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Genetic variations of mitochondrial antiviral signaling gene (*MAVS*) in domestic chickens

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ABSTRACT

Mitochondrial antiviral signaling (*MAVS*) gene plays a key role in antiviral regulation in mammals potentially by activating IRF3/7 and NF-κB and leading to the induction of type I interferon (IFN)-mediated antiviral and inflammatory responses. In this study, we screened genetic polymorphisms of the *MAVS* gene in various Chinese domestic chicken breeds/populations and evaluated its potential effect on gene expression. Among the sequenced fragment (4678 bp), a total of 75 single nucleotide polymorphisms (SNPs) were identified in 46 chickens from 10 breeds/populations, including 30 coding SNPs and 45 non-coding SNPs. Extremely high haplotype diversity (37 nucleotide haplotypes, 18 amino acid haplotypes) was observed in the coding region (CDS), and a similar pattern of high polymorphisms was also observed for the 3′-untranslated region (3′-UTR). Luciferase assays of two representative 3′-UTR haplotypes were performed in both HEK293 cells and DF-1 chicken fibroblast cells, and we found that they were differentially associated with different abilities on regulating mRNA expression level ($P < 0.05$). Collectively, we observed a considerably high genetic variability of the *MAVS* gene, and the 3′-UTR variants had an ability to regulate mRNA expression. These results would cast some clues on understanding the potential role of *MAVS* on viral resistance in chicken.

1. Introduction

Over the past years, the incidence and prevalence of infectious diseases have become an important factor to hinder the rapid development of animal husbandry in China. Avian viral infectious disease is one of the most important diseases that restricts the development of poultry industry, and causes huge economic losses. The innate immune system is the first line of defense against viral infection and of key importance early in viral infections. Knowledge of the innate immune recognition of avian viral infection is crucial to protect against viruses. The recognition of viruses by host cells is mediated by pathogen recognition receptors (PRRs) sensing virus-specific pathogen-associated molecular pattern (PAMP) (Liniger et al., 2012). PRR activation results in type I interferon (IFN) induction, cytokine secretion and the activation of antigen-presenting cells promoting adaptive immune responses (Takeuchi and Akira, 2007, 2009). Three classes of PRRs have been recognized, including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). The RLRs family members are conserved among vertebrates and contained three members: RIG-I, MDA5 (melanoma differentiation-associated gene 5) and LGP2 (laboratory of genetics and physiology 2) (Kawai and Akira, 2007; Ting et al., 2008; Yoneyama and Fujita, 2008). RIG-I and MDA5 interact with mitochondrial antiviral signaling gene (MAVS, also known as IPS-1, Cardif or VISA) via CARD–CARD domains at their N-terminus (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005), which is essential for the activation of downstream NF-κB and IRF3/7 signaling pathway (West et al., 2011). MAVS not only plays a central role in antiviral response and inflammatory responses, but also regulates other pathways and processes such as apoptosis, mitochondrial dynamics and autophagy (Belgnaoui et al., 2011). Though MAVS function has been extensively studied, its genetic variation information was seldom analyzed. Some SNPs in human *MAVS* gene were identified as a risk factor for systemic lupus erythematosus (Liu et al., 2011; Pothlichet et al., 2011), and SNPs of grass carp *MAVS* gene were reported to be associated with the susceptibility/resistance to RNA virus infection (Wan et al., 2013).

Abbreviations: MAVS, mitochondrial antiviral signaling gene; IFN, type I interferon; SNPs, single nucleotide polymorphisms; CDS, coding region; UTR, untranslated region; PRRs, pathogen recognition receptors; PAMPs, pathogen-associated molecular patterns; TLRs, toll-like receptors; RLRs, retinoic acid-inducible gene I like receptors; NLRs, nucleotide-binding oligomerization domain like receptors; *RIG-I*, retinoic acid-inducible gene I; *MDA5*, melanoma differentiation-associated gene 5; *LGP2*, laboratory of genetics and physiology 2; AIV, avian influenza virus; *MHC*, major histocompatibility complex; *Mx*, myxovirus resistance gene; RBPs, RNA binding proteins; miRNA, microRNA.

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Similar to mammals, several PRRs have been identified in chicken, but the RIG-I homologue is absent in chicken (Barber et al., 2010; Karpala et al., 2011). The lack of RIG-I in chicken may result in increasing susceptibility to AIV compared with duck (Barber et al., 2010). Recently, chicken MDA5 (chMDA5) was believed to be chicken PRR responsible for avian influenza virus (AIV) infection (Karpala et al., 2011). During this process, chicken MAVS (chMAVS) is essential to mediate chMDA5-dependent type I IFN production (Liniger et al., 2012). In view of the importance of chMAVS, it is necessary to analyze its genetic variations and to test whether these polymorphisms can be used as markers for selective breeding. In this study, we found a high level of genetic variation of the *MAVS* gene in chickens from different breeds/populations and 3′-UTR region variants of this gene have the ability to regulate mRNA expression.

2. Materials and methods

2.1. Sample collection

Forty-six blood samples were collected from local conservation farms in Sichuan, Guangxi and Guangdong provinces (Table 1). The protocol was approved by the Committee on the Care and Use of Laboratory Animals of the State-level Animal Experimental Teaching Demonstration Center of Sichuan Agricultural University and the institutional review board of Kunming Institute of Zoology. Samples were stored at −20 °C upon further processing.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA from blood samples was extracted by using the standard phenol/chloroform method. Eight primer pairs were designed to cover the entire *MAVS* gene region (Table 2) according to genomic sequence of red jungle fowl (*Gallus gallus*, GenBank accession number: NC_006091.3). PCR amplification was performed in a volume of 50 μL reaction mixture containing 50–100 ng of DNA, 400 μM dNTP, 0.2 μM of each forward and reverse primer, 1× LA Taq™ PCR buffer, 2.5 units of LA Taq polymerase (TaKaRa, China). We used the following PCR conditions: one denaturation cycle at 95 °C for 2 min, 35 cycles of 94 °C for 30 s, appropriate annealing temperature (Table 2) for 30 s, and 72 °C for 1 min, followed by a final extension cycle at 72 °C for 10 min. PCR products were purified on spin columns (Tiangen Biotech Co., China) and were directly sequenced by using PCR primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) on a 3730XL DNA sequencer (Applied Biosystems) at the Kunming Biodiversity Large-Apparatus Regional Center, Kunming Institute of Zoology.

2.3. Sequence data analysis

Sequences were edited and aligned by DNAstar software (DNAstar Inc. Madison, WI, USA). Sequence variations, composition and variable sites were identified using MEGA5.0 (Tamura et al., 2011). Haplotypes were reconstructed by using Phase v2.1.1 (Li and Stephens, 2003) with default options except that the program was run five times and the last iteration was 10 times longer, as suggested by the instruction. We constructed a phylogenetic tree of the inferred 3′-UTR haplotypes based on the maximum likelihood (ML) method by using MEGA5.0. For estimating nucleotide diversity (π) and for neutrality tests (*Tajima's* D (Tajima, 1989) and *Fu's* Fs test (Fu, 1997)), all polymorphisms were considered and DnaSP V5 (Librado and Rozas, 2009) was used.

2.4. Plasmid construction

To confirm whether different haplotypes of the 3′-UTR in *chMAVS* gene have a potential function, we selected two representative haplotypes based on the clustering pattern of the ML tree. Haplotype 3h.18, which bears characteristic motif of haplotypes belonging to clade C1, and haplotype 3h.22, representative for haplotypes belonging to clade C2, were selected for luciferase assay. The two *chMAVS* 3′-UTR haplotypes were amplified by primer pair 5′-CCG**CTCGAG**CAGTAGAGAGAAGATGAG-3′/5′-ATAAGAAT**GCGGCCGC**GTGTGGCAAACATGTTT-3 (the endonuclease restriction enzyme sites are marked in bold and underlined). The amplified fragment has a length of 2666 bp. PCR fragments were digested by *Xho* I and *Not* I. After gel purification, digested products were ligated into pGL3-promoter luciferase vector (Promega, USA). All inserts were confirmed by direct sequencing.

2.5. Cell culture, transient transfection and luciferase reporter assay

HEK293 cells and DF-1 chicken fibroblast cells were bought from the Kunming Cell Bank, Kunming Institute of Zoology, which was initially introduced from ATCC. Cells were cultured in DMEM medium with 10% FBS (Invitrogen, USA) at 37 °C in 5% CO_2. Cells (1×10^4) were seeded in 24-well plates and grown overnight. Cells were co-transfected with 500 ng of each reporter vector (3h.18 and 3h.22) and 50 ng of Renilla luciferase reporter pRL-TK (Promega, USA). Plasmids were transfected using Lipofectamine 2000 (Invitrogen, USA). After 48 h, cells were harvested in 100 μL passive lysis buffer (Promega, USA) for 1 h and were stored at −80 °C or were immediately detected for luciferase activity by using the Dual-Luciferase Reporter Assay System (Promega, USA) on an Infinite M1000 Pro multimode microplate reader (Tecan, Switzerland). Unpaired Student's t-test was used to quantify potential statistical significance between reporter vectors with inserts of haplotypes 3h.18 and 3h.22 by using GraphPad software (GraphPad software, La Jolla, CA, USA). A *P* value less than 0.05 was considered statistically significant.

3. Results

3.1. Genetic variation of the chMAVS gene

We sequenced 46 chickens for the entire CDS region and 22 chickens for the 3′-UTR region of the *chMAVS* gene. A total of 75 SNPs were

Table 1
Sample information and nucleotide haplotype distribution of the *chMAVS* CDS region in this study.

ID	Breed	Location	Number of samples	CDS nucleotide haplotype distribution (number)
1	Arbor Acres Broilers	Guangdong	3	h1 (1), h18 (2), h32 (1), h35 (2)
2	Caoke Chicken	Sichuan	6	h1 (1), h8 (1), h11 (1), h17 (1), h18 (3), h28 (2), h29 (1), h33 (1), h34 (1)
3	Erlang Mountainous Chicken	Sichuan	4	h1 (2), h15 (1), h18 (1), h21 (1), h28 (1), h30 (1), h33 (1)
4	Huiyang Bearded Chicken	Guangdong	2	h1 (3), h3 (1)
5	Jiuyuan Black Chicken	Sichuan	3	h18 (2), h21 (1), h22 (1), h24 (1), h28 (1)
6	Lingnanhuang Dwarf Chicken	Guangdong	7	h1 (4), h2 (2), h5 (1), h9 (3), h16 (1), h18 (3)
7	Qingyuan Ma Chicken	Guangdong	4	h1 (1), h2 (1), h6 (1), h13 (1), h14 (1), h18 (2), h29 (1)
8	Silkies	Sichuan	7	h1 (2), h5 (1), h18 (5), h20 (1), h26 (2), h27 (1), h32 (1), h33 (1)
9	Tibetan Chicken	Sichuan	4	h4 (1), h18 (1), h19 (1), h21 (1), h22 (1), h23 (1), h26 (1), h37 (1)
10	Xiayan Chicken	Guangxi	4	h1 (1), h7 (1), h18 (1), h25 (1), h31 (2), h32 (1), h36 (1)
11	Xinghua Chicken	Guangdong	2	h1 (1), h10 (1), h12 (1), h14 (1)
Total	–	–	46	–

Table 2
Primer and PCR conditions for amplifying and sequencing all 6 exons of the *MAVS* gene in chickens.

Region	Primer name	Sequence (5′–3′)	Production (bp)	Anneal temperature (°C)
Exons 1 & 2	MAVS1-1F	CATGTGAGATTTCCCGTGTG	1201	62
	MAVS2-1R	TGAGAGAAACAGGCACCCAA		
Exon 3	MAVS3-1F	GACTCCTTGGTTTTCCTGGG	341	56
	MAVS3-2R	AACACCAACCCTCCAGCCTA		
Exon 4	MAVS4-1F	GTGGGGCTGCATGGTGATT	631	65
	MAVS4-3R	CTCCATCAGCACCAGCTACA		
Exon 5	MAVS5-1F	AGTTCTGGCTTATGCAGCGT	233	56
	MAVS5-1R	CCTGAAGGAAATGCAACTGA		
Exon 6	MAVS6-1F	GTTAGTGGCTGGAGGAAATG	718	54
	MAVS6-5R	AATCAGAGCGATGCCAACAG		
	MAVS6-5F	CATGTGGAGCATCACCCCAG	1034	54
	MAVS6-11R	TAGCACTGGTGTATGGCCTC		
	MAVS6-11F	TACCAGCATGAGCCAGACTT	1235	55
	MAVS6-19R	ACATGACTCAACAACCTGGG		
	MAVS6-19F	GCACTCCTCTGACAGCTCTG	1016	55
	MAVS6-24R	ATAAATGCCCGATAGTCACT		

detected, including 30 SNPs in CDS region (1926 bp), 2 SNPs in 5′-UTR region (146 bp) and 43 SNPs in the long 3′-UTR region (2606 bp). Based on the CDS region of 46 chickens, the nucleotide diversity and haplotype diversity were 0.00316 ± 0.00019 (π) and 1.000 ± 0.006 (*h*), respectively. The nucleotide diversity (0.00471 ± 0.00021) and haplotype diversity (0.989 ± 0.0001) estimated based on the *chMAVS* 3′-UTR of 22 chickens yielded a similar level of diversity.

A total of 37 haplotypes (h1–h37) were reconstructed on the basis of 30 SNPs in the CDS region (Fig. 1A). The network profile of these 37 haplotypes presented a dual core pattern, with haplotypes h1 (17.4%) and h18 (21.7%) being located in the respective central locations. However, haplotype h18 had 7-mutation distance to haplotype h1. The remaining haplotypes had a low frequency and were shared by no more than 5 samples; all of them were radiated from the central location with a star-like pattern (Fig. 1B). Among the 30 coding SNPs, 13 SNPs caused amino acid changes. We subsequently defined 18 haplotypes based on the amino acid variation (H1–H18, Fig. 2A). Similar to nucleotide haplotypes, several main haplotypes were identified, such as H5 (30.4%), followed by haplotypes H1 (19.6%) and H6 (9.8%). The network profile of amino acid sequence haplotypes was in general consistent with the

A

```
                 111111111 1111111111
     112334556 8022333333 3455577889
     268775027 2148111355 9601114122
    9953587125 5677678257 2395651670
h1   CCAACCCGGC GTGAGTGCCA CCCGACGGAG   16
h2   .......... .......... .T........    3
h3   .......... ........T. ..........    1
h4   .......... .....CA... ....G.....    1
h5   .......... .....CA... .T........    2
h6   .......... .....CA... T....T....    1
h7   .......... ....AC.... .T........    1
h8   .......... ...G.CA... .TTA......    1
h9   .......... A....CA... T....T....    3
h10  .......... A....CA... T....T..G.    1
h11  .......... A..G.CA... .TTA......    1
h12  .......... AC.G.CA... .T........    1
h13  ........A. A....CA... T....T....    1
h14  .......A.. .....C.... .T........    2
h15  .......A.. .....CA... .T........    1
h16  .......A.. ...G.CA... .T....A...    1
h17  .......A.. ...G.CA... .TT.......    1
h18  .......A.. ...G.CA... .TTA......   20
h19  .......A.. ...G.CA... .TTA.....A    1
h20  .......A.. ...G.CA... .TTA...A..    1
h21  .......A.. ..AG.CA... .TTA......    3
h22  .......A.. ..AG.CA... .TTA.....A    2
h23  .......A.. A.....A... .T..G..A..    1
h24  ......T... .....CA... ..........    1
h25  ......T... .....CA... ...A......    1
h26  ......T... ..A..CA... ..........    3
h27  .....T.A.. ...G.CA... .TTA......    1
h28  ....T..... A....CA... T....T....    4
h29  ..G....... .......... ..........    2
h30  ..G....... ..A..CA... ..........    1
h31  ..G.....AT .......... .T........    2
h32  ..G....A.. A..G.CA... ..........    3
h33  ..G...T... ..A..CA... ..........    3
h34  ..GG...... ..A....T.. ....G....A    1
h35  .TG.....A. A.....A... ..........    2
h36  .TG.....A. A....CA..G ..........    1
h37  T......A.. ...G.CA... .TTA...A..    1
h38  ...TG..... .......... ..........   reported
```

B

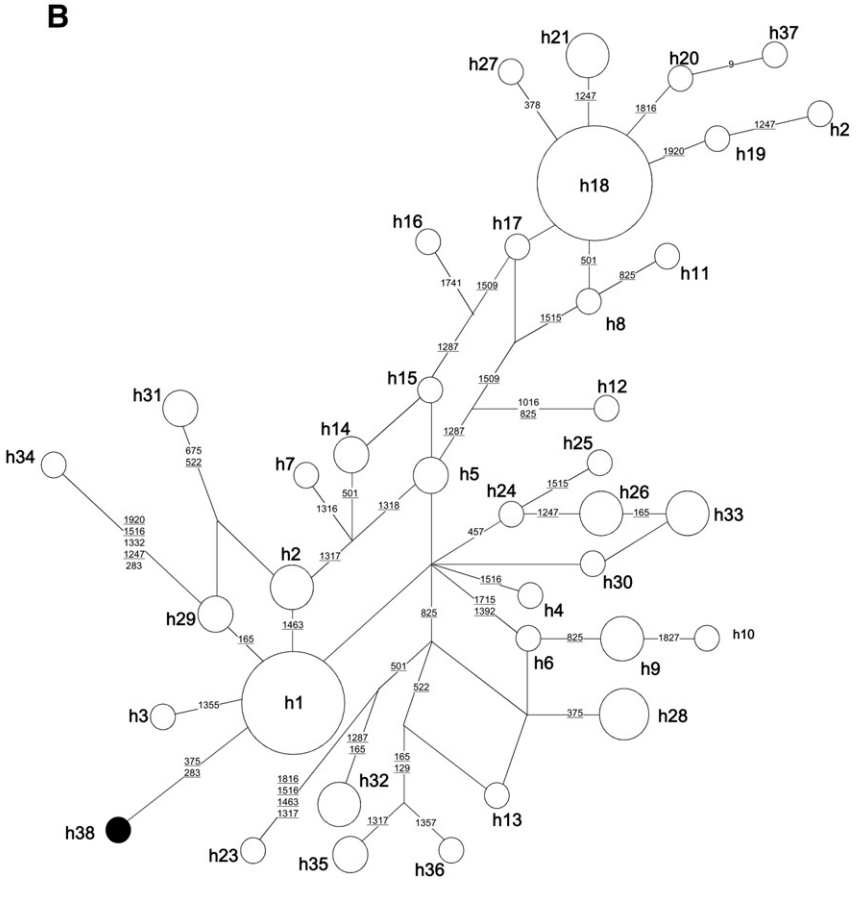

Fig. 1. Sequence variations (A) and network profile (B) of *chMAVS* coding region (1926 bp) of 46 chickens from South and Southwest China, and one reported red jungle fowl outside China. (A) The number of individuals sharing the same haplotype was listed in the right column. Dots (·) denote identical sites. The *MAVS* genomic sequence of red jungle fowl (GenBank accession number: NC_006091.3, marked with "reported") was also included for comparison. (B) The links are labeled by nucleotide positions to designate variations. The order of the variants on a branch is arbitrary. Each circle represents a haplotype, with the area of a circle being proportional to the frequency of the haplotype.

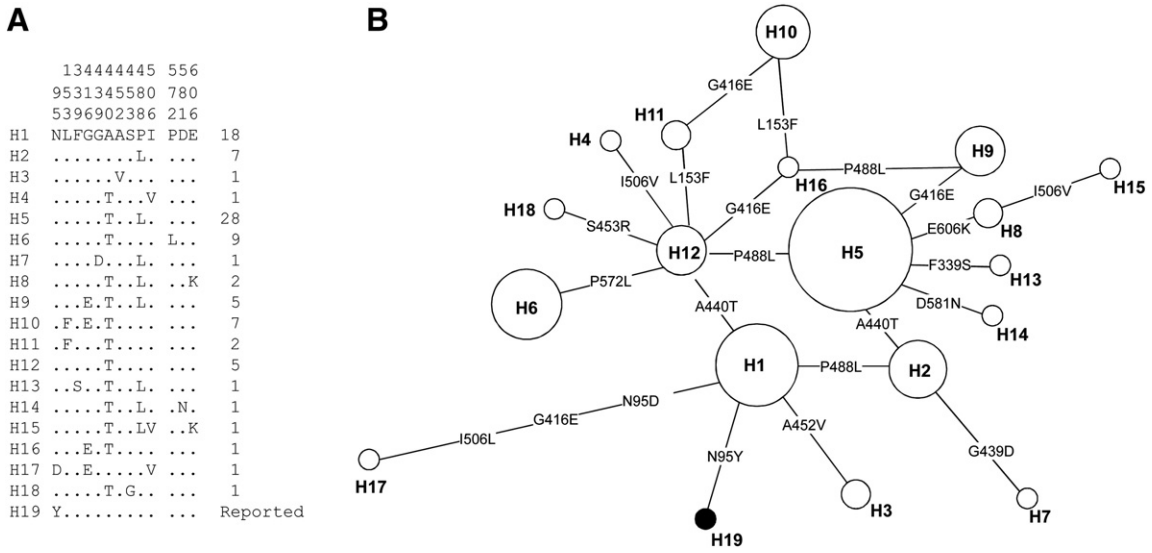

Fig. 2. Amino acid changes (A) and network profile (B) of the predicted chMAVS protein (642 amino acids) of 46 chickens sequenced in this study. (A) Dots (·) denote identical sites. The red jungle fowl (GenBank accession number: NC_006091.3, marked with "reported") was also included for comparison. The number of individuals sharing the same haplotype was listed in the right column. (B) The links are labeled by amino acid changes. The order of the variations on a branch is arbitrary. Each circle represents a haplotype, with the area of the circle being proportional to frequency of the haplotype.

pattern of nucleotide sequence haplotypes (Fig. 2B), with several central nodes.

Under the neutral null model, the expected value of the D statistics is zero, whereas directional selection would cause a negative D-value and balancing selection would lead to a positive value (Tajima, 1989). We found a negative *Tajima's* D value (Tajima, 1989) and *Fu's* Fs test (Fu, 1997) for the whole chicken sample (*Tajima's* D value = −0.54; *Fu's* Fs test, −46.358), although the *P*-values were not significant ($P > 0.05$).

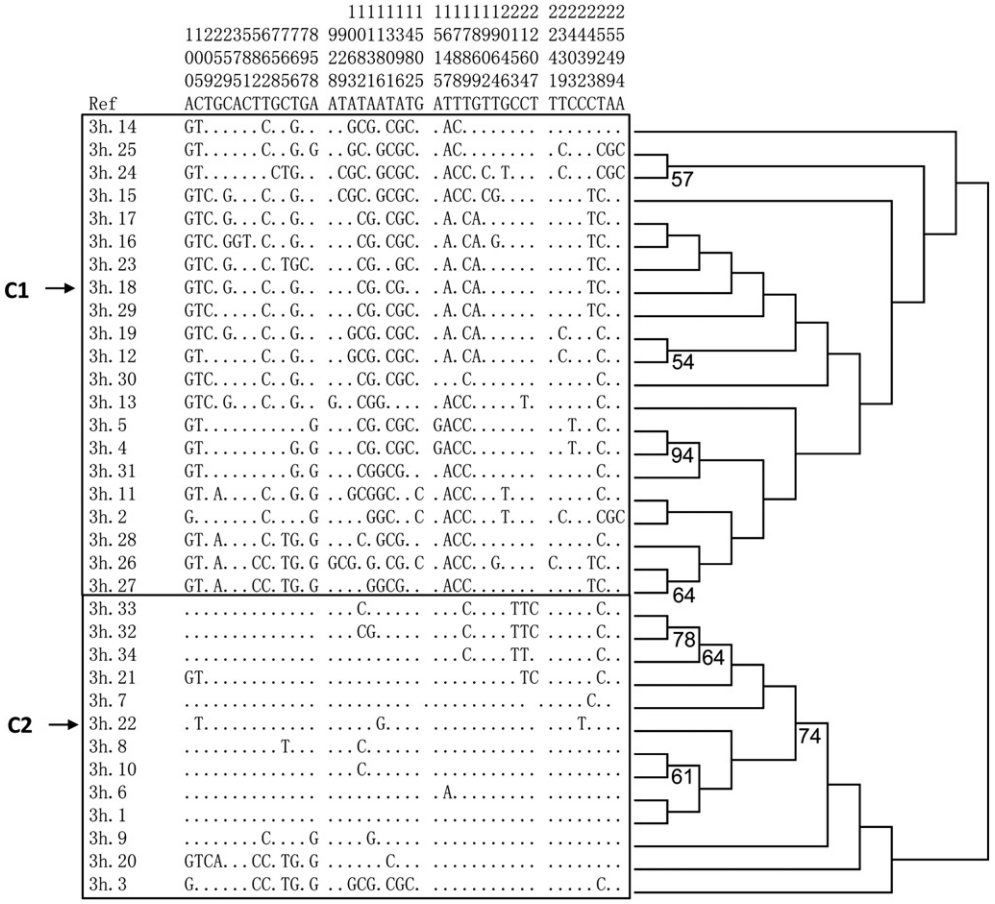

Fig. 3. Phylogenetic tree of 34 *chMAVS* 3′-UTR haplotypes identified in 22 chickens. The haplotypes were estimated by Phase v2.1.1 (Li and Stephens, 2003) and the maximum likelihood tree was reconstructed by using MEGA5.0 (Tajima, 1989). Haplotype 3h.1 was used as the reference to score the sequence variation for each haplotype. Bootstrap support less than 50% was not marked on the tree.

The *chMAVS* 3′-UTR was highly polymorphic and defined 34 haplotypes. These haplotypes were clustered into two divergent clades in the ML tree (Fig. 3). Haplotypes in clade C1 ($\pi = 0.00425 \pm 0.00037$) had a slightly higher level of variation than those of clade C2 ($\pi = 0.00271 \pm 0.00050$).

3.2. Effect of the chMAVS 3′-UTR on luciferase expression

In order to investigate potential effects of different 3′-UTR haplotypes on the translation of chMAVS protein, each of the two *chMAVS* 3′-UTR sequences (haplotypes 3h.18 and 3h.22 as representatives of clades C1 and C2 in the ML tree, respectively) was fused in frame to firefly luciferase. The luciferase expression of the chimeric reporter gene could determine the effect of 3′-UTR haplotypes. As shown in Fig. 4A, both *chMAVS* 3′-UTR constructs could restrain the firefly luciferase expression in HEK 293 cells and chicken DF-1 fibroblast cells. This result indicated that *chMAVS* 3′-UTR was required to suppress the expression of the chMAVS protein. Moreover, the luciferase expression level of 3h.18 construct was 2.8-fold higher than that of 3h.22 construct in HEK293 cells. We found that the inhibition effect and the difference between the two 3′-UTR haplotypes were more remarkable in chicken DF-1 cells: luciferase expression level of 3h.18 construct had a 43-fold increase compared to that of 3h.22 construct (Fig. 4A). These results suggested that genetic changes at different loci of the 3′-UTR might be responsible for regulation of the chMAVS protein expression.

RNA structures play active roles in nearly every step of gene expression, including transcription, splicing, RNA localization, translation and RNA decay (Wan et al., 2011). We further analyzed the RNA secondary structure of haplotypes 3h.18 and 3h.22 by using the energy minimization program of Mfold web server (http://mfold.rna.albany.edu//?q=mfold/RNA-Folding-Form) (Zuker, 2003). Compared with haplotype 3h.18, variations in haplotype 3h.22 would induce four major branched changes (Fig. 4B). These changes altered local stem–loop structure, which could be of potential functional significance (Chen et al., 2006).

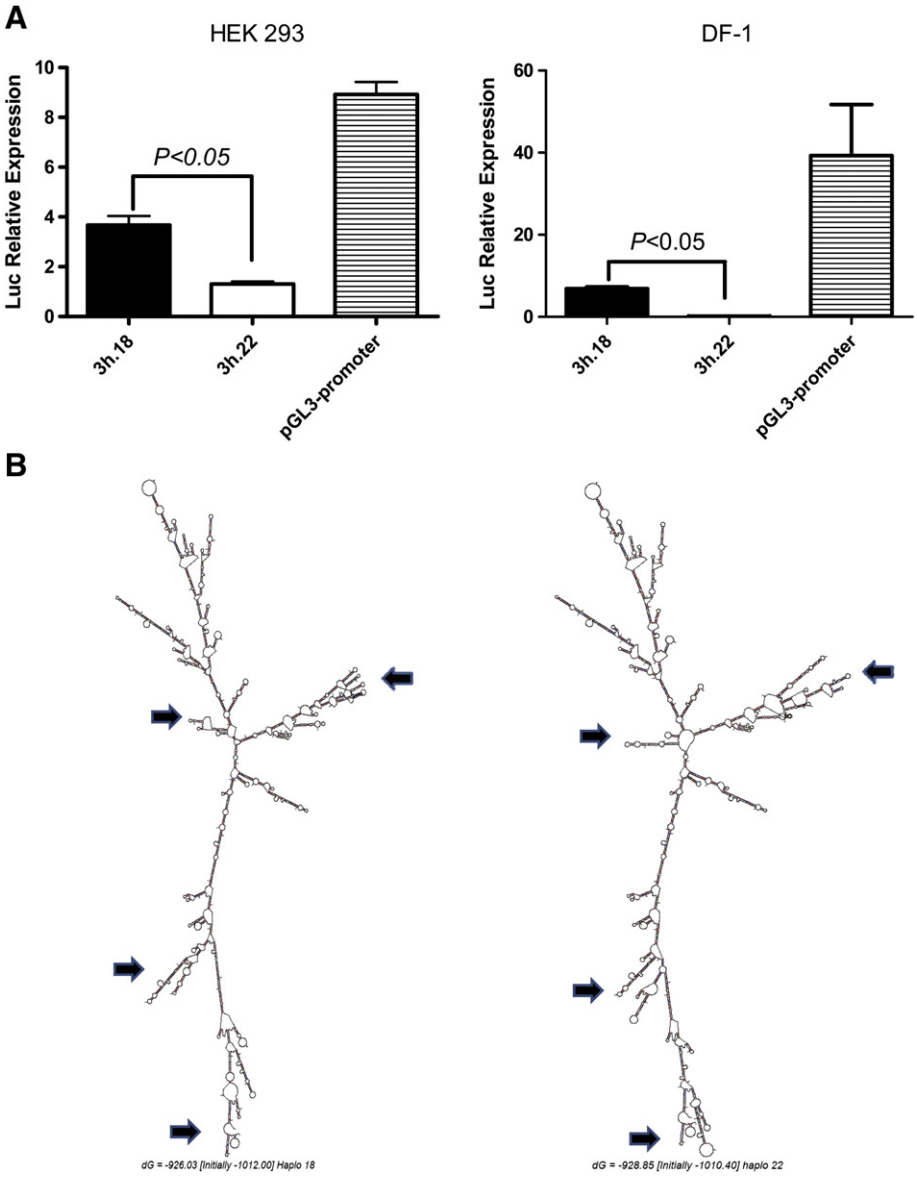

Fig. 4. Prediction of secondary change and functional characterization of two representative *chMAVS* 3′-UTR haplotypes. (A) HEK293 cells and DF-1 chicken fibroblast cells (1×10^4) were transfected with plasmid containing haplotype 3h.18 or 3h.22 (500 ng) and 50 ng pRL-TK (as inner control). Cells were harvested 48 h after transfection and luciferase assays were performed. A representative result of three experiments was shown. Graphs show mean ± SD, n = 3. (B) Predicted secondary structure of the 3′-UTR haplotypes 3h.18 and 3h.22 by using Mfold program. The regions with structure change introduced by different variants in haplotypes 3h.18 and 3h.22 were marked by arrows.

Furthermore, these variants occurred in the region with secondary structural changes. We speculated that these structural changes in the 3′-UTR region could potentially affect mRNA abundance.

4. Discussion

As the main source of meat and egg, chicken is one of most important farm animals with worldwide distribution (de et al., 2011). However, chicken becomes the carrier in the spread of some emerging infectious diseases, such as avian influenza, that can impact on human health. Along with the rapid development of modern poultry industry, the individual ability for disease resistance has significantly decreased (Swaggerty et al., 2009). Over the past decades, the intensive artificial selection has been applied to domestic chicken populations, which would largely decrease genetic variability. In fact, weak correlations (though both positive and negative) between production traits and the resistant allele have been observed in commercial lines (Livant et al., 2007). Therefore, to understand the genetic basis of disease susceptibility has important implications for breeding and intensive farming.

It is widely acknowledged that the immune system associated genes always have a higher diversity at non-synonymous relative to synonymous sites in mammals and *Drosophila* (Hughes et al., 2005; Schlenke and Begun, 2003). In chicken, polymorphisms of the innate immunity genes were previously reported to be associated with resistance or susceptibility to many diseases (Netea et al., 2012), including major histocompatibility complex (*MHC*) (Chaves et al., 2010; Eimes et al., 2010), myxovirus resistance gene (*Mx*) (Haller et al., 2009), and *TLR* gene (Ruan and Zheng, 2011; Ruan et al., 2012). As one critical innate immune gene by mediating RLR signal pathway, to understand the genetic variability of *MAVS* is significant for dissecting its biological implication against viral infection. In this study, we found that chicken *MAVS* gene has a considerably high genetic polymorphism among chickens from South and Southwest China. Furthermore, we observed a relatively high genetic divergence among different haplotypes (Figs. 1 and 3), which would suggest a high diversity of the chicken gene pool.

The classical viewpoint considered domestication as a single event potentially resulting in strong bottleneck, which was further followed by additional bottlenecks caused by breed formation and modern breeding (Clutton-Brock, 1987). Although chicken has multiple domestication events as we had previously found (Liu et al., 2006), intensive artificial selection would cast comprehensive determination on the present gene pool within these widely used populations for commercial production. Along with this process, the selection signal could be present for these essential genes connected with selection traits of interest. In this study, we tested that whether selection signature was present in *chMAVS* in these commercial lines, but we did not detect a significant signal as revealed by the *Tajima's* D and *Fu's* Fs tests ($P > 0.05$). Further analysis with more chicken samples should be carried out to confirm the pattern.

Previous *in silico* prediction analysis showed that 3′-UTR might contain AU-rich elements, RNA binding proteins (RBPs), microRNA (miRNA) and other potent motifs, which may have biological functions (Barreau et al., 2005; Cherry et al., 2008). We investigated the regulating effect of 3′-UTR of *chMAVS* on mRNA by choosing two representative 3′-UTR haplotypes (haplotypes 3h.18 and 3h.22) and performing firefly luciferase assay. Our results showed that both 3′-UTR haplotypes inhibited the expression levels of firefly luciferase in human and chicken cell lines, which would link to a normal physiology condition because excessive immunological and inflammatory responses might trigger many immunological diseases. Intriguingly, we found that expression levels of these two haplotypes were significantly different (Fig. 4). Furthermore, these 3′-UTR variants would cause a change of secondary structure. This result provided direct evidence for connecting the 3′-UTR genetic variations to potential biological function.

In conclusion, we detected a considerably high genetic variation of the *chMAVS* gene for both coding sequence and 3′-UTR region among chickens from different breeds/populations. We further showed that polymorphisms in the *chMAVS* 3′-UTR region might play a role on regulating chMAVS protein expression via luciferase assay. Taken together, our results would cast some clues on understanding the potential role of *MAVS* on viral resistance in chicken. Future study is essential for understanding the biological roles and molecular underpinning of the *MAVS* gene against viral infection in chicken.

Conflict of interest

The authors declare that they have no conflict of interest.

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