POLYMORPHISM IN EXON 14 OF ANTIVIRAL RESISTANT MX GENE IN VIETNAMESE INDIGENOUS CHICKEN BREEDS

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Received date: 05.05.2013 Accepted date: 25.08.2013

ABSTRACT

The study was conducted to analyze the nucleotide polymorphisms of exon 14, Mx gene of seven Vietnamese indigenous chicken breedsL viz. Ac, Dong Tao, H'mong, Ho, Mia, Mong and Ri. The results revealed that the polymorphisms of exon 14 occured at two sites (2032th and 2159th). The non-synonymous substitution A/G at nucleotide 2032th of Mx gene was found in all of seven indigenous breeds used in this study. In the same breed, both of alleles A and G were present, however, allele A (viral resistance) was at a higher frequency than allele G (viral susceptiblity). Particularly, the allele A frequency of Ri and H'mong was highest among 7 breeds examined (80.91% and 74.04%, respectively). Comparative analysis of the deduced amino acids showed that 2032 A/G polymorphism altered amino acid substitution at site 631 of Mx protein of Asparagine (related to viral resistance) by Serine (lack of potent for viral resistance).

Keywords: Exon 14, indigenous chickens, Mx gene, nucleotide polymorphism, Vietnam.

Nghiên cứu tính đa hình Exon 14, gen MX ở một số giống gà bản địa của Việt Nam

TÓM TẮT

Nghiên cứu tính đa hình của exon 14, gen Mx được thực hiện trên 7 giống gà bản địa của Việt Nam (bao gồm: gà Ác, Đông Tảo, H'Mông, Hồ, Mia, Móng và Ri). Kết quả cho thấy tính đa hình của exon 14 xảy ra tại hai vị trí (2032 và 2159). Sự thay thế A hoặc G tại vị trí 2032 của gen Mx có ở tất cả 7 giống gà bản địa được sử dụng trong nghiên cứu này. Trong cùng một giống đều có cả hai alen A và G, tuy nhiên, alen A (liên quan đến tinh kháng virus) có tần số xuất hiện cao hơn so với alen G (liên quan đến tính mẫn cảm với virus). Đặc biệt, tần số của alen A ở giống gà Ri và gà H'mong là cao nhất trong số 7 giống được kiểm tra (với tỉ lệ tương ứng là 80,91% và 74,04%). Phân tích so sánh các axit amin cho thấy sự thay đổi nucleotide A hoặc G tại vị trí 2032 sẽ dẫn đến sự thay đổi axit amin tại vị trí 631 của protein Mx từ Asparagine (liên quan tới khả năng kháng virus của giống) sang Serine (không liên quan tới khả năng kháng virus của giống).

Từ khóa: Đa hình nucleotide, Exon 14, gen Mx, giống gà bản địa, Việt Nam.

1. INTRODUCTION

Mx proteins, part of the dynamin family of large GTPases, interfere with the replication of RNA viruses by inhibiting trafficking or activity of viral polymerases. Mx gene of Gallus gallus located on chromosome 14, comprising intron- exon regions, for approximately 21 kb in length. Under the simulation of interferon, Mx protein (a product of Mx mRNA) is synthesized, predominantly presents in cytoplasm. During the splicing process, intron regions in Mx gene are removed and left Mx mRNA with the total length of 2545 nucleotides. Of which, the first 140 nucleotides and region from nucleotide 2259th to 2545th are 5' and 3' un-translated region, respectively. Thus, the protein

coding region of Mx mRNA contains 2118 nucleotides, spanning from nucleotide 141th to 2258th (the nucleotide position is based on sequence Z23168 from GenBank).

There have been several reports regarding genetic polymorphism and antiviral activity against avian influenza virus (Ko et al., 2002, Sironi et al., 2008, Berlin et al., 2008, Seyama et al., 2006). The study on Mx cDNA of different chicken breeds (Ko et al., 2002) showed that in many natural variations of chicken Mx gene, only the mutation of S631N (serine to asparagine), which was caused by a single nucleotide substitution in 2032 position, had antiviral activity. The Mx protein had activity only when nucleotide G took place of A in 2032th that led the 631st amino acid changed from serine to asparagine (Ko et al., 2002, Seyama et al., 2006).

As Vietnam has rich resources of local chicken breeds, some of them are known having a certain level of infectious disease resistance. Knowledge of the single-nucleotide polymorphism in exon 14 of the chicken Mx gene will facilitate further researches on the resistant activity of Mx gene and its use for breeding of influenza virus resistant chicken varieties. This study aimed to investigate the exon 14 polymorphisms of the Mx gene in some representative indigenous chicken breeds collected from different locals in Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

A total of 783 samples in 7 breeds was collected from different provinces of Vietnam. They are Ac, Dong Tao, H'mong, Ho, Mia, Mong and Ri breeds. Information of indigenous chicken breeds used in this study is shown in Table 1.

2.2. DNA extraction

Genomic DNA was extracted from the chicken venous blood by using the Nucleospin Tissue Kit (Macherey Nagel, Germany) according to the manufacturer's protocol. The quality of extracted DNA was checked by agarose electrophoresis, stained with EtBr, and visualized under UV. The concentration of extracted DNA was then measured by UV-VIS spectrophotometer (Nanodrop, USA).

2.3. Primer design and PCR protocol

The specific primers(E14F1: 5' CCGTGTTTTAATAGTGCACTGTCACCT 3', E14R1: 5' CAGCTGAAGGCTCCCCCTCCTT 3') were designed to amplified the entire region ofexon 14 and two neighboring regions based on Blast Nucleotide of available Mx sequences in GenBank. The PCR product was amplified by using DreamTaq™ Green DNA Polymerase kit (Fermentas) in Eppendorf MasterCycler. PCR reaction master-mix was a 10X PCR buffer 2µl, 10 µmol/l dNTP 2 µl, 10 pmol/µl primers 1 µl, 5U/µl DreamTaq 0,5 µl, DNA template (100 ng/µl) 1 µl and ultra pure distilled water up to 20 µl. The thermal cycle was carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturizing at 94°C for 45 sec, annealing at 59°C for 60 sec, and extension at 72°C for 60 sec, with a final extension at 72°C for 5 min. Subsequently, 10 µL of PCR products were visualized on 1.0% agarose gel staining with ethidium bromide under UV light.

Table 1. Information of indigenous chicken breeds used in this study

Indigenous breeds	Number of individual/breed	Place of sample collection
Ac	120	National Institute of Animal Husbandry
Dong Tao	107	Khoai Chau, Hung Yen
H'mong	104	National Institute of Animal Husbandry and Yen Bai
Ho	120	Thuan Thanh, Bac Ninh
Mia	105	Son Tay, Ha Noi
Mong	117	Duy Tien, Ha Nam
Ri	110	Soc Son, Ha Noi

2.4. Sequencing of PCR product

The PCR products were purified with the QIAquick Gel extraction kit (Qiagen, Germany) according to the recommended protocol. The purified product was sequenced with specific primers. To minimize the errors, the sequencing reaction was done in both forward and reverse directions.

2.5. Exon 14 sequence analysis

The chromatograms of exon 14 sequencing were analyzed using BioEdit and DNAstar program. The nucleotide identity of the exon 14 sequence of the Vietnamese chicken breeds in comparison with other sequences were performed using Megablast tool at http://blast.ncbi.nlm.nih.gov and using the information of published sequence Mx-Chick ENSGALT00000025999 as reference.

3. RESULTS

3.1. Nucleotide polymorphism of exon 14

In this analysis, only 234 bp of coding region was analyzed (3' un-translated region was excluded). The sequence alignment of exon 14 Mx gene of 7 chicken breeds was shown in Figure 1 in which all identity sequences of the same breed were eliminated from the alignment. It was obvious that (i) exon 14 of 7 breeds used in this study highly conserved, and (ii) substitutions could be observed at the two positions of 2032th and 2159th. Sequences of exon 14 of total 7 breeds used in this study were further compared to available sequences in GenBank. Megablast search revealed an extreme high level of similarity (over 98%) between 7 Vietnamese indigenous chicken breeds with the others published on Genbank (not shown). At closer inspection (Figure 2), the chromatograms of every samples showed only single peak (A or G) at site 2032th which relatedto antiviral resistance. The polymorphism of allele A/G at site 2032th of the Mx gene was summarized in Table 2 and showed that the number of resistant allele A reached maximum in Ri breed, followed by H'mong breed with the rate of 80.91% and 74.04%, respectively. That result partially reflected that the Ri, H'mong and Dong Tao breeds expressed better resistance than others while Mia breed had highest rates of infection and died when the avian influenza outbreaks swept over in 2009.

3.2. Amino acid polymorphism of exon 14

In line with the nucleotide alignment (Figure 1), the alignment of amino acid sequences between investigated 7 indigenous chicken breeds (Figure 3) showed high level of sequence conservation. It was shown that point mutation at the site 2032th was nonsynonymous substitution which led to amino acid alteration S631N as reported elsewhere (Ko et al., 2002). The mutation G/A at site 2159th which was seen in DongTao-41f2 was synonymous substitution.

Table 2. Summary of nucleotide polymorphism at site 2032th (exon 14, Mx gene) of 7 indigenous chicken breeds

Indigenous breeds	Number of sequencing samples	Number of samples having			
		2032-A	Rate (%)	2032-G	Rate (%)
Ac	120	76	63.33	44	36.67
Dong Tao	107	77	71.96	30	28.04
H'mong	104	77	74.04	28	26.92
Ho	120	79	65.83	41	34.17
Mia	105	53	50.48	52	49.52
Mong	117	82	70.09	35	29.91
Ri	110	89	80.91	19	17.27

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                2030      2040      2050      2060      2070      2080      2090      2100      2110      2120      2130      2140
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....
Z23168          GGAGCAAGTAAACGCCTGAGCAATCAGATTCCTCTGATCATCCTCTCTACTGTCCTTCATGACTTTGGAAATTATTTGCAGACCTCAATGTTGCATCTCTTGCAAGGAAAAGAAGAAATA
Ac-26f1         .......A..................................................................................................................
Ac-28r14        ........................................................................................................................
DongTao-41f2    .......A..................................................................................................................
DongTao-42r8    ........................................................................................................................
Hmong-34f14    ........................................................................................................................
Hmong-35f17    .......A..................................................................................................................
Ho-16r3        .......A..................................................................................................................
Ho-20r20       ........................................................................................................................
Mia-14f13      .......A..................................................................................................................
Mia-15r20      ........................................................................................................................
Mong-03f6     .......A..................................................................................................................
Mong-05f13    ........................................................................................................................
Ri-40f17       .......A..................................................................................................................
Ri-36f1        ........................................................................................................................

                2150      2160      2170      2180      2190      2200      2210      2220      2230      2240      2250
                |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Z23168          AACTATTTACTCCAGGAAGATCATGAAGCTGCTAACCAGCAGAAGTTACTGACCAGCAGAATTAGTCACCTCAACAAAGCCTACCAATACCTGGTAGACTTTAAGTCTCTGTAG
Ac-26f1         ........................................................................................................................
Ac-28r14        ........................................................................................................................
DongTao-41f2    ............A...........................................................................................................
DongTao-42r8    ........................................................................................................................
Hmong-34f14    ........................................................................................................................
Hmong-35f17    ........................................................................................................................
Ho-16r3        ........................................................................................................................
Ho-20r20       ........................................................................................................................
Mia-14f13      ........................................................................................................................
Mia-15r20      ........................................................................................................................
Mong-03f6     ........................................................................................................................
Mong-05f13    ........................................................................................................................
Ri-40f17       ........................................................................................................................
Ri-36f1        ........................................................................................................................
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Figure 1. Alignment of exon 14, Mx gene of 7 indigenous chicken breeds. The A/G polymorphism at site 2032th could be seen in all breeds

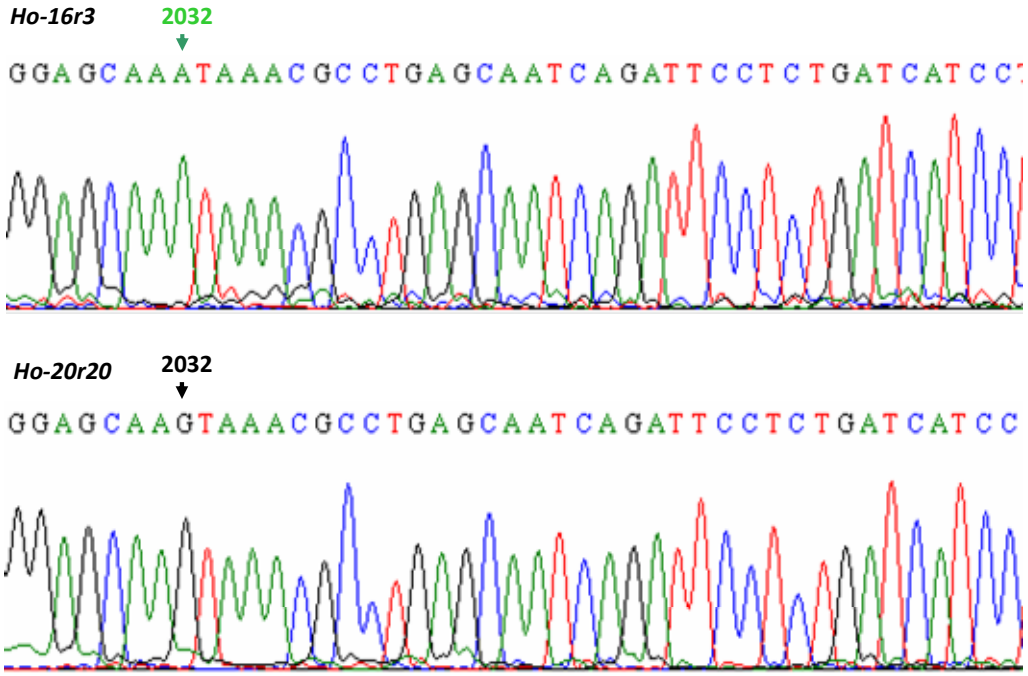

Figure 2. Chromatograms of partial exon 14 sequences of Ho breed. At polymorphism position (site 2032th), only single peak A or G was observed (arrows).

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                630       640       650       660       670       680       690       700
                .|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
Z23168          GASKRLSNQIPLIILSTVLHDFGNYLQTSMLHLLQGKEEINYLLQEDHEAANQQKLLTSRISHLNKAYQYLVDFKSL*
Ac-26f1         ..N.............................................................................*
Ac-28r14        ................................................................................*
DongTao-41f2    ..N.............................................................................*
DongTao-42r8    ................................................................................*
Hmong-34f14     ................................................................................*
Hmong-35f17     ..N.............................................................................*
Ho-16r3         ..N.............................................................................*
Ho-20r20        ................................................................................*
Mia-14f13       ..N.............................................................................*
Mia-15r20       ................................................................................*
Mong-03f6       ..N.............................................................................*
Mong-05f13      ................................................................................*
Ri-40f17        ..N.............................................................................*
Ri-36f1         ................................................................................*
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Figure 3. Alignment of deduced amino acid of exon 14 of 7 indigenous chicken breeds. Non-synonymous substitution from AAT to AGT induced changes of encoded amino acid N (Asn) to S (Ser) at site 631. Asterics represent stop codons.

4. DISCUSSION

In the current study, a total of 783 individuals of 7 Vietnamese indigenous chicken breeds was sequenced. Of the all, the high level of sequence homology could be observed between breeds. The A/G polymorphisms at the site 2032th were observed in all of 7 breeds in the analysis. In the previous studies, (i) the difference in the Mx allele frequency in the same chicken lines (Sironi et al., 2008) and (ii) the low frequency of the Mx allele for viral resistance (Balkissoon et al., 2007) were confirmed. This study was also reflected the dissimilarity in A/G allele frequency between 7 indigenous chicken breeds and allele A was at higher frequency in comparing to allele G. However, there was somewhat of small sample size in order to accurately estimate allele frequencies having a potency of susceptibility/resistance to virus infection of 7 breeds in this study. We also recommended that it should be extended for full Mx mRNA sequencing to get a clear interpretation of why those indigenous chicken breeds could show a certain level of resistance against certain pathogens in general and influenza virus in particular.

5. CONCLUSION

This study aimed at determining the polymorphisms of exon 14 of the Mx gene in 7 indigenous chicken breed from different provinces in Vietnam. The analysis of exon 14 sequences revealed two polymorphic positions of 2032th and 2159th. The frequencies of resistant allele 2032-A was found at higher frequencies in compare to the susceptible allele 2032-G in all of investigated breeds.

ACKNOWLEDGMENTS

We are grateful for the scientific expertise provided by Anne Cornet. We also thank Trinh Thi Thu Thuy and Ngo Thu Ha for excellent technical skills and enthusiasm.

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