 DOI: 10.5114/ceji.2014.43715

Molecular cloning and sequencing analysis of the interferon receptor (IFNAR-1) from *Columba livia*

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Abstract

Objective: *Partial sequence cloning of interferon receptor (IFNAR-1) of Columba livia.*

Material and methods: *In order to obtain a certain length (630 bp) of gene, a pair of primers was designed according to the conserved nucleotide sequence of Gallus (EU477527.1) and Taeniopygia guttata (XM_002189232.1) IFNAR-1 gene fragment that was published by GenBank. Special primers were designed by the Race method to amplify the 3'terminal cDNA.*

Results: *The Columba livia IFNAR-1 displayed 88.5%, 80.5% and 73.8% nucleotide identity to Falco peregrinus, Gallus and Taeniopygia guttata, respectively. Phylogenetic analysis of the IFNAR1 gene showed that the relationship of Columba livia, Falco peregrinus and chicken had high homology.*

Conclusions: *We successfully obtained a Columba livia IFNAR-1 gene partial sequence. Analysis of the genetic tree showed that the relationship of Columba livia and Falco peregrinus IFNAR-1 had high homology. This result can be used as reference for further research and practical application.*

Key words: *Columba livia, IFNAR-1, gene cloning, gene sequence analysis.*

(Centr Eur J Immunol 2014; 39 (2): 159-164)

Introduction

Interferon was identified by Isaacs and Lindenmann during their seminal studies on virus interference 50 years ago. They reported that influenza virus-infected chick cells produced a secreted factor that transferred a virus-resistant state to previously uninfected cells. Importantly, the resistance was displayed against both the homologous inducing virus and heterologous viruses. The factor was designated interferon (IFN) because of its ability to interfere with virus growth [1]. Since then IFN has been implicated in a wide variety of biological functions, including anti-proliferative effects, induction of human leukocyte antigen (HLA), increase in natural killer (NK) activity and others [2, 3].

Interferon proteins are historically characterised as type I (α, β) or viral IFNs and type II (γ) or immune IFN [4]. Type I IFN induces antiviral, anti-proliferation, and immunomodulatory effects through interaction with specific receptor complexes on the surface of target cells, which consists of two chains: The high-affinity ligand-subunit (IFN-α, β receptor α-chain, IFNAR-1) and the unknown function subunit (IFN-α, β receptor β-chain, IFNAR-2) [5, 6]. Similarly to other IFN receptors, IFNAR-1 is a single transmembrane protein of the class II cytokine receptor family (CRF2), defined by structural similarities in the extracellular domain. Interferon receptor-mediated signalling leads to the activation of latent cytoplasmic factors, the signal transducers and activators of transcription (STAT) family of proteins, which are activated by a process that involves members of the Janus tyrosine kinase (JAK) family [7-9].

The objective of this study was to clone a sequence of *IFANR-1* gene and provide a basic understanding of the gene mutations between *Columba livia* and animals closely related to it through phylogenetic analysis. Physiological and biochemical property studies of the obtained IFANR-1 gene and protein were conducted to provide valuable reference for further research on the gene.

Material and methods

Sample collection and tissue preparation

Spleen samples were collected from *Columba livia.* The *Columba livia* was brought as a live, mature *Columba livia* from a common farm and kept under inspection for 5 days to be sure that it was free from any clinical infection, then the sample was harvested and stored at –20°C.

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Table 1. Conserved sequence amplification PCR and 3'RACE products using forward and reverse primer sequences

Primer name	Primer sequences
A	5'-GCTCAGATTGGTCCCCCTGA-3'
	5'-CAGGAGGGCCAATTTCATTTGTT-3'
3'RACE 1	5'-GCACCAGACACTACCTATT-3'
	5'-CTAGCGCTCCTGACTCAGCGGTTTTTTTTTTTTTTTTT-3'
3'RACE 2	5'-ACTCCACAGAGTGGCTCAGTGT-3'
	5'-CTAGCGCTCCTGACTCAGCGG-3'

Total RNA isolation extraction and synthesis of cDNA

Total RNA samples were extracted from spleens using Trizol (TransGen), and the cDNA was obtained using the PrimScript RT reagent Kit (TaKaRa) according to the manufacture's protocol.

RT-PCR, 3'RACE and sequencing

A pair of homologous primers (Table 1A) was designed with DNASTAR 5.0 software in the conserved region of Gallus (EU477527.1) and Taeniopygia guttata (XM_002189232.1). All primers used in this study are listed in Table 1. With the primers, a cDNA fragment was amplified by RT-PCR using the first strand cDNAs as templates. The PCR was performed at 94°C for 5 minutes, followed by 30 cycles at 94°C for 45 seconds, 58°C for 40 seconds and 72°C for 1 minute, with a final extension at 72°C for 10 minutes, and then stopped at 4°C. Polymerase chain reaction products were detected by electrophoresing 5 µl aliquots through 1% agarose in 1 × TAE and purified by Agarose Gel DNA Extraction Kit (Shanghai Sangon Biotech Co., Ltd.). The products were cloned by into pEASY-T1 vector (TransGen) and sent to Shanghai Sangon Biotech Co., Ltd. for sequencing. Two pairs of specific primers (Table 1: 3'RACE 1 and 2) were designed

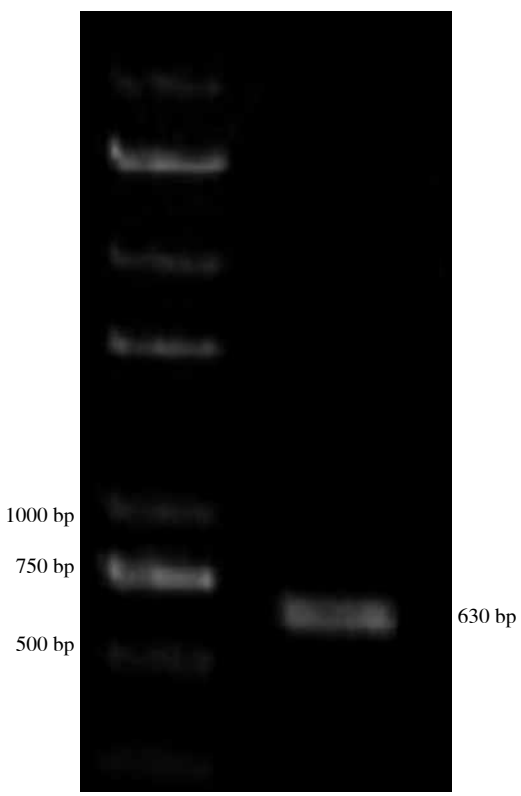

Fig. 1. RT-PCR amplification result of the conserved sequence

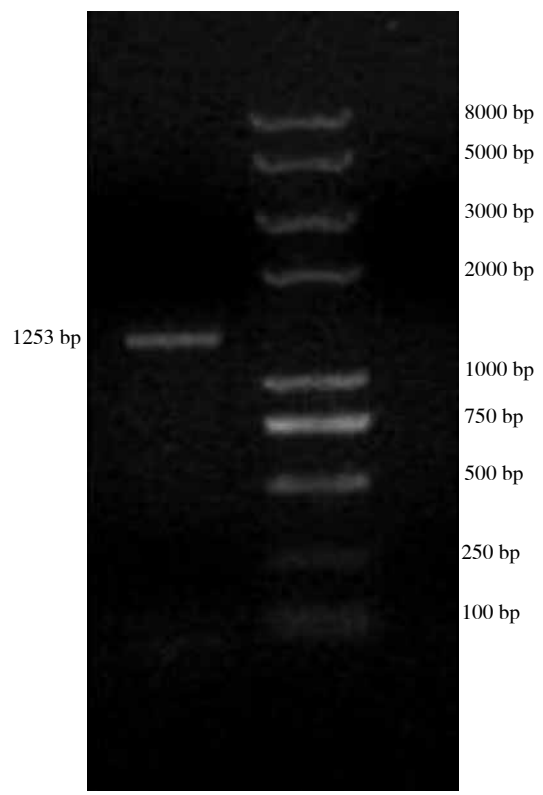

Fig. 2. Results of 3'RACE amplification

according to the obtained sequences. In the 3'RACE and the second pair for the second round PCR products were analysed by electrophoresis in 1% agarose then purified and cloned into pEASY-T1 vector, detected and sequenced as mentioned above.

IFNAR1 nucleotide sequence homology comparison and phylogenetic analysis

Sequencing results of *Columba livia* IFNAR-1 and the nucleotide sequence of *Gallus* (EU477527.1), Falco peregrinus (XM_005234387.1), *Melopsittacus undulatus* (XM_005151790.1), *Anas platyrhynchos* (XM_005013847.1), *Ficedula albicollis* (XM_005037095.1), Taeniopygia guttata (XM_002189232.1), *Papio Anubis* (NM_001168783.1), *Macaca mulatta* (NM_001266513.2), *Homosapiens* (XM_005260964.1), *Geospiza fortis* (XM_005427794.1) that registered in GenBank were subtyped and put up for homologous analysis.

IFNAR1 amino acid analysis

Phylogenetic analysis was made using DNASTAR software. Sequence analysis of the predicted *Columba livia* IFNAR-1 protein translated from the nucleotide sequence of the Columba livia IFNAR1 fragment was performed using NCBI and ExPaSy software. Signal peptides were predicted using the Signal P 3.0 server. The domain structure of the Caprine protein was analysed on the SMART server. TMHMM Server v2.0 was used to predict transmembrane regions of IFNAR-1 protein.

Results

Molecular cloning and analysis of *Columba livia* IFNAR-1

A cDNA fragment of *Columba livia* IFNAR-1 of 630 bp (Fig. 1) was obtained from single RT-PCR that was

	1	2	3	4	5	6	7	8	9	10	11	
1		88.5	73.8	80.5	74.0	55.8	55.9	86.0	56.2	73.8	80.3	1
2	12.6		72.1	79.9	71.5	56.4	55.9	86.3	57.1	70.3	81.6	2
3	32.8	35.5		68.3	89.9	46.3	46.2	71.3	49.3	88.2	66.7	3
4	22.8	23.7	42.0		68.6	54.5	54.3	77.9	55.5	67.2	79.2	4
5	32.3	36.6	10.9	41.6		48.9	49.1	71.5	49.4	90.5	69.6	5
6	68.3	66.9	99.6	71.9	90.2		90.6	55.4	95.4	46.6	51.4	6
7	67.9	68.4	100.0	72.4	89.6	10.1		54.7	98.9	47.9	52.2	7
8	15.8	15.3	36.9	26.5	36.5	69.5	71.6		57.1	68.7	81.3	8
9	67.2	65.4	88.5	69.0	88.2	4.7	1.1	65.0		49.5	57.0	9
10	32.7	38.4	12.8	43.7	10.2	98.1	92.5	41.2	87.4		68.4	10
11	23.1	21.3	44.8	24.7	39.6	79.9	77.6	21.7	65.4	41.6		11
	1	2	3	4	5	6	7	8	9	10	11	

1 Columba livia.seq
2 Falco peregrinus.seq
3 Ficedula albicollis.seq
4 gallus.seq
5 Geospiza fortis.seq
6 Homo sapiens.seq
7 Macaca mulatta.seq
8 Melopsittacus undulatus.seq
9 Papio anubis.seq
10 Taeniopygia guttata.seq
11 Anas platyrhynchos.seq

Fig. 3. Homology comparisons of nucleotide sequences among *Gallus*, *Columba livia*, *Falco peregrinus* and other animals IFNAR-1 from GenBank

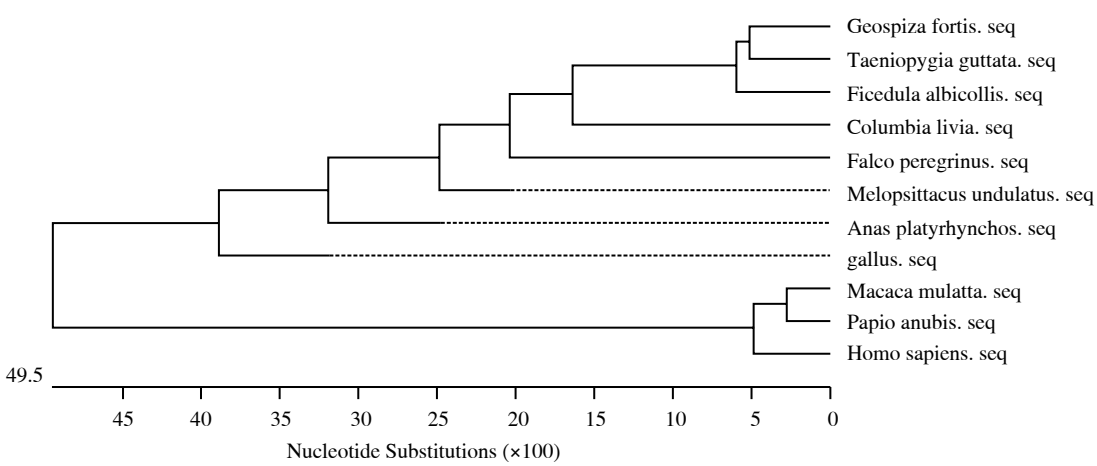

Fig. 4. The evolutionary tree of *Columba livia* compared with other animals from GenBank

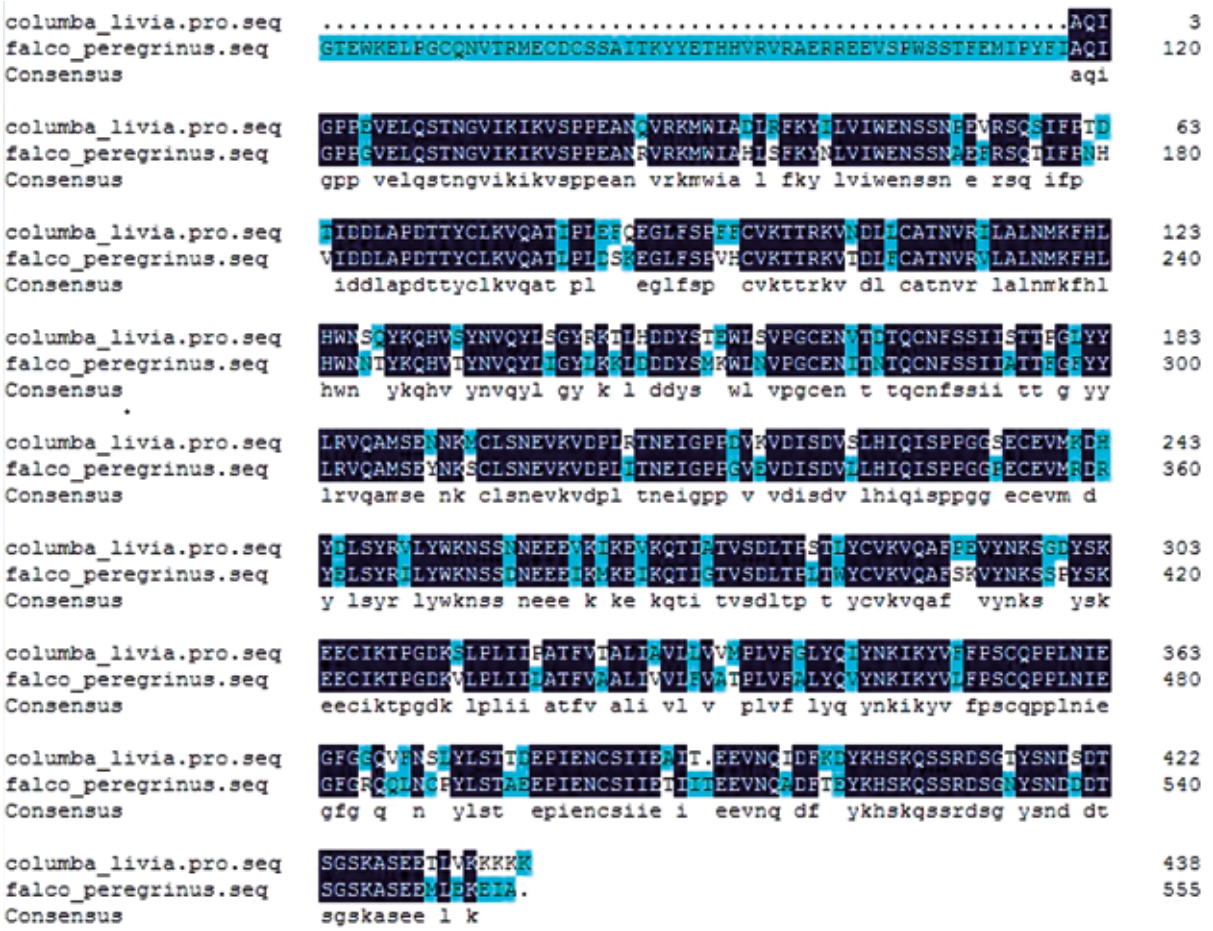

Fig. 5. Comparison of the predicted amino acid sequences of *Columba livia* and *Falco peregrinus* IFNAR-1. The blue shade reveals the conserved amino acid residues and the green shade reveals the non-conserved amino acid residues

	1	2	3	4	5	6	7	8	9	10	11	
1		69.7	72.7	57.6	69.3	61.6	32.5	34.7	71.2	39.8	61.7	1
2	38.7		80.4	67.1	68.0	68.5	36.8	36.4	77.3	37.1	68.8	2
3	33.9	22.8		66.7	68.5	66.5	38.8	39.2	77.8	40.4	65.7	3
4	61.5	43.1	43.9		59.5	85.7	30.4	31.8	64.7	35.0	80.4	4
5	39.5	41.7	40.8	57.7		60.0	35.7	36.4	67.0	37.3	58.8	5
6	53.3	40.8	44.3	15.9	56.6		32.9	34.3	65.1	34.2	83.9	6
7	145.5	124.6	115.9	157.5	129.4	143.2		83.4	36.7	91.4	31.2	7
8	134.1	126.0	114.6	149.0	126.4	135.8	18.9		36.9	97.3	32.5	8
9	36.2	27.0	26.3	47.5	43.3	46.7	124.8	124.2		38.4	62.0	9
10	112.3	123.2	110.1	132.7	122.1	136.3	9.1	2.7	117.8		34.6	10
11	53.1	40.3	45.7	22.8	59.0	18.2	152.8	145.5	52.6	134.3		11
	1	2	3	4	5	6	7	8	9	10	11	

1 Anas platyrhynchos.pro
2 columba livia.pro
3 falco peregrinus.pro
4 Ficedula albicollis.pro
5 gallus.pro
6 Geospiza fortis.pro
7 Homo sapiens.pro
8 Macaca mulatta.pro
9 Melopsittacus undulatus.pro
10 Papio anubis.pro
11 Taeniopygia guttata.pro

Fig. 6. Homology comparisons of amino acid sequences among *Gallus*, *Columba livia*, *Falco peregrinus* and other animals IFNAR-1 from GenBank

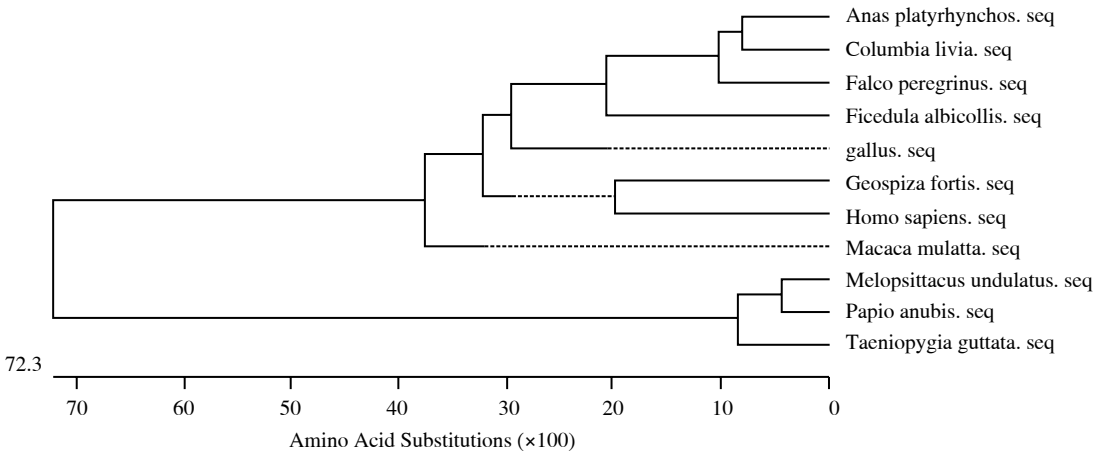

Fig. 7. The evolutionary tree of *Columba livia* compared with other animals from GenBank

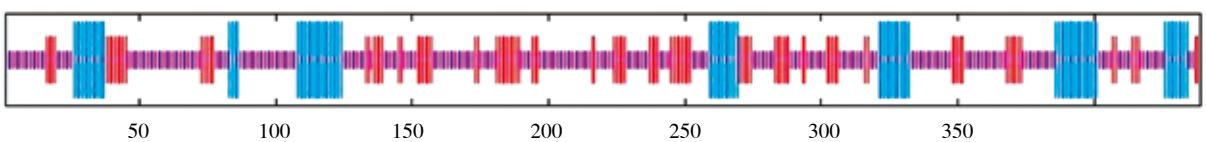

Fig. 8. The predicted secondary structure of *Columba livia* IFNAR-1amino acid sequence. The blue line represents α-helix, the red line represents extended strand and the purple line represents random coil

identified from the spleen cDNA library of *Columba livia*, which are homologous to *Gallus* and *Taeniopygia guttata*. Based on this sequence, the 3'terminal cDNA was cloned using 3'RACE PCR. After splicing, a 1253 bp fragment (Fig. 2) of Columba livia IFNAR-1 was obtained.

IFNAR-1 sequence analysis

The *Columba livia* IFNAR-1 displayed 88.5%, 80.5% and 73.8% nucleotide identity to *Falco peregrinus*, *Gallus* and *Taeniopygia guttata*, respectively (Figs. 3 and 4). Analysis of the phylogenetic tree showed that the relationship of *Columba livia* and *Falco peregrinus* IFNAR-1 had high homology.

IFNAR1 amino acid analysis

The deduced amino acid (aa) sequences of *Columba livia* IFNAR-1 had an estimated isoelectric point and Mr of 5.55 and 50.0 KD, respectively. Multiple sequence alignment was carried out using the software package MegAlign (Lasergene 7.0, DNAStar Inc., Madison, WI). The Columba livia IFNAR1 displayed 80.4% and 68% aa identity to Falco peregrinus and Gallus, respectively. The inferred aa sequences of the IFNAR-1 of *Columba livia* and Falco peregrinus were aligned (Fig. 5). There were few amino acid mutations between *Columba livia* and *Falco peregrinus*; therefore, they had high homology. Figures 6 and 7 show the distance-based neighbour-joining tree displaying the phylogenetic relationships among *Columba livia* and other animals based on the *IFNAR1* gene. *Columba livia* has a closer relationship with birds such as *Falco peregrinus* and *Gallus*, and it has a more distant relationship with mammals. The secondary structure of the sequence was predicted to be mainly random coil, separated by extended strand and α-helix (Fig. 8). Transmembrane regions were predicted to be outside between the first and

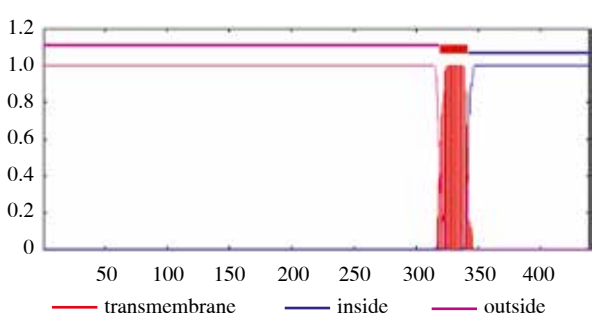

Fig. 9. Analysis of transmembrane regions of *Columba livia* IFNAR-1. The blue line represents inside, the red line represents transmembrane and the purple line represents outside

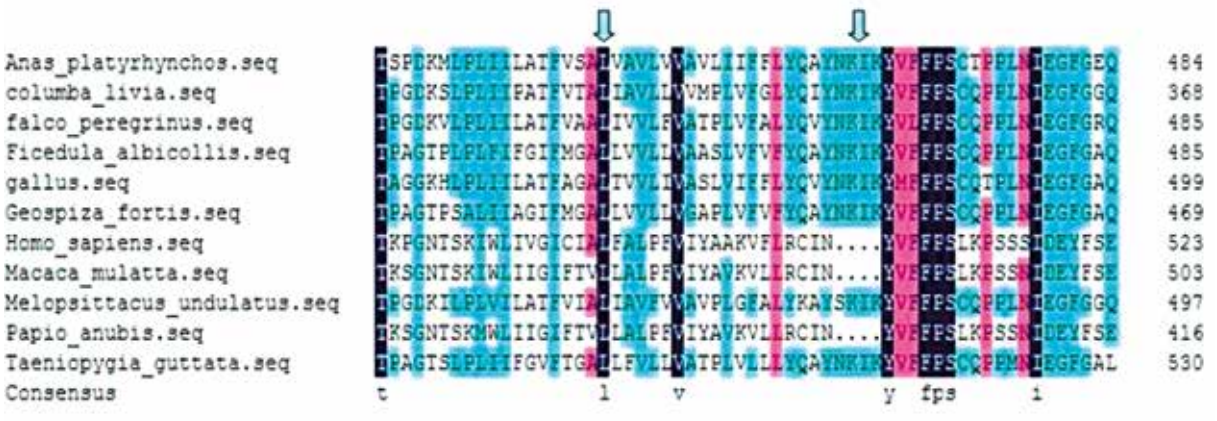

Fig. 10. Comparison of the amino acid sequences of several avian and mammalian species. The region between the two arrows represents the transmembrane region

318th amino acid, and transmembrane between 319th and 341th, and inside between the 342th and 439th (Fig. 9).

Discussion

According to the results, we can determine that *Columba livia* IFNAR-1 had high homology with avian, while its identity to mammalian was low. The highest homology was with *Falco peregrinus* (88.5%), the second was with *Melopsittacus undulates* (86%) and the third was with *Gallus* (80.5%).

According to the analysis of the amino acid sequences of the species by TMHMM Server v2.0, the transmembrane regions are similar. They all include approximately 20 amino acids that are all hydrophobic amino acids, such as Ala, Leu (Fig. 10).

The meat of *Columba livia*, known as "Dragon-meat", is delicious in taste and well received in China, especially as squab. The number of *Columba livia* being raised is about 50 million and it is worldwide in distribution. *Columba livia* is an important part of China's special economic animal production. But the occurrence of avian influenza came to be a serious threat to the production of *Columba livia*. It is necessary for *Columba livia* breeding to establish an immune mechanism, to find a new and an efficient security immune route. By contrast, the research of interferon receptor lags behind other areas of interferon research. In this study, we successfully obtained a *Columba livia* IFNAR-1 partial sequence and analysed the gene sequence and the amino acid sequences. The result can be used as reference for further research and practical application.

The authors declare no conflict of interest.

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