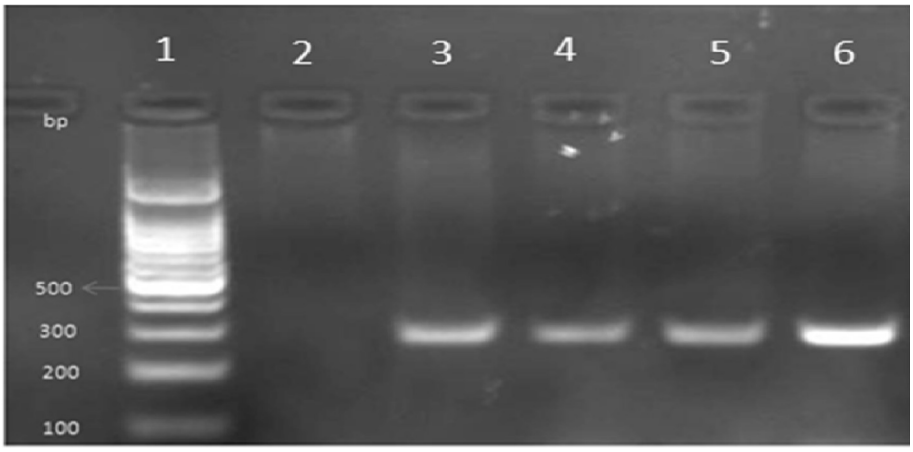

Fig. 1. Representatives of the PCR amplified fragment of the *Mx* gene in Egyptian Chickens. Lane 1: DNA size marker, 100 bp ladder. Lane 2: negative control sample. Lanes 3–6: successful PCR giving the band of interest: 299 bp or 301 bp.

of 94 °C for 1 min, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. PCR successful products were identified on 2% agarose gel in 1X TBE buffer and visualization after staining with ethidium bromide (EtBr). All polymerase chain reactions were conducted in duplicates.

2.5. Genotyping

The PCR products were digested with restriction enzyme Hpy81 (Fermentas). The digestion reaction mixture was prepared using 10 µl of PCR product, 10 U restriction enzyme, dH_2O and Tango buffer according to the manufacturer instructions, the mixture was incubated at 37 °C for 15 min. then the enzyme was deactivated by incubating at 65 °C for 10 min. In case of the G allele presence, the enzyme cuts the sequence 5′-GTN|NAC-3′, 2 bp downstream of the target *Mx* dimorphism, whereas the fragment containing the A allele is not cleaved. Digestion products were subjected to electrophoresis on 3% agarose gel in 1X TBE buffer and visualized after staining with ethidium bromide. Genotype and allele frequencies for the two alleles A and G were calculated in all the Egyptian chickens. Genotype frequencies were calculated according to Brooker et al. [32]. The allele frequencies were calculated from the genotype frequencies according to Gillespie [33].

2.6. Sequencing

PCR products were purified using the Exo SAP-IT PCR Purification Kit (Applied Biosystems) following the manufacturer's recommended protocol. Sequencing reactions were performed using Big Dye TM terminator Cycle Sequencing Kit (Applied Biosystems). Sequences were determined using ABI3700 and 3730 automated DNA sequencers (Applied Biosystems).

2.7. Protein translation and sequence analysis

DNA sequences were translated to proteins by using the ExBASy-Translate tool http://web.expasy.org/translate/. Nucleotide sequence alignments were performed using CLUSTALW program http://www.genome.jp/tools/clustalw/.

3. Results

3.1. The Mx gene amplified fragment

The size of the *Mx* gene PCR amplified fragment that containing the target dimorphic position G/A was 299 bp or 301 bp (Fig. 1). The exact size of each amplified fragment was determined from sequencing results.

3.2. Genotype and allele frequencies

Cutting the PCR products with the restriction enzyme Hpy81 produced two bands (at approximately 200 bp and 100 bp) in the homozygous samples that possess allele G only (GG genotype). The uncut PCR products were at approximately 300 bp representing the homozygous samples with allele A only (AA genotype). The three bands at approximately 100 bp, 200 bp, 300 bp represented heterozygous samples (AG genotype) (Fig. 2).

The genotype and allele frequencies of the A and G alleles in the different studied chickens are presented in Table 2.

The three genotypes AA, AG, GG of the *Mx* gene were represented in all the tested Egyptian chicken breeds and strains except the Balabi strain which showed only one genotype AA, with a genotype frequency value of 1.000. The highest frequencies of GG and AG genotypes were 0.473 in the El-Salam strain and 0.842 in the Red egg commercial strains, respectively. The average AA, AG and GG genotype frequencies in all the tested chickens breeds and strains were 0.44, 0.45 and 0.11 respectively. The obtained results indicated that the A allele was presented in all the tested chicken breeds and strains. The calculated allele A frequency values ranged from 0.417 in El-Salam strain to 1.000 in Baladi strain. The G allele was also detected in all the tested chickens, expect Baladi strains, with allele frequency values that ranged from 0.197 in Fayoumi strain to 0.583 in El-Salam strain. Results also indicated that the average A allele frequency in all the tested chicken breeds and strains (0.67) was higher than the average G allele frequency (0.33).

3.3. Sequence pattern

Sequences of the amplified *Mx* gene fragment which obtained from the different Egyptian chicken breeds and strains samples were grouped in five sequence patterns. The five sequence patterns were submitted to the International genbank database under the

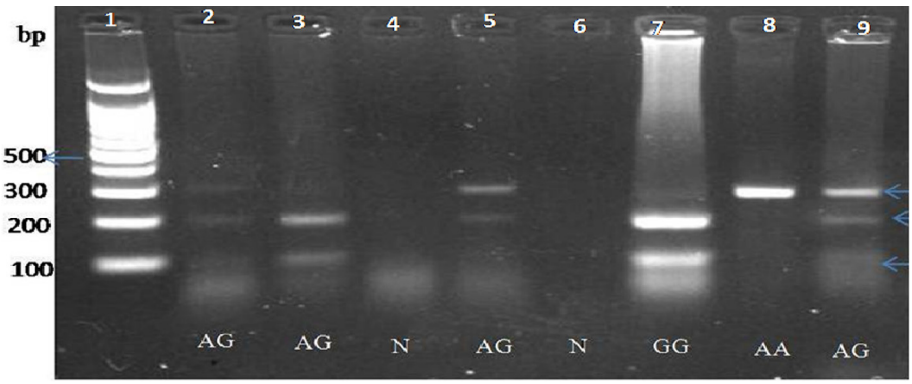

Fig. 2. Restriction cut of the PCR products with Hpy81 enzyme. Lane 1: DNA size marker, 100 bp ladder. Lane 2, 3, 5, 7, 8, 9: showing different genotypes as written. Lanes 4, 6: are blank wells (N).

Table 2
Genotype and allele frequencies of A and G alleles in the different studied chickens.

Chickens		No.	Genotype frequency			Allele frequency	
			AA	AG	GG	A	G
Dandarawi breed	Male	8	0.750	0.250	0.000	0.875	0.125
	Female	30	0.567	0.333	0.100	0.733	0.267
	Total	38	0.605	0.316	0.079	0.763	0.237
Fayoumi breed	Male	10	0.500	0.400	0.100	0.700	0.300
	Female	28	0.714	0.250	0.036	0.840	0.160
	Total	38	0.658	0.289	0.053	0.803	0.197
El-Salam strain	Male	6	0.333	0.333	0.333	0.500	0.500
	Female	30	0.300	0.200	0.500	0.400	0.600
	Total	36	0.305	0.222	0.473	0.417	0.583
Golden Montazah strain	Male	14	0.429	0.429	0.143	0.643	0.357
	Female	18	0.222	0.611	0.167	0.528	0.472
	Total	32	0.313	0.531	0.156	0.578	0.422
Dokki-4 strain	Total	20	0.250	0.750	0.000	0.625	0.375
White egg commercial strain	Total	17	0.530	0.411	0.059	0.735	0.264
Red egg commercial strain	Total	19	0.105	0.842	0.063	0.526	0.474
Gemmizah strain	Male	1	0.000	0.000	1.000	0.000	1.000
	Female	24	0.208	0.750	0.042	0.583	0.417
	Total	25	0.200	0.720	0.080	0.560	0.440
Baladi strain	Male	11	1.000	0.000	0.000	1.000	0.000
	Female	10	1.000	0.000	0.000	1.000	0.000
	Total	21	1.000	0.000	0.000	1.000	0.000

accession numbers: KY584063, KY584064, KY584065, KY584066 and KY584067. Alignment result of the five detected sequence patterns is presented in Fig. 3.

The results indicated full identity between the data collected by PCR-RFLP and sequencing techniques for the presence of A or G alleles at the target dimorphic position in *Mx* gene exon 14. This position is at the nucleotide No. 99 in the amplified *Mx* gene fragment and corresponds to the nucleotide number 2032 in the previously mentioned *Mx* cDNA reference sequence. Representatives of sequencing data from the AG, GG and AA genotypes at the nucleotide position No. 99 are shown in Fig. 4.

In addition to the previous G/A dimorphism, four variable sites were also detected within the *Mx* gene amplified fragment from different samples (Fig. 3). Three of which were in the intronic region: one C/T dimorphism is at nucleotide number fourteen and two successive insertion/deletion are after the nucleotide number 71, of the amplified fragment. The fourth one was another G/A dimorphism in the *Mx* exon 14 at the nucleotide position No. 226 of the amplified fragment. This position corresponded to the nucleotide number 2159 in the *Mx* cDNA reference sequence.

Protein translation of the coding exonic region in the different detected *Mx* gene sequence patterns indicated that the dimorphism G/A at the position that corresponding to nucleotide number 2032 in *Mx* cDNA is the only non-synonemous dimorphism. It results in a change at amino acid 631 of the *Mx* protein from serine to asparagine and vice versa. The other detected dimorpfism G/A in the same amplified coding region was found to be synonemous.

4. Discussion

Due to the severe threats of infectious spread to large numbers of livestock, animal breeding focused on innate immune-associated genes such as *Mx* that may play an important role in reducing the incidence of infection. The *Mx* pathway is one of the most powerful pathways. The *Mx* protein has a direct antiviral activity that inhibits a wide range of viruses by blocking an early stage of the viral replication cycle [22]. In chickens, the *Mx* gene protein product has been reported to confer antiviral activity against the influenza viruses, the recombinant vesicular stomatitis virus (VSV) and Newcastle Disease virus (NDV) [17–23].

Sequence analysis of *Mx* cDNA from Japanese and Egyptian chickens revealed that the non-synonymous dimorphism (G/A) in *Mx* gene exon 14 leads to the change at aa 631 of the chicken *Mx* protein. This aa change was found to affect *Mx* protein antiviral activity [19]. The amino acid 631 is found in the C-terminal GTPase effector domain of the *Mx* protein (GED) that is very important for the activity of the protein. The change at the aa 631 is from

```
pattern1   1 GCACTGTCACCTCTTAATAGAGTACCTTCAGCCTGTTTTTTCTTCTTTTAGGAAAAAAGT
pattern2   1 GCACTGTCACCTCTTAATAGAGTACCTTCAGCCTGTTTTTTCTTCTTTTAGGAAAAAAGT
pattern3   1 GCACTGTCACCTCCTAATAGAGTACCTTCAGCCTGTTTTTTCTTCTTTTAGGAAAAAAGT
pattern4   1 GCACTGTCACCTCTTAATAGAGTACCTTCAGCCTGTTTTTTCTTCTTTTAGGAAAAAAGT
pattern5   1 GCACTGTCACCTCCTAATAGAGTACCTTCAGCCTGTTTTTTCTTCTTTTAGGAAAAAAGT
             ************** ************************************************

pattern1      CTTCACTCTTTTTTTTTCCCTCTCCTTGTAGGGAGCAARTAAACGCCTGAGCAATCAGAT
pattern2      CTTCACTCTTTTTTTTTCCCTCTCCTTGTAGGGAGCAAGTAAACGCCTGAGCAATCAGAT
pattern3      CTTCACTCTTTTTTTTTCCCTCTCCTTGTAGGGAGCAAGTAAACGCCTGAGCAATCAGAT
pattern4      CTTCACTCTTT--TTTTCCCTCTCCTTGTAGGGAGCAAATAAACGCCTGAGCAATCAGAT
pattern5      CTTCACTCTTT--TTTTCCCTCTCCTTGTAGGGAGCAAATAAACGCCTGAGCAATCAGAT
              ***********  ************************* ***********************

pattern1      TCCTCTGATCATCCTCTCTACTGTCCTTCATGACTTTGGAAATTATTTGCAGACCTCAAT
pattern2      TCCTCTGATCATCCTCTCTACTGTCCTTCATGACTTTGGAAATTATTTGCAGACCTCAAT
pattern3      TCCTCTGATCATCCTCTCTACTGTCCTTCATGACTTTGGAAATTATTTGCAGACCTCAAT
pattern4      TCCTCTGATCATCCTCTCTACTGTCCTTCATGACTTTGGAAATTATTTGCAGACCTCAAT
pattern5      TCCTCTGATCATCCTCTCTACTGTCCTTCATGACTTTGGAAATTATTTGCAGACCTCAAT
              ************************************************************

pattern1      GTTGCATCTCTTGCAAGGAAAAGAAGAAATAAACTATTTACTCCARGAAGATCATGAAGC
pattern2      GTTGCATCTCTTGCAAGGAAAAGAAGAAATAAACTATTTACTCCAGGAAGATCATGAAGC
pattern3      GTTGCATCTCTTGCAAGGAAAAGAAGAAATAAACTATTTACTCCAGGAAGATCATGAAGC
pattern4      GTTGCATCTCTTGCAAGGAAAAGAAGAAATAAACTATTTACTCCAAGAAGATCATGAAGC
pattern5      GTTGCATCTCTTGCAAGGAAAAGAAGAAATAAACTATTTACTCCAAGAAGATCATGAAGC
              ********************************************* ***************

pattern1      TGCTAACCAGCAGAAGTTACTGACCAGCAGAATTAGTCACCTCAACAAAGCCTACCAATA
pattern2      TGCTAACCAGCAGAAGTTACTGACCAGCAGAATTAGTCACCTCAACAAAGCCTACCAATA
pattern3      TGCTAACCAGCAGAAGTTACTGACCAGCAGAATTAGTCACCTCAACAAAGCCTACCAATA
pattern4      TGCTAACCAGCAGAAGTTACTGACCAGCAGAATTAGTCACCTCAACAAAGCCTACCAATA
pattern5      TGCTAACCAGCAGAAGTTACTGACCAGCAGAATTAGTCACCTCAACAAAGCCTACCAATA
              ************************************************************

pattern1      C 301
pattern2      C 301
pattern3      C 301
pattern4      C 301
pattern5      C 301
              *
```

Fig. 3. Alignment of the five detected *Mx* gene sequence patterns from the different Egyptian chicken samples. R: means a heterozygous position that contains the two nucleotides A and G. * means identical nucleotides.

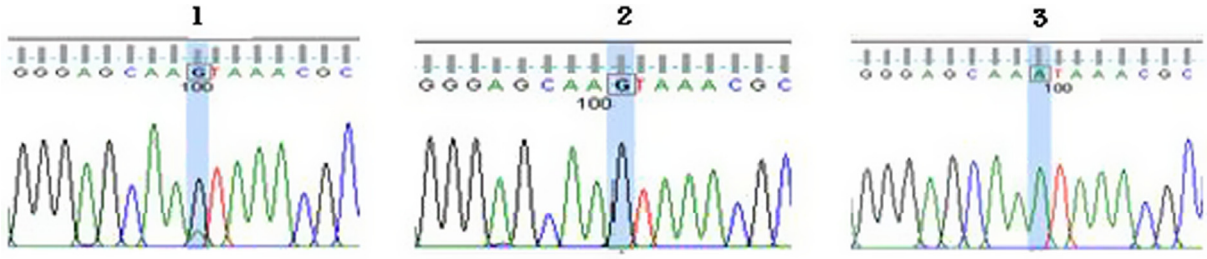

Fig. 4. Representatives of the three genotypes AG, GG and AA at the nucleotide No. 99 in sequence charts of the *Mx* gene fragment. 1: AG genotype, 2: GG genotype and 3: AA genotype.

asparagine to serine and vice versa. This aa substitution is a semi-conservative substitution that means the two amino acids share some properties but not all the physiochemical properties. Semi-conservative amino acids substitution may affect protein folding, stability, function, expression as well as protein-protein interactions and sub-cellular localization of the protein molecules [34–37]. Several studies confirmed that the variant having asparagine instead of serine at position 631 is responsible for the raise of the antiviral activity of the *Mx* protein against VSV, NDV as well as influenza viruses H5N1, H5N2 and H5N3 [19,20,24,22,26,27,23].

Several studies have investigated the frequencies of the two alleles A and G that are responsible for the change at aa 631 in chickens *Mx* gene using a variety of methods: mismatched polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [38], electrophoresis of PCR products on polyacrylamide gels [39], and sequencing [40]. In the present study a

PCR-RFLP protocol [31] was applied for the two alleles A and G genotyping. This genotyping protocol simply does not require the creation of mismatches as the protocol of Seyama et al. [38].

The PCR-RFLP results indicated that the three genotypes AA, AG, GG at the target nucleotide position in the *Mx* gene were represented in all the tested Egyptian chicken breeds and strains except the Baladi strain which showed only one genotype AA. The average AA, AG and GG genotype frequencies in all the tested chickens were 0.44, 0.45 and 0.11 respectively. The present results also indicated that the average allele frequency of the resistant A allele in all the tested birds (0.67) was higher than the sensitive G allele average frequency (0.33). Similar to our results were obtained by Sartika et al. [41]; who reported that indigenous chickens in Indonesia have more frequency of resistant type *Mx* gene allele. In other studies, it was reported that the GG genotypic frequency was significantly higher than the AA genotypic frequency in Chinese and Bangladesh chickens [15,16].

The sequencing results indicated complete matching between the results of PCR-RFLP and sequencing techniques in identifying the A or G alleles at the target nucleotide position, that responsible for the change at amino acid 631 in the *Mx* protein. The other G/A dimorphism at the nucleotide position, that corresponds to the nucleotide number 2159 in the *Mx* cDNA reference sequence was found to be synonymous. This dimorphism was also identified before by Ko et al. [19] in Japanese and Egyptian chickens *Mx* cDNA among 25 polymorphic positions.

The interesting finding of the present study is the highest genotype and allele frequencies for the resistant A allele that was detected in the Baladi chicken strain which showed AA genotype only. The Baladi strain originated from hybridization among exotic and Egyptian autochthonous chickens continued along with different times of old trade dispersal and colonization to Egypt [42]. Appling PCR-RFLP technique in the breeding programs to select chickens that carry the *Mx* resistant allele A from the different Egyptian chicken breeds and strains and especially from the Baladi strain will help in raising chickens antiviral activity.

5. Conclusion

In the present study the genotype and allele frequencies of the resistant and the sensitive alleles of the *Mx* gene were identified in tested Egyptian chicken breeds and strains. The results indicated that the average allele frequency of the resistant A allele is higher than the sensitive G allele average frequency. The highest genotype and allele frequencies for the resistant A allele was detected in the Baladi strain which showed AA genotype only. Selection of chickens that carry the *Mx* resistant allele as a genetic marker for high antiviral activity in the breeding programs could help in improving poultry breeding in Egypt by producing infectious disease-resistant chickens.

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