

Research Article

Identification of Type II Interferon Receptors in Geese: Gene Structure, Phylogenetic Analysis, and Expression Patterns

Hao Zhou,¹ Shun Chen,^{1,2,3} Yulin Qi,¹ Qin Zhou,¹ Mingshu Wang,^{1,2,3} Renyong Jia,^{1,2,3} Dekang Zhu,^{2,3} Mafeng Liu,¹ Fei Liu,³ Xiaoyue Chen,^{2,3} and Anchun Cheng^{1,2,3}

¹Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan 611130, China ²Avian Disease Research Center, College of Veterinary Medicine of Sichuan Agricultural University, Chengdu, Sichuan 611130, China ³Key Laboratory of Animal Disease and Human Health of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 611130, China

Correspondence should be addressed to Shun Chen; sophia_cs@163.com and Anchun Cheng; chenganchun@vip.163.com

Received 10 March 2015; Accepted 9 July 2015

Academic Editor: Niwat Maneekarn

Copyright © 2015 Hao Zhou et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Interferon γ receptor 1 (IFNGR1) and IFNGR2 are two cell membrane molecules belonging to class II cytokines, which play important roles in the IFN-mediated antiviral signaling pathway. Here, goose IFNGR1 and IFNGR2 were cloned and identified for the first time. Tissue distribution analysis revealed that relatively high levels of goose IFN γ mRNA transcripts were detected in immune tissues, including the harderian gland, cecal tonsil, cecum, and thymus. Relatively high expression levels of both IFNGR1 and IFNGR2 were detected in the cecal tonsil, which implicated an important role of IFN γ in the secondary immune system of geese. No specific correlation between IFN γ , IFNGR1, and IFNGR2 expression levels was observed in the same tissues of healthy geese. IFN γ and its cognate receptors showed different expression profiles, although they appeared to maintain a relatively balanced state. Furthermore, the agonist R848 led to the upregulation of goose IFN γ but did not affect the expression of goose IFNGR1 or IFNGR2. In summary, trends in expression of goose IFN γ and its cognate receptors showed tissue specificity, as well as an agerelated dependency. These findings may help us to better understand the age-related susceptibility to pathogens in birds.

1. Introduction

The interferon (IFN) γ cytokine can be induced by pathogens or artificial stimulation, which subsequently activates antiviral, antiproliferative, and immunomodulatory effects through recognizing specific receptors on the surface of target cells [1, 2]. The IFN γ receptor (IFNGR), a heterodimer consisting of two chains, IFNGR1 and IFNGR2, can be activated by IFN γ to transduce the downstream antiviral signal [3]. IFNGR1 and IFNGR2 are single transmembrane (TM) proteins belonging to the class II cytokine family, which likely function as the gateway to the control of IFN-mediated cellular signaling. As the ligand-binding subunit, IFNGR1 possesses an intracellular binding site for Janus tyrosine kinase (JAK) 1, a signal transducer and activator of transcription 1 (STAT1) [1]. The JAK2 binding site is located in an intracellular domain of IFNGR2, which serves as a signal-transducing subunit [1]. All of these sites are essential for the recruitment and activation of JAK1/JAK2 and subsequent phosphorylation of STAT1. The activated STAT1 homodimer then translocates to the nucleus and binds to the regulatory sequence (IFN γ -activated sequence) to promote gene transcription [4, 5]. Moreover, IFN γ can also regulate the antiviral gene transcription via IFN-stimulated gene factor 3 (ISGF3), thus inducing an effective immune response [6].

To date, studies have focused mainly on mammalian IFN γ systems, but little attention has been paid to avian IFN γ and its receptors. Chicken IFNGR1 was cloned from peripheral blood lymphocytes (PBLs) using the rapid amplification of cDNA ends (RACE), and the three-dimensional structure of its extracellular region was identified [7]. The extracellular region of chicken IFNGR2 also shares a similar structure with its human IFNGR counterpart [8]. In chickens, assessment of age-related expression of IFN, IFN receptors, and pattern

recognition receptors (PRRs) has indicated that the IFN system is somewhat immature during the early developmental stage of chick embryonic cells [9]. The development of IFN γ in the intestinal immunity of juvenile chickens has been characterized as well [10].

Based on a comprehensive review of reports on the gene structure, evolutionary analysis, and crosstalk between IFN and its cognate receptors in birds [11], studies of the IFN system in waterfowl appear to be lagging behind. In addition, the development and immune characteristics of avian IFNy are still poorly understood. Moreover, the duck IFNGR1 and IFNGR2 genes are only predicted sequences. Up to now, no information has been made available on the identification of goose IFN receptors. Given these considerations, this study was conducted to examine the expression level of goose IFNy and its associated receptors throughout the embryogenesis phase and posthatch period. Herein, for the first time, goose IFNGR1 and IFNGR2 cDNA sequences were identified, and the corresponding amino acid sequences as well as structural characteristics were analyzed. Comparative analysis of goose IFNGR sequences with those in birds, mammals, fish, and reptiles may shed light on the evolutionary position of goose genes among vertebrates. The tissue distribution and agerelated expression of goose IFNy and IFNy receptors also were analyzed in this study. The results of this study will extend existing information on the age-related development of goose IFN γ and its cognate receptors, which may shed further light on IFN antiviral responses in this species.

2. Methods

2.1. Animals. The study was conducted with Sichuan White Geese (Chinese goose, *A. cygnoides*). Goose embryos at 20 embryonic incubation days (EID20), goslings (1 week of age), and adult geese (3 months of age) were chosen. All animals in this study were purchased from the farm at Sichuan Agricultural University (Ya'an city, Sichuan province). One-week-old goslings and adult geese were maintained for 3 days in laboratory animal rooms for acclimation prior to experiments, and water and fodder were provided. The welfare of the animals was ensured during the sampling process.

2.2. RNA Extraction and cDNA Synthesis. The birds were euthanized, and then tissues were collected and snap-frozen in liquid N_2 . The chosen tissues included cecal tonsil, liver, lung, kidney, harderian gland, brain, bursa of Fabricius, cecum, heart, small intestine, spleen, thymus, gizzard, and proventriculus. Total RNA was extracted from various tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cDNA was synthesized using the QuantScript RT kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Finally, cDNA templates of all different samples were stored at -80° C until use.

2.3. Molecular Cloning of Goose IFNGR. A partial sequence of goose IFNGR was amplified by the degenerate primers F1, R1, F2, and R2 (all primer sequences used in this

study are listed in Table 1), which were designed based on the conserved regions among its counterparts in birds (all reference sequences used in this study are listed in Table 2). The resultant PCR fragments were subcloned into the pGEM-T Easy Vector (Promega), followed by transformation of DH5 α cells. The positive clones were sequenced by using the ABI 3730 XL sequencer (Applied Biosystems, Foster City, CA, USA). Subsequently, 3' and 5' rapid amplification of cDNA ends (RACE) was performed to obtain the full-length cDNA sequence of target genes. Based on the partial sequence obtained, Gene Specific Primers (GSPs), including 3GSP1, 3GSP2, 5GSP1, 5GSP2, and 5GSP3, were designed to obtain the full-length goose IFNGR cDNA. For 3'-RACE, the first strand cDNA was synthesized using the Adapter Primer (AP). The 3'-end of goose IFNGR was amplified by nested PCR using the primers 3GSP1 and 3GSP2 with AP1 and AP2. For 5'-RACE, the first strand cDNA was synthesized by using the primer 5GSP1 and M-MLV Reverse Transcriptase (Promega). A homopolymeric tail was then added to the 3'-end of the cDNA using TdT and dCTP (TaKaRa, Kyoto, Japan). The 5'-end of goose IFNGR was also obtained by two nested PCRs with the primer pairs 5GSP2/Abridged Anchor Primer (AAP) and 5GSP3/Abridged Universal Amplification Primer (AUAP). Finally, the full-length coding sequence of goose IFNGR was amplified by using Primer STAR Max DNA polymerase (TaKaRa).

2.4. Bioinformatic Analysis of Sequences. Potential open reading frames (ORFs) were analyzed by using the ORF finder program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and translated into the corresponding amino acids using DNA-MAN. N-Glycosylation sites were predicted with online software (http://www.cbs.dtu.dk/services/NetNGlyc/). Afterwards, the TM region was examined with the TMHMM server version 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). The potential protein domains of amino acid sequences were forecasted via the SMART server (http://smart.embl-heidelberg.de/). Pairwise identity analysis was performed with the Species Demarcation Tool [12]. Alignment of putative amino acid sequences of IFNGR1 and IFNGR2 was performed using the Clustal program, and sequence similarities were calculated with the MegAlign program. Secondary structures were analyzed using the I-TASSER program (http://zhanglab.ccmb.med.umich.edu/). To analyze the evolutionary relationships between type II IFN receptors in birds and other vertebrates, a phylogenetic tree was constructed using amino acid sequences via the neighbor-joining (NJ) method in MEGA4 with bootstrap analysis based on 1000 repetitions [13].

2.5. Tissue Distribution and Age-Related Expression Analysis of Goose IFNGR mRNA. The tissue distribution of IFNGR in healthy 1-week-old goslings was studied by real-time quantitative qPCR (RT-qPCR) using the Bio-Rad CFX96 Real-Time Detection System. The age-related expression analysis of goose IFN γ receptors at the mRNA level in certain tissues of geese (embryonic incubation 20 days and adult) was also detected by RT-qPCR. Where possible, the primers were designed across intron and extron boundaries.

		1	1						
Methods	Gene name	Primer name	Nucleotide sequence (5′-3′)						
Reverse transcription		Oligo(dT)18	TTTTTTTTTTTTTTTTT						
	IFNGR1	F1	TTAAAGCTGTTGTTGGATCA						
Partial sequence	II'NGKI	R1	CAATCACASGYTGTTCTTC						
r ai tiai sequence	IFNGR2	F2	CTGAGGTGGTCTCCTGTTA						
	II'NGK2	R2	TCAAATACTCTTCAAWGTGTG						
		AP	CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGC (T)18						
3RACE		AP1	CCAGTGAGCAGAGTGACG						
		AP2	GAGGACTCGAGCTCAAGC						
5RACE		AAP	GGCCACGCGTCGACTACGGGIIGGGIIGGGIIGGGIIG						
JIACE		AUAP	GGCCACGCGTCGACTAGTAC						
	IFNGR1	G1-3GSP1	GGCACCAGACAAAGTGGAAGAGTC						
3RACE-GSP	mon	G1-3GSP2	TGCAGAAGATTACAGAGGAGGTCC						
	IFNGR2	G2-3GSP1	TGGACTGCGGAGAATCCCCGGAATG						
	11110112	G2-3GSP2	CAATGAGTGAGACAACCAGAGCTG						
5RACE-GSP		G1-5GSP1	ATCCCAAAAAGTCACC						
	IFNGR1	G1-5GSP2	GAAATACAGGATGGTAAATATCAAC						
		G1-5GSP3	GAGAGATCCAGTTTTGGAGGTC						
		G2-5GSP1	CATTCTCCCAGTAG						
	IFNGR2	G2-5GSP2	AGTCACGCTGTTCACTTTAGGG						
		G2-5GSP3	ATTCCACCCAGTCAGAAGTCAT						
	IFNGR1	goqRT-G1-F	GCATTCAGGTTCCTCTTG						
	minond	goqRT-G1-R	AAGCGTTATCCATGTTCAG						
Real-time PCR	IFNGR2	goqRT-G2-F	AATCTTCTCCACGTTTACCG						
Real time I OR	1111012	goqRT-G2-R	CAGTAGAAGTAATTCATGGTG						
	β-actin	goqRT- β actin-F	TCCCTGGAGAAGAGCTACGA						
	r menn	goqRT-βactin-R	GTGTTGGCGTACAGGTCCTT						

TABLE 1: List of primers and sequences.

Degenerate bases: Y = C + T; W = A + T; and S = C + G.

TABLE 2: List of reference sequences.

Gene name	Organism	GenBank accession number				
	Ficedula albicollis	XM005043892				
IFNGR1	Taeniopygia guttata	XM002194727				
in region	Gallus gallus	NM001130387				
	Anas platyrhynchos	XM005017754				
	Ficedula albicollis	XM005037096				
IFNGR2	Taeniopygia guttata	XM002189208				
	Gallus gallus	AY820753				
	Anas platyrhynchos	XM005013846				

Reactions were carried out in triplicate each in a total reaction volume of 10 μ L, including 0.8 μ L cDNA sample, 5 μ L SYBR Green PCR master mix (QuantiFast SYBR Green PCR Kit), 0.3 μ L of each primer (listed in Table 1), and 3.6 μ L ddH₂O. The amplification program was 94°C for 4 min, followed by 40 cycles of 94°C for 10 s and 58°C for 30 s. After the amplification phase, a melting curve analysis (from 65°C to 95°C with a heating rate of 0.5°C per second and a continuous fluorescence measurement) was routinely performed to

confirm the presence of a single and specific PCR product. Standard curves were generated for each gene from 10-fold serial dilutions of PCR products to estimate amplification efficiency. Finally, RT-qPCR data were analyzed by the $2^{-\Delta\Delta CT}$ method using Bio-Rad CFX Manager Software.

2.6. Transcriptional Levels of IFN γ and IFNGR in Goose Mononuclear Cells (MNCs) after R848 Stimulation. Goose (3 months of age) spleen MNCs were collected, cultured in RPMI1640 (Gibco, Gaithersburg, MD, USA), and then seeded into 24-well cell culture plates in 10% serumcontaining RPMI1640 medium. Thereafter, the cells were stimulated with R848 (20 μ g/mL) (Invivogen, San Diego, CA, USA) for 10 h, while PBS-treated cells were chosen as a control. IFN γ and IFNGR transcripts were detected by RTqPCR according to methods described above.

3. Results

3.1. Sequence Analysis of Goose IFNGRI. The full-length (1322 bp) cDNA of goose IFNGRI [GenBank: KM457284] contains a 117 bp 5'-UTR, a 1134 bp single open reading frame encoding 377 amino acids, and a 71 bp 3'-UTR (Figure 1). Three potential N-glycosylation sites were found in the

1	$\verb+agcagccgccgcagtgccttcaccaacaaaccttgtagtaacatcccaaaatttcaaaaccgtcttgagttggcagtaccagcctacg$
91	tctgaaactccttatttgttgtggaaATGAAACCTTACAGCCCAGGTACCTATATGACTGTTTCAACTTGTGTGAACATCTCAACTAAT
1	M K P Y S P G T Y M T V S T C V N I S T N
181 22	TCTTGTGATCTCTCACGGGAAGTAAAGGAAACTTTTTCTCCTTACTGGTTTACGAGTAAAGCTGTTGTTGGATCAGAACAGTCTGAGTAT S C D L S R E V K E T F S P Y W F R V K A V V G S E O S E Y
271	GTTGAAACAAATGAGTTTATTTTGCAAAAGCATGGAAAAATAGGACCTCCAAAACTGGATCTCTCAAGGCATGCTGATAAAATCATAGTT
52	VETNEFILQKHGKIGPPKLDLSRHADKIIV
361	GATATTTACCATCCTGTATTTCCATCTATGGAGTTTCAGCCTTGGATCACAGACAATTTAGATTTCATGTACAAGGTGACTTTTTGGGAT D I Y H P V F P S M E F Q P W I T D N L D F M Y K V T F W D
82 451	AATGAAACTCAGCGTAAAGAAGAGGTTTTTGCAGAAGACTGTCAGGTGGATAAATGTAGCCTAGACATCCCAGTTACTCCTAATGGTTCT
112	N E T Q R K E E V F A E D C Q V D K C S L D I P V T P N G S
541	ATTTACTGTGTTTCGGCAAAGAGCAGTTTGTTTGAAAATCTGATAGTTGGTGCCCCGTCCGAAGAAAGCTGCATTCAGGTTCCTCTTGAG
142 631	I Y C V S A K S S L F E N L I V G A P S E E S C I Q V P L E CAAACTACGAGTACACAAAACATTGTCATTGTGTGGTGGCGGGGGGGG
172	Q T T S T Q N I V I V C V A V V I M G I I L T L C C G F K K
721	CTAAGGGAGAGGAATATAAAGCTGCCTAAATCCTTGGTCACTGTGATAAGAAACCTGAACATGGATAACGCTTTAGAATCAAAATCAGAG
202	L R E R N I K L P K S L V T V I R N L N M D N A L E S K S E
811 232	GGAAAATACATCTCTATAGTAAGCGTCATGCCAGTCCAGTCAGCGTTGCCTTTGAATAGCAAGCCTTGCTGAATATAGAGCCAGAA G K Y I S I V S V M P V Q S A L P L N S K E A L L N I E P E
232 901	GARGAAGCTGTCAGTCTTGATAATTTCAGTGAAGGAGCACTCTTTTTTCCCTCCGCCAGAGGGCACCAGACGAGGGGAGAGAGTCCTCTTGTG
262	E E A V S L D N F S E G A S S C P P P E A P D K V E E S S V
991	CAGAAGATTACAGAGGAGGTCCCCTTCTGATGATGAACAGAATTGTAAAGTAAAAGAGAGTTACTTTATTTCGGACAGTAACCAAACAGGT
292 1081	Q K I T E E V P S D D E Q N C K V K E S Y F I S D S N Q T G ATAAGTAGTAACTCTTCAGGTCCAGAGGTTTCTGCCACAGAAATACAACAACAGTCATTCCAAGAAGCTGTCCCAAATTTTCTGGCTAT
322	ISSNSSGPEVSATEIQQTVIPRSCPKFSGY
1171	$\tt GACAAGCCCCACGTGCCATTAGATATGTTGATAGATGTTGGTGAAGAACAACCTGTGATTGCTTACAGGCCTACTGACTAAccaggatag$
352	DKPHVPLDMLIDVGEEQPVIAYRPTD*
1261	atgaaatgtttaataaaagctcatgaagaacagcaaaaaaaa
	(a)
1	${\tt aggtcggtgccgggcttcgggcgaagaatcttctccacgtttaccggcaccaaaggatgtaaaggtttattcctataacttccacagcgc$
91 181	gctgaggtggtctcctgttaaagtagatagaggggtgtgtatatacagtccattttaaaacaggggcctttaaccagtgggatggat
271	aaactgcactcgtatcgcccggactgagtgcagtttccccctgtcacttaatgagcgtctctggacttttgttttgcgtgtgaggtctga gctggggcaaATGACTTCTGACTGGGTGGAATCGGATCCATTTGTGGCAGAGAGAG
1	M T S D W V E S D P F V A E R D T T I G P P K V N S V
361	GACTGTAAGCTCTGACTCACTGCTCATTAGTGTCTCACCCCCTTTTGAATTCGAAGAAGGTACTCTCCAGTATCATGTGTCCTACTGGGA
28 451	T V S S D S L L I S V S P P F E F E E G T L Q Y H V S Y W E GAATGCAACGACTACTACTAAAGAGATGTTGGTGAATAATGCACTATTCAAAATTGAAAATCTAAAGCAAATGACACTTTATTGTTTTAC
58	NATTTTKEMLVNNA LFKIENLKQMATHIGA
541	AATTGAAATAGAACTGAAAATGCATTTATATGACCGGATCCCTGGACTGCAGAGAATCCCCGGAATGTTACAGAACTCCAATGAGTGAG
88	I E I E L K M H L Y D R I P G L Q R I P E C Y R T P M S E T
631 118	AACCAGAGCTGCATATATTATAACAACATTTACACTGGTCGGTC
721	T K K K Y T T T T T T T T T T T T T T T
148	H K T I K Y L C Q P P L K I P S H I E E Y L R D P G M P H L
811	AGAAGCGTTGGAGAATTACCACGAGGAAGCTCCCACACGATTCTTTATCTGTTTTGTGTTTTGAAGAAGGAAG
178 901	E A L E N Y H E E A P H D S L S V L C F E E G S E A Y D D T TTTGGATGGTAACACTCGTTCACAGCAGCAGCTCCGGTGACTGTGAAGTAACTTAAgcagtgccccagtgagaatgcctgtttccagtcgt
208	L D G N T R S H S S S G D C E V T *
991	tgcagaggctcctgcgagtggtgctgtgcagctccatgcaggacagaca
1081	${\tt aagactttgcttcctgagaataatatggaacctgagcctttttaaaaatattttctgctgtgttacgacaaccttctttct$
1171 1261	actgaaaacgtggaagctgaagggaagtgaacactaaattgctttcagacaga
1351	cttctactggggtttttgtttgtttgtttgtttgtttgtgaagcttgtgttgccacttaaagtgtattatatccaaaaaaaa
	(b)
1	177 199 377
	(c)
1	19 97 123 145 224

(d)

ТМ

FN3

FIGURE 1: Nucleotide sequences of goose IFNGRs and deduced amino acid sequence structure. (a) Nucleotide sequence of goose IFNGR1 and the deduced amino acid sequence. The 5'-UTR and 3'-UTR sequences are shown in lowercase letters, while the ORF is presented in uppercase letters. The putative amino acid sequence is highlighted in blue and presented below the capital letters. Potential N-glycosylation sites are boxed. (b) Nucleotide sequence of goose IFNGR2 and deduced amino acid sequence. (c) Predicted protein domains characteristic of IFNGR1 and their alignment with counterparts from other birds and mammals. Conserved sequences are represented by the graph under the alignment. TM domains are marked in light yellow. (d) Predicted protein domains characteristic of IFNGR2. TM domains are marked in light yellow, while the fibronectin type III domain (FN3) is marked in light red.

>AAI67921_Xenopus_Silurana_tropicalis																								
	100																							
>goose_IFNGR1	27.9 100																							
>XP_005017811_Anas_platyrhynchos		100																						
>NP_001123859_Gallus_gallus		59.5 10																						
>XP_005489284_Zonotrichia_albicollis >XP_005420190_Geospiza_fortis	30.7 59.8 1 33.3 58.4 1																							
>XP_002194763_Taeniopygia_guttata		59.4 57 55.7 5			100																			
>XP_002194705_Tachlopygla_guttata >XP_005043949 Ficedula albicollis		57.4 53				100																		
>XP_005518707_Pseudopodoces_humilis		57.2 55					00																	
>XP_005509491_Columba_livia		50.2 58					61 100																	
>XP_005154885_Melopsittacus_undulatus		63.5 6					0.6 68.3	3 100																
>XP_005435859_Falco_cherrug	31.2 61.6	56.3 57	.5 59.6	59.7	56.6	58.8 6	0.7 63.1	72.9	100															
>XP_005230295_Falco_peregrinus	31.3 60.9	55.8 56	.3 59.4	59.3	56.5	58.3 e	0.8 62.7	7 71.5	96.4															
>XP_005280397_Chrysemys_picta_bellii	34.3 45.7	44.9 45	.8 48.3	46.1	46.7	44.2 4	8.4 45.6	5 48.7	45.7	45	100													
>XP_006112786_Pelodiscus_sinensis		45.4 45		45.1			2.5 44.1					100	_											
>XP_006030325_Alligator_sinensis		39 41					3.6 42.7					60.4 10												
>EDL93784_Rattus_norvegicus		31.9 28					1.2 30.1					0.1 31												
>EDL03452_Mus_musculus		31.9 29					8.6 29.6 5.1 31.7		29.8 33.6	29.4		31.7 34 35.3 37		100 53.3	100									
>XP_003898168_Papio_anubis >NP_001253229_Macaca_mulatta		33.8 35 33.8 35					5.1 31.5					35 37				100								
>AAH05333_Homo_sapiens		32.2 34					5.4 30.8					33 37 34.8 3					00							
>XP_006188549_Camelus_ferus		35.9 35					35 34.8					38.3 38					2.3 100							
>BAN09008_Sus_scrofa		33 32					32 29.9			33.5		35.5 34					0.3 75.							
>AAI03297_Bos_taurus	30.6 32.2	32.3 33	.5 31.9			29.8 3	2.5 31.9	31.9	31.1	31.5	36.5 3	33.1 37	.3 49.6	48.7	60.9	61.3 6	1.2 72.	5 73	100					
>XP_004011420_Ovis_aries	30.9 33.3	33.7 33	.7 30.9	35.2	34	31.6 3	1.5 31.6					35.2 36				59.6 59				100				
>AGU16999_Anolis_carolinensis		32.5 34					34 33.2					34.6 32				32.3 33				28.8				
>ETE71008_Ophiophagus_hannah		32.5 28					7.4 32.3					31.3 28					1.1 29.			27.6		100		
>AAI63407_Danio_rerio		25.3 25					2.6 22.9						.7 21.1						21.5					
>NP_001117888_Oncorhynchus_mykiss	23.5 21.9	24 22	.8 22.8	25.4	23.5	23.1 2	1.5 24.8	5 20.7	23.3	24.1	25.5 2	23.9 23	.0 22.3	24.1	24.5	24.1 23	0.9 22	2 23.0	25.0	25 .	23.7	20.5 2	5.8 100	
								(0)															
								(a)															
ACI7647 Andia ambianaia	100							(a)															
>AGL76447_Anolis_carolinensis	100	100						(a)															
>JAA96806_Crotalus_horridus	47.1	100	100	1				(a)															
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata	47.1 30.2	37.4	100					(a)															
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis	47.1 30.2 30.8	37.4 34.7	79.7	100		_		(a)															
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis	47.1 30.2 30.8 34.6	37.4 34.7 34	79.7 74.8	74.7	100		_	(a)															
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis	47.1 30.2 30.8	37.4 34.7	79.7		100 52.5	100		(a)															
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis	47.1 30.2 30.8 34.6	37.4 34.7 34	79.7 74.8	74.7	_	100 40.8	100	(a)															
>JAA96806_Crotalus_horridus >XP_002189244 Taeniopygia_guttat >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis	47.1 30.2 30.8 34.6 28.2	37.4 34.7 34 27.2	79.7 74.8 57.8	74.7 60.2	52.5	_	100 71.1	(a 100)															
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005497041_Zonotrichia_albicollis >XP_005151848_Melopsittacus_undulatus	47.1 30.2 30.8 34.6 28.2 32.6	37.4 34.7 34 27.2 36.8	79.7 74.8 57.8 48.8	74.7 60.2 49.8	52.5 50.9	40.8)	1														
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis >XP_005151848_Melopsittacus_undulatus >XP_005511438_Columba_livia >XP_005511438_Columba_livia	47.1 30.2 30.8 34.6 28.2 32.6 34.2	37.4 34.7 34 27.2 36.8 40	79.7 74.8 57.8 48.8 47.7	74.7 60.2 49.8 51.2	52.5 50.9 57.8	40.8 41	71.1	100	,	100														
>JAA96806_Crotalus_horridus >XP_002189244 Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis >XP_005151848_Melopsittacus_undulatus >XP_005151848_Melopsittacus_undulatus >XP_005438664_Falco_cherrug >XP_005234445_Falco_peregrinus	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3	37.4 34.7 34 27.2 36.8 40 38.7	79.7 74.8 57.8 48.8 47.7 51	74.7 60.2 49.8 51.2 52.1	52.5 50.9 57.8 52.1	40.8 41 41.3	71.1 72.1	100 74.8	100	100 62.2	100													
>JAA96806_Crotalus_horridus >XP_002189244 Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005497041_Zonotrichia_albicollis >XP_005111848_Melopsittacus_undulatus >XP_00511438_Columba_livia >XP_0051348664_Falco_cherrug >XP_005234445_Falco_peregrinus >goose_JFNGR2	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3 36.9 29	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3	79.7 74.8 57.8 48.8 47.7 51 51 46.4	74.7 60.2 49.8 51.2 52.1 52.8 44.1	52.5 50.9 57.8 52.1 52.1 49.8	40.8 41 41.3 41.7 44.8	71.1 72.1 72.3 57.8	100 74.8 75.2 60.3	100 91.3 62.2		100	100												
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005497041_Zonotrichia_albicollis >XP_005511438_Columba_livia >XP_005511438_Columba_livia >XP_005511438_Columba_livia >XP_005234445_Falco_cherrug >XP_005234445_Falco_peregrinus >goose_FFNGR2 >XP_005013903_Anas_platyrhynchos	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3 36.9 29 31.5	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9	52.5 50.9 57.8 52.1 52.1 49.8 45.9	40.8 41 41.3 41.7 44.8 35.6	71.1 72.1 72.3 57.8 59	100 74.8 75.2 60.3 63.2	100 91.3 62.2 62.7	62.2 61.4	84.4	_	100											
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis >XP_00551848_Melopsittacus_undulatus >XP_005511438_Columba_livia >XP_005511438_Columba_livia >XP_005234445_Falco_peregrinus >goose_FIFNGR2 >XP_005013903_Anas_platyrhynchos >AAV67776_Gallus_gallus	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3 36.9 29 31.5 35.2	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5 50	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1	40.8 41 41.3 41.7 44.8 35.6 42.1	71.1 72.1 72.3 57.8 59 62.9	100 74.8 75.2 60.3 63.2 65.7	100 91.3 62.2 62.7 65.6	62.2 61.4 66.1	84.4 67.7	61	100	100										
>JAA96806_Crotalus_horridus >XP_002189244 Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005516480_Melopsittacus_undulatus >XP_005511848_Melopsittacus_undulatus >XP_005511848_Melopsittacus_undulatus >XP_005518464_Falco_cherrug >XP_005234445_Falco_peregrinus >goose_IFNGR2 >XP_0057076_Gallus_gallus >NP_001101783.1_Rattus_norvegicus	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3 36.9 29 31.5 35.2 35.2 30.7	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2 34.3	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5 50 31.7	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6	71.1 72.1 72.3 57.8 59 62.9 32.8	100 74.8 75.2 60.3 63.2 65.7 37.7	100 91.3 62.2 62.7 65.6 35.5	62.2 61.4 66.1 36.1	84.4 67.7 33.8	61 34.5	33.7	100	100									
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005427852_Geospiza_fortis >XP_005497041_Zonotrichia_albicollis >XP_00511484_Melopsittacus_undulatus >XP_005511438_Columba_livia >XP_005314386_falco_cherrug >XP_0052438664_Falco_cherrug >XP_005214445_Falco_peregrinus >goose_IFNGR2 >XP_00513903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_001107831_Rattus_norvegicus >AAC52938_Mus_musculus	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3 36.9 29 31.5 35.2 30.7 30.6	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2 34.3 35.3	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5 50 31.7 36.6	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8 34	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4	71.1 72.1 72.3 57.8 59 62.9 32.8 34.4	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2	100 91.3 62.2 62.7 65.6 35.5 36.5	62.2 61.4 66.1 36.1 36.9	84.4 67.7 33.8 36.8	61 34.5 35.6	33.7 33.8	81.5	100	100								
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis >XP_00511848_Melopsittacus_undulatus >XP_00511438_Columba_livia >XP_005211438_Columba_livia >XP_005214345_Falco_peregrinus >goose_IFNGR2 >XP_005013903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_001101783.1_Ratus_norvegicus >AAC62398_Mus_musculus >ABW97193_Papio_anubis	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3 36.9 29 31.5 35.2 30.7 30.6 33	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2 34.3 35.3 36.1	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5 50 31.7 36.6 33.5	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9 30.8	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8 34 32.9	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4 25.4	71.1 72.1 72.3 57.8 59 62.9 32.8 34.4 33.5	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2 37	100 91.3 62.2 62.7 65.6 35.5 36.5 34.3	62.2 61.4 66.1 36.1 36.9 34.4	84.4 67.7 33.8 36.8 30.1	61 34.5 35.6 34	33.7 33.8 35.2	81.5 60.5	59.9	100	100							
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_00551648_Melopsittacus_undulatus >XP_005511848_Melopsittacus_undulatus >XP_005514438_Columba_livia >XP_005438664_Falco_cherrug >XP_005234445_Falco_peregrinus >goose_IFNGR2 >XP_00513903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_001101783.1_Rattus_norvegicus >AAU672938_Mus_musculus >ABW67193_Papio_anubis >JAA36651_Pan_troglodytes	47.1 30.2 30.8 34.6 28.2 32.6 34.2 36.3 36.9 29 31.5 35.2 30.7 30.6 33 35.2 30.7	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2 34.3 35.3 36.1 36	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5 50 31.7 36.6 33.5 33.5	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9 30.8 31.2	52.5 50.9 57.8 52.1 49.8 45.9 51.1 30.8 34 32.9 31.2	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4 25.4 25.4	71.1 72.1 72.3 57.8 59 62.9 32.8 34.4 33.5 33.2	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2 37 36.2 37 36.6	100 91.3 62.2 62.7 65.6 35.5 34.3 34.3 34	62.2 61.4 66.1 36.1 36.9 34.4 35.2	84.4 67.7 33.8 36.8 30.1 31.1	61 34.5 35.6 34 34.1	33.7 33.8 35.2 35.3	81.5 60.5 60.6	59.9 59.4	95.8	100	100						
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_0054278852_Geospiza_fortis >XP_0054278852_Geospiza_fortis >XP_00551690_Pseudopodoces_humilis >XP_005151848_Melopsittacus_undulatus >XP_005511438_Columba_livia >XP_00531438_Galumba_livia >XP_005234445_Falco_cherrug >XP_005234445_Falco_cherrug >XP_005013903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_001101783.1_Rattus_norvegicus >AAC52938_Mus_musculus >ABW97193_Papio_anubis >JAA36651_Pan_troglodytes >NP_005525_Homc_sapiens	47.11 30.2 30.8 34.6 28.2 22.6 34.2 29 31.5 35.2 30.7 30.6 33 31.7,7 30.6 33 31.7,7 32.6	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2 34.3 35.3 36.1 36 36.5	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5 50 31.7 36.6 33.5 33.5 33.9	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9 30.8 31.2 31.5	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8 34 32.9 31.2 31.9	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4 25.4 25.4 25.4 25	71.1 72.1 72.3 57.8 59 62.9 32.8 34.4 33.5 33.2 33.8	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2 37 36.6 37.4	100 91.3 62.2 62.7 65.6 35.5 36.5 34.3 34 34.2	62.2 61.4 66.1 36.1 36.9 34.4 35.2 34.2	84.4 67.7 33.8 36.8 30.1 31.1 31.1	61 34.5 35.6 34 34.1 34.9	33.7 33.8 35.2 35.3 35.4	81.5 60.5 60.6 61.4	59.9 59.4 60.5	95.8 95.2	98.8	100						
>JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis >XP_005511438_Columba_livia >XP_005511438_Columba_livia >XP_005511438_Columba_livia >XP_005511438_Columba_livia >XP_00551445_Falco_peregrinus >goose_IFNGR2 >XP_005013903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_0052392_Mus_musculus >ABW97193_Papio_anubis >JAA36651_Pan_troglotytes >NP_00525_Homo_sapiens >ACA51056_Callicebus_moloch	47.11 30.2 30.8 34.6 32.2 36.3 36.9 31.5 35.2 35.2 30.7 35.2 30.7 33.3 30.7 33.3 31.7 32.6 33.3 31.7 32.6 34.3	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2 34.3 35.3 36.1 36 36.5 36.5 36.7	79.7 74.8 57.8 48.8 47.7 51 51 46.4 43.5 50 31.7 36.6 33.5 33.5 33.9 34.7	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9 30.8 31.2 31.5 30.9	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8 34 32.9 31.2 31.9 32.1	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4 25.4 25.4 25.4 25 26.2	71.1 72.3 57.8 59 62.9 32.8 34.4 33.5 33.2 33.8 31.8	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2 37.3 36.2 37.3 36.6 37.4 37.9	100 91.3 62.2 62.7 65.6 35.5 34.3 34 34.2 36.2	62.2 61.4 66.1 36.9 34.4 35.2 34.2 35.2	84.4 67.7 33.8 36.8 30.1 31.1 31.1 30.1	61 34.5 35.6 34 34.1 34.9 32.7	33.7 33.8 35.2 35.3 35.4 35	81.5 60.5 60.6 61.4 60.4	59.9 59.4 60.5 59.1	95.8 95.2 86.2	98.8 86.3	86.5	100					
> AA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis >XP_00511848_Columba_livia >XP_00511438_Columba_livia >XP_005211438_Columba_livia >XP_00521445_Falco_peregrinus >goose_IFNGR2 >XP_005013903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_001101783.1_Rattus_norvegicus >AAC52938_Mus_musculus >ABW97193_Papio_anubis >JAA36651_Pan_troglodytes >NP_005526_Gallucebus_moloch >AAC559772_Bos_taurus	47.11 30.2 30.8 34.6 34.2 32.2 36.3 36.9 36.9 35.2 35.2 30.7 31.5.5 35.2 30.7 31.7 32.6 33.3 31.7 32.6 34.3 34.3 34.3 30.3	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.3 35.3 36.4 36.2 34.3 35.3 36.1 36 36.5 36.7 31	79.7 74.8 57.8 48.8 47.7 51 46.4 43.5 50 31.7 36.6 33.5 33.9 34.7 30.3	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9 30.8 31.2 31.5 30.9 30.2	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8 34 32.9 31.2 31.9 32.1 28.6	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4 25.4 25.4 25.4 25.2 26.2 23.5	71.1 72.3 57.8 59 62.9 32.8 34.4 33.5 33.2 33.8 31.8 33.3	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2 37.7 36.2 37.3 36.6 37.4 37.9 35.1	100 91.3 62.2 65.6 35.5 34.3 34 34.2 36.2 35.7	62.2 61.4 66.1 36.9 34.4 35.2 34.2 35.2 35.3	84.4 67.7 33.8 36.8 30.1 31.1 31.1 30.1 29.4	61 34.5 35.6 34 34.1 34.9 32.7 33.7	33.7 33.8 35.2 35.3 35.4 35 33.3	81.5 60.5 61.4 60.4 51.8	59.9 59.4 60.5 59.1 50.4	95.8 95.2 86.2 63.7	98.8 86.3 64.4	86.5 65.1	62.6	100				
 >JAA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_00551648_Melopsittacus_undulatus >XP_005511848_Melopsittacus_undulatus >XP_00551848_Melopsittacus_undulatus >XP_00551848_Log_cherrug >XP_005234445_Falco_peregrinus >goose_IFNGR2 >XP_005013903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_001101783.1_Rattus_norvegicus >AAC52938_Mus_musculus >JAA36651_Pan_troglodytes >NP_00525_Homo_sapiens >ACA51056_Callicebus_moloch >AAS9772_Bos_taurus >XP_00402812_Ovis_aries 	47.11 30.2 30.8 34.6 34.6 33.6 36.9 29 31.5 35.2 30.7 30.7 30.7 30.7 31.7 32.6 34 31.7 32.6 34 30.3 31.7 32.6 34 30.3 30.8 31.7 31.7 32.6 31.7 31.7 31.7 31.7 31.7 31.7 31.7 31.7	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.4 38.2 34.3 35.3 36.4 35.3 36.1 36 36.5 36.7 31 35.5	79.7 74.8 57.8 48.8 47.7 51 46.4 43.5 50 31.7 36.6 33.5 33.9 34.7 30.3 32.7	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9 30.8 31.2 31.5 30.9 30.2 31.5	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8 34 32.9 31.2 31.9 32.1 28.6 31.1	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4 25.4 25.4 25.4 25.2 26.2 23.5 25.6	71.1 72.1 72.3 57.8 59 62.9 32.8 34.4 33.5 33.2 33.8 31.8 33.3 33.9	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2 37 36.6 37.4 37.4 37.9 35.1 35.6	100 91.3 62.2 62.7 65.6 35.5 34.3 34 34.2 36.2 35.7 36.9	62.2 61.4 66.1 36.9 34.4 35.2 34.2 35.2 35.3 36.6	84.4 67.7 33.8 36.8 30.1 31.1 31.1 30.1 29.4 29.5	61 34.5 35.6 34 34.1 34.9 32.7 33.7 33.7	33.7 33.8 35.2 35.3 35.4 35 33.3 31.4	81.5 60.5 60.6 61.4 60.4 51.8 52.3	59.9 59.4 60.5 59.1 50.4 51.9	95.8 95.2 86.2 63.7 63.6	98.8 86.3 64.4 64.5	86.5 65.1 65.2	62.6 63.5	93.1	1000		_	
> AA96806_Crotalus_horridus >XP_002189244_Taeniopygia_guttata >XP_005427852_Geospiza_fortis >XP_005526690_Pseudopodoces_humilis >XP_005526690_Pseudopodoces_humilis >XP_00511848_Columba_livia >XP_00511438_Columba_livia >XP_005211438_Columba_livia >XP_00521445_Falco_peregrinus >goose_IFNGR2 >XP_005013903_Anas_platyrhynchos >AAV67776_Gallus_gallus >NP_001101783.1_Rattus_norvegicus >AAC52938_Mus_musculus >ABW97193_Papio_anubis >JAA36651_Pan_troglodytes >NP_005526_Gallucebus_moloch >AAC559772_Bos_taurus	47.11 30.2 30.8 34.6 34.2 32.2 36.3 36.9 36.9 35.2 35.2 30.7 31.5.5 35.2 30.7 31.7 32.6 33.3 31.7 32.6 34.3 34.3 34.3 30.3	37.4 34.7 34 27.2 36.8 40 38.7 39 35.3 36.3 35.3 36.4 36.2 34.3 35.3 36.1 36 36.5 36.7 31	79.7 74.8 57.8 48.8 47.7 51 46.4 43.5 50 31.7 36.6 33.5 33.9 34.7 30.3	74.7 60.2 49.8 51.2 52.1 52.8 44.1 45.9 48.3 32.4 34.9 30.8 31.2 31.5 30.9 30.2	52.5 50.9 57.8 52.1 52.1 49.8 45.9 51.1 30.8 34 32.9 31.2 31.9 32.1 28.6	40.8 41 41.3 41.7 44.8 35.6 42.1 24.6 28.4 25.4 25.4 25.4 25.2 26.2 23.5	71.1 72.3 57.8 59 62.9 32.8 34.4 33.5 33.2 33.8 31.8 33.3	100 74.8 75.2 60.3 63.2 65.7 37.7 36.2 37.7 36.2 37.3 36.6 37.4 37.9 35.1	100 91.3 62.2 65.6 35.5 34.3 34 34.2 36.2 35.7	62.2 61.4 66.1 36.9 34.4 35.2 34.2 35.2 35.3	84.4 67.7 33.8 36.8 30.1 31.1 31.1 30.1 29.4	61 34.5 35.6 34 34.1 34.9 32.7 33.7	33.7 33.8 35.2 35.3 35.4 35 33.3 31.4 33.2	81.5 60.5 61.4 60.4 51.8	59.9 59.4 60.5 59.1 50.4	95.8 95.2 86.2 63.7	98.8 86.3 64.4	86.5 65.1	62.6		100 75.5 26.3	5 10		

(b)

FIGURE 2: Heat map of IFNGR sequences in different species. The 2D color-coded matrix, decorated with a full color spectrum scheme, of IFNGR1 (a) and IFNGR2 (b) based on pairwise identity scores was constructed using the Species Demarcation Tool (STD).

goose IFNGR1 amino acid sequence (Figure 1). Only one TM domain was identified in goose IFNGR1, indicating that it is a single membrane protein (Figure 1).

Additionally, the deduced amino acid sequence of goose IFNGR1 was compared with those of avian and mammalian species. According to the 2D color-coded matrix generated based on a pairwise sequence alignment analysis (Figure 2), goose IFNGR1 shared the highest identity with its counterpart in *Anas platyrhynchos* [GenBank: XP005017811] (87.5%), which is much higher than that of *Homo sapiens* [GenBank: AAH05333] (32.3%) and *Danio rerio* [GenBank: AAI63407] (25.7%). Notably, the IFNGR1 amino acid sequence of *Gallus gallus* [GenBank: NP001123859] showed a lower identity with that of goose (63.2%) than that of duck (87.5%).

The multiple sequence alignment analysis showed that five cysteine sites and five tyrosine sites are completely conserved in birds and mammals (Figure 3). Furthermore, the JAK1 binding site (LPKSLV) and STAT1 binding site (YDKPH) were found in goose IFNGR1, which is highly similar to those of human and mouse (Figure 3). 3.2. Sequence Analysis of Goose IFNGR2. In this study, goose IFNGR2 was also cloned for the first time. The full-length cDNA of goose IFNGR2 [GenBank: KM461716] obtained was 1438 bp, with an open reading frame of 675 bp encoding for 224 amino acids (Figure 1). The 5'-UTR and 3'-UTR of IFNGR2 were 280 bp and 483 bp in length, respectively. IFNGR2 was predicted to have only one N-glycosylation site at the 58th amino acid (Figure 1). Unlike goose IFNGR1, goose IFNGR2 was found to have a TM domain and a fibronectin type III domain (FN3).

The color-coded matrix based on amino acid sequence alignment (Figure 2) showed that goose IFNGR2 shared the highest identity with *A. platyrhynchos* IFNGR2 [GenBank: XP005013903] (84.4%). Meanwhile, it shared 67.7% identity with *G. gallus* IFNGR2 [GenBank: AAV67776], 62.2% identity with *Falco cherrug* IFNGR2 [GenBank: XP005438664], and 60.3% identity with *Columba livia* IFNGR2 [GenBank: XP005511438].

The multiple sequence alignment analysis of IFNGR2 showed that two cysteine sites and four tyrosine sites were

Goose Anas platyrhynchos -MQADVLAYSGKMREWHYRRSVRPGFLLGGTTKQVKASACRPPTDVTQKA Gallus gallus -----MGAPLALMVLTALV Taeniopygia guttata MGSTRHLPSRALYRNDPGPSAAGGARPRVATAARCGRREPILEDKVCGQG Homo sapiens -----MALLFLLPLVMQ Mus musculus -----MGPQAAAGRMILLVVLMLSAK Goose _____ Anas platyrhynchos AKQAAGAGFSSTLQP-VPSPTDLVVTSQNFKTVLSWQYQPMSETPYFVVE Gallus gallus APGQNAASLQERLPA-VPSPTGTSVKSKNFRTVLYWQYPSMSETPHFVVE Taeniopygia guttata EEDLEAVVVVQVLAAHLPSPTGIVVTSENFKTVLHWQYPTMSKTPHFIVE **GVSRAEMGTADLGPSSVPTPTNVTIESYNMNPIVYWEYQIMPQVPVFTVE** Homo sapiens Mus musculus VGSGALTSTEDPEPPSVPVPTNVLIKSYNLNPVVCWEYQNMSQTPIFTVQ MKPYSPGTYMTVSTCVNISTNSCDLSREVKETFSPYWFRVKAVVGSEQSE Goose Anas platyrhynchos IKPYIPGTYMTVSTCVNISTNSCDLSREVKETFSPYWFRVKAVVGSEESE Gallus gallus VKPYLSGKYQTVSTCVNISATSCDLSEEINEIFHSYWFRIKAIVGSQQSQ Taeniopygia guttata IKPYNLGHYKNVSTCVNTSAHFCDLSKEICDPYSSHWLRVKAVVGSQESE Homo sapiens VKNYGVKNSEWIDACINISHHYCNISDHVGDPSNSLWVRVKARVGQKESA Mus musculus VKVY---SGSWTDSCTNISDHCCNIYEQIMYPDVSAWARVKAKVGQKESD :* * . : * * * *:: . : . * *:** **.::* Goose YVETNEFILQKHGKIGPPKLDLS-RHADKIIVDIYHPVFPS-----MEF Anas platyrhynchos YVETNEFILQKHGKIGPPKLDLS-RHADKIIVDIYHPVFP-----MEL Gallus gallus YVETDEFVLQKHGKIGPPKLNLS-RHGAEIIVDVYHPEFPS----VEV Taeniopygia guttata YVEANEFILQRHGKIGPPKLNIS-RHGDKIMVDIYHPVFP------Homo sapiens YAKSEEFAVCRDGKIGPPKLDIR-KEEKQIMIDIFHPSVFVNGDEQEVDY Mus musculus YARSKEFLMCLKGKVGPPGLEIRRKKEEQLSVLVFHPEVVVNGESQGTMF *..:.** : .**:*** *:: :. :: : ::** Goose QPWITDN-LDFMYKVTFWDNETQRKEEVFAEDCQ--VDKCSLDIPVTPNG Anas platyrhynchos QPWITDN-SDITYQVTFWDNETQHKNEVFADDCLQFTNKCSIDIPVTPNG Gallus gallus RPWMREIYSELSYSVIFRNSENESRKNFTVADCE--MNECNLSIPVPSEG LSCIEDIYSNLAYLVTVQGSENE-TEELYEDNCT--VHKCSLKIPVLTES Taeniopygia guttata Homo sapiens DPETTCYIRVYNVYVRMNGS-EIQYKILTQKEDDCDEIQCQLAIPVSSLN Mus musculus **GDGSTCYTFDYTVYVEHNRSGEILHTKHTVEKEECNETLCELNISVSTLD** * *.: *.* . . SIYCVSAKSSLFENLIVGAPSEESCIQVPLEQTTSTQNIVIVCVAVVIMG Goose Anas platyrhynchos STYCVSAKGILFQNLIVGAPSEESCIQVPLEQTTSTEKMVIVCVAVVIMG Gallus gallus STYCVSAKGHFFDDLIVGASSEESCIWVPITQAWSTQVTIAVSSIVLVVS Taeniopygia guttata STYCVSAKG-IFDSLMVGTPSEESCTPAPLRQTSSTHGIIILCVVIGILT Homo sapiens SQYCVSAEGVLHVWGVTTEKSKEVCITIFNSSIKGSLWIPVVAALLLFLV SRYCISVDGISSFWQVRTEKSKDVCIPPFHDDRKDSIWILVVAPLTVFTV Mus musculus * **:*... : *:: * . . : : . Goose IILTLCCGFKKL----RERNIKLPKSLVTVIRNLNMDNALESKSE-----Anas platyrhynchos VIFTLFCGFKKL----REKNIKLPKSLVTVIRNLNTDNTFESKSE----LILTVCYGCKKL----RKKNIKLPKSLVSVIRSLNADNSFESRSE-----Gallus gallus Taeniopygia guttata VLLTVYCGCKKL----RKNNIQLPKSLVSVMRNLNTGALMGPRSE----Homo sapiens LSLVFICFYIKKINPLKEKSIILPKSLISVVRSATLETKPESKYVSLITS Mus musculus VILVFAYWYTKK-NSFKRKSIMLPKSLLSVVKSATLETKPESKYS-LVTP : :.. :...* *****::*::. . * -----GKYISIVSVMPVQSALP---LNSKEALLNIEPEEEAVSLDNFS Goose -----GKYISVVSIMPVQSVSP---LNSKETLLNIEPEEEAVSPENFS Anas platyrhynchos Gallus gallus -----AKGICAASVMPVPSVSVPLTVNDDEALLNVES-AEDVSPEDFS -----GKYISVTSRLSDLPVIG-----EVTLLEIEPKEQTVSPVNSC Taeniopygia guttata Homo sapiens YQPFSLEKE-VVCEEPLSPATVPGMHTEDNPGKVEHTEELSSITEVVTTE Mus musculus HQPAVLESETVICEEPLSTVTAP----DSPEAAEQ-EELSKETKALEAG : : . :. .. . * . . . Goose EGASSCPP--PEAPDKVEESSVQKITEEVPSD-DEQNCKVKESY-----EEASSCPL--PETPDKVEESSVQKITEEVPSD-DEQNCKVKESY-----Anas platyrhynchos EGTSSGPP--LEASHKLEETSVQEN-TEVPSD-VEQSHKEKESD-----Gallus gallus Taeniopygia guttata DGESSVPS--PEAPAKVEEVPIQESTEEVSVDTDEQNCEVKESY-----Homo sapiens ENIPDVVPGSHLTPIERESSSPLSSNQSEPGSIALNSYHSRNCSESDHSR GSTSAMTPDSPPTPTQRRSFSLLSSNQS--GPCSLTAYHSRNGS-----Mus musculus . :: :. :

(a)

FIGURE 3: Continued.

Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	FISDSNQTGISSNSSGPEVSATEIQQTVIPRSCPKFSGYDK FISSSNQTDTSSNSSGPEISATEIHQTVMPRSCPKFSGYDK FISDSSQTDVCSNSSGPVVSATEIRQAVIPSSCPKFSGYDK FISNSSQVDICSKSSESEISTTETQSTVTPSRCFKFSGYDK NGFDTDSSCLESHSSLSDSEFPPNNKGEIKTEGQELITVIKAPTSFGYDK DSGLVGSGSSISDLESLPNNNSETKMAEHDPPPVRKAPMASGYDK
	. * * : ****
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	<pre>PH VPLDML - IDVGEEQPVIAYRPTD PH VPIDML - IDVGEEQRVIAYRPTD PH VPLDVL - IDVGEEQPVIAYRSTE PH VPLDVLMIDVGEEQPVNAYRPTE PH VLVDLL - VDDSGKESLIGYRPTEDSKEFS PH MLVDVL - VDVGGKESLMGYRLTGEAQELS **: :*:* :* :* . :: : .** *</pre>
	(b)

FIGURE 3: Multiple alignment analysis of IFNGR1 amino acid sequences from geese, birds, and mammalians. Selected species and GenBank accession numbers are as follows: *A. platyrhynchos* [XP005017811], *G. gallus* [NP001123859], *Taeniopygia guttata* [XP002194763], *H. sapiens* [AAH05333.1], and *M. musculus* [EDL03452.1]. The alignment was generated with ClustalW and modified manually. Amino acids conserved among all species are indicated as identical (*), highly conserved (:), or weakly conserved (.). The light green shade highlights the JAK1 binding site, while the light red shade indicates the STAT1 binding site.

completely conserved in birds and mammals (Figure 4). Consistent with the human and mouse counterparts, goose IFNGR2 also had a JAK2 binding site (PLKIPSHIEEYL) located in a span from position of 158 to 169 (Figure 4).

3.3. Secondary Structural Model of Goose IFNGR1 and IFNGR2. As depicted in Figure 5, the secondary structure of goose IFNGR1 protein was predicted to contain 3 α -helices and 17 β -sheets. Meanwhile, the goose IFNGR2 amino acid sequence was predicted to contain 2 α -helices and 12 β -sheets. Although the IFNGR1 amino acid sequence was longer than that of IFNGR2, their secondary structures were observed to be similar.

3.4. Phylogenetic Analysis of Goose IFNGR. To clarify the evolutionary relationship between IFNGR of geese and other species, a phylogenetic tree was constructed with the amino acid sequences based on a Poisson model as shown in Figure 6. These sequences were mainly separated into four clusters of avian, mammalian, fish, and amphibian/reptilian groups. The phylogenetic analysis showed that the IFNGR1 and IFNGR2 clusters were divergent subgroups. Furthermore, goose IFNGR1 appeared to be closely related to its counterparts among birds, especially duck IFNGR1. Analysis of the bird group also revealed that the goose IFNGR1 and duck IFNGR1 sequences were located in the same monophyletic group, which was distinct from other birds, such as chickens, pigeons, and sparrows. Similar results also were observed with goose IFNGR2. Furthermore, the genetic distance of fish sequences analyzed was relatively far from those of avian species, and goose IFNGR1 and IFNGR2 showed the farthest distance from the fish IFNGR molecules.

3.5. *Tissue Distribution of Goose IFNy and IFNGR*. The quantitative analysis showed that the relative expression levels

of IFN γ , IFNGR1, and IFNGR2 mRNA varied in different tested tissues (Figure 7). Relatively high levels of IFN γ were detected in the harderian gland, cecal tonsil, and cecum, followed by thymus, liver, bursa of Fabricius, and spleen, and the IFN γ expression was lowest in the brain. The goose IFNGR1 gene was highly expressed in the cecal tonsil, moderately expressed in the lung, bursa of Fabricius, heart, and proventriculus, and minimally expressed in the brain and gizzard. In addition, goose IFNGR2 was strongly detected in the immune-associated tissues, especially in the cecal tonsil and bursa of Fabricius. In most immune-related tissues, the relative mRNA transcriptional levels of IFN γ , IFNGR1, and IFNGR2 were similar at the same time point, and the ubiquitous expression of these genes in immune tissues of healthy goslings was observed.

3.6. Age-Related Expression Analysis of Goose IFNy and *IFNGR.* To understand the expression patterns of $IFN\gamma$ and its receptors, their mRNA levels in ten tissues of goose embryos, goslings, and adult geese were assessed by RT-qPCR (Figure 8). In goose embryos, the highest level of IFN γ was found in the cecum, while it was barely expressed in the brain. Meanwhile, IFNGR1 was detected at high levels in the cecum, small intestine, and liver and at lower levels in the heart, kidney, harderian gland, and bursa of Fabricius. In the embryonic stage, goose IFNGR2 was strongly transcribed in the harderian gland and small intestine. In the adult goose, IFNy was strongly detected in the kidney and harderian gland. The highest level of IFNGR1 was seen in the liver, while IFNGR2 was strongly transcribed in the liver and spleen. However, no significant differences were observed in the expression of IFNGR2 in the heart, lung, and thymus.

Obvious decreases in IFN γ expression were observed in the cecum, small intestine, and lung during goose development. Notably, in the cecum, heart, harderian gland, kidney,

Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	MAIFELWTCRPNVPCGVVYLNVLIIIFISFHVLDSSPCLPAPKDVKVYSY MPWRPLLLFLVGIFLLGPARAPGTEASPHLPAPEDVMVYSF MRPTLLWSLLLLGVFAAAAAAPPDPLSQLPAPQHPKIRLY MRPLPLWLPSLLLCGLGAAASSP-DSFSQLAAPLNPRLHLY
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	NFHNTLRWSPVKVERGVVLYTVHFKTGAFNQWDEMNCTRIAR NFCNSLRWSPVKVDGGSVSYTVQFKTGAFNHWSEMDCTRITQ MGCAQTPR NAEQVLSWEPVALSNSTRPVVYQVQFKYTDSKWFTADIMSIGVNCTQITA NDEQILTWEPSPSSNDPRPVVYQVEYSFIDGSWHRLLEPNCTDITE
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	MTSDWVESDPFVAERDTTIG TECSFPLSLNERLWTFILRVRSELGQMTSDWVETDPFVAERDTTIG TECSFLKSVKERRWTVVLRVRAEMGPRTSAWVETDPFVAERNTTIG TWCPFPPELRRRWTILLRLRAERGALASPWVLTPPFVAETNTTLG TECDFTAASPSAGFPMDFNVTLRLRAELGALHSAWVTMPWFQHYRNVTVG TKCDLTGGGRLKLFPHPFTVFLRVRAKRGNLTSKWVGLEPFQHYENVTVG * ** * :.*:*
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	PPKVNSVTVSSDSLLISVSPPFEFEEGTLQYHVSYWENA-TTTTK-E PPKVNSVIVSSDSLLISVSPPFESKEGTVQYKVSYWENA-TTATKEE PPKVNSVIVSSDSLLISVTPPFGPEPGYHLQYHVSYWENT-TITTKKE PPRVNNVSARPDSLLVGVSPPFTPEPGDLLQYLVSYWENS-SSPTEKK PPENIEVTPGEGSLIIRFSSPFDIADTSTAFFCYYVHYWEKGGIQQVK PPKNISVTPGKGSLVIHFSPPFDVFHGATFQYLVHYWEKSETQQEQVE *** .**:: .:.** .** ****: :
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	MLVNNALFKIENLKQMTLYCFTIEIELKMHLYDRIP-GLQRIPECYRTPM MWVNNALFKIENLKQMTLYCFTIEIELVKYLHEQIP-GLQRIPECYRTPM IKTSNTLFKIKDLKQSTLYCFTIQIELMTYSRFHLI-GLQTVPECYRTTI LSESKTRFEIGNLKESTLYCFSIQVQLKIYSGHLLE-GQQSAPECHRTAL GPFRSNSISLDNLKPSRVYCLQVQAQLLWNKSNIFRVGHLSNISCYETMA GPFKSNSIVLGNLKPYRVYCLQTEAQLILKNKKIRPHGLLSNVSCHETTA . :::** :**: ::* * .**:
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	SETTRAAYIITTFTLVGLVLILIIIGLFCLWRHHK-TIKYLCQPPLKIPS NETTRVVYIITTFTLVGLVLILMIIGLFFLSRHHK-TIKYLCQPPLKIPS SEATKAGYIVAIFMSVGLLIVIIVGFFCLWRNQK-AIKYLSQPPLRIPS SEATRAWYIIFLFSVGFVALNLVVAASLFLWKYHQ-KIKYWAQPPLEIPS DASTELQQVILISVGFFSLLSVLAGACFFLVLKYRGLIKYWFHTPPSIPL NASARLQQVILIPLGIFALLLGLTGACFTLFLKYQSRVKYWFQAPPNIPE . ::. :: * : * : :* * : :** :.* **
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	HIEEYLRDPGMPHLEALENYHEEAPHDSLSVLCFEEGSEAYDDTLDGNTR HIEEYLRDPSMPHLEALENHPEEALPDSYSVLYFEEGSKAYGDTLAEDTR HFEEYLRDPSMPQLEVLENHDED-PQDLLTVVYTGEGSSAYGDMLDGNTC HFREFLRDPDVAGLEELYSPAEEEPQALVLGGEGGQEGEDPSPNTSR QIEEYLKDPTQPILEALDKDSSPKDDVWDSVSIISFPEKEQEDVLQTL QIEEYLKDPDQFILEVLDKDGSPKEDSWDSVSIISSPEKERDDVLQTP ::.*:** ** ** *. :: *
Goose Anas platyrhynchos Gallus gallus Taeniopygia guttata Homo sapiens Mus musculus	SHSSSGDCEVT SHSSSSESEVT SHSSSSSRDVT ARAASEGPPQ-

FIGURE 4: Multiple alignment analysis of IFNGR2 amino acid sequences from several birds and mammals. Selected species and GenBank accession numbers are as follows: *A. platyrhynchos* [XP005013903], *G. gallus* [AAV67776], *T. guttata* [XP002189244], *H. sapiens* [NP005525], and *M. musculus* [AAC52938]. The alignment was generated with ClustalW and modified manually. Amino acids conserved among all species are indicated as identical (*), highly conserved (:), or weakly conserved (.). The light yellow shade highlights the JAK2 binding site.

liver, and small intestine, the transcriptional level of IFNGR1 in 1-week-old goslings was obviously lower compared with that in goose embryos. Furthermore, in the liver and spleen, the IFNGR2 transcriptional level was obviously increased, while it was apparently decreased in the small intestine and harderian gland. 3.7. Effect of R848 on Transcriptional Levels of Goose IFN γ and IFNGRs. As shown in Figure 9, R848 caused a highly significant upregulation of goose IFN γ (P < 0.05) compared to the PBS control, but no significant change in expression of IFNGR1 (P = 0.25) and IFNGR2 (P = 0.07) was detected. These results indicated that the R848 agonist could activate

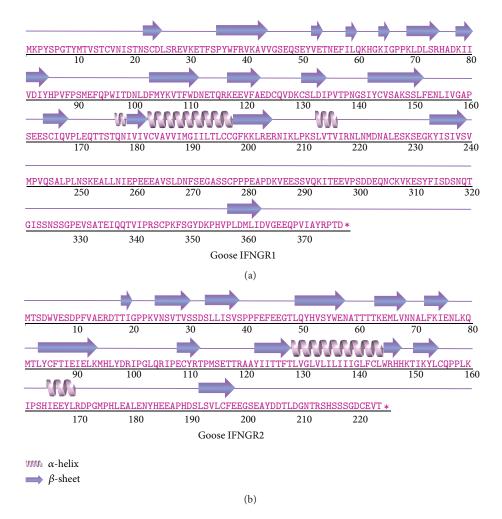


FIGURE 5: Secondary structures of goose IFNGR1 and IFNGR2. Secondary structures of goose IFNGR1 and IFNGR2 were analyzed using the I-TASSER online server. Both α -helices and β -sheets are shown in corresponding positions above the sequence.

IFN γ but did not affect the expression of IFNGR1 or IFNGR2 in geese. The results above may facilitate further studies of the goose IFNGR-mediated immunological signaling pathway.

4. Discussion

IFN γ is a pleiotropic cytokine secreted by T-helper-1 (Th1) cells, promoting both innate and adaptive responses to infection within the host [14, 15]. The major producers of this cytokine are activated T cells, natural killer (NK) cells, and professional antigen-presenting cells (APCs) [16-18]. IFN γ binds to constitutively expressed IFN γ receptors, a heterodimer consisting of two chains, IFNGR1 and IFNGR2, which then activates the downstream JAK-STAT signaling pathway. The phosphorylated STAT proteins move into the nucleus, bind specific DNA response elements, and directly transcribe IFN-stimulated genes to induce an antiviral immune response. As reviewed elsewhere [19], IFN γ can contribute to the protection against infection with some viruses, such as hepatitis B virus, herpes simplex virus, and lymphocytic choriomeningitis virus. The antiviral response

may rely on the expression levels of IFNGR1 and IFNGR2, as well as the interaction between IFNGR and IFN γ .

Until now, comparatively little was known about avian IFN_γ receptors at the molecular level other than those of chickens. Herein, we described the molecular cloning of goose IFNGR1 (1322 bp) and IFNGR2 (1438 bp) cDNA for the first time. Goose IFNGR1 and IFNGR2 were found to both possess a TM region, which demonstrated that they are single membrane proteins. The JAK1 binding site (positions 209–214) and STAT1 binding site (positions 351–355) of IFNGR1 were localized to the intracellular region, which can recruit JAK1 and STAT1 for signal transduction. The amino acids of these binding sites in birds have been reported to be relatively conserved in both humans and mice [3]. Similarly, the JAK2 binding site (positions 158–169) of IFNGR2 was also located at the intracellular region. These specific motifs are relatively conservative between birds and mammals [3].

In this study, the goose IFNGR1 and IFNGR2 amino acid sequences were analyzed at the structural and phylogenetic levels. Prior to this study, the secondary structures of IFN receptors of geese were largely unknown. We found that

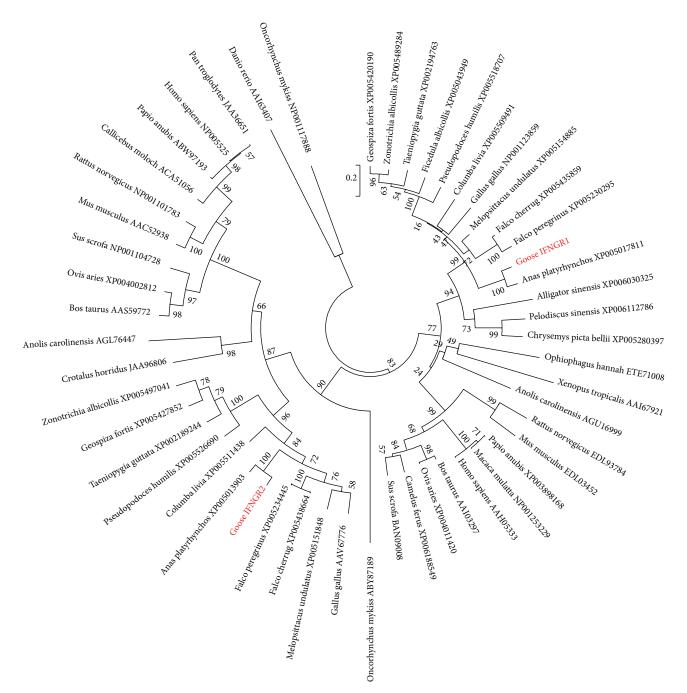


FIGURE 6: Phylogenetic analysis based on IFNGR1 and IFNGR2 amino acids. The phylogenetic tree of partial vertebrate IFNGR1 and IFNGR2 amino acid sequences was constructed using the NJ method in MEGA5. Numbers at branch nodes indicate the confidence level with 1000 bootstrap replications. IFNGR1 and IFNGR2 of birds are indicated with a green oval, and those of geese are indicated with red circles.

the secondary structure of the goose IFNGR1 protein contained 5.6% α -helices, 23.6% β -sheets, and 70.8% random coils, which was different from that predicted for the counterpart protein in chickens [7]. Additionally, the goose IFNGR2 protein contained 9.8% α -helices, 28.6% β -sheets, and 61.6% random coils, suggesting certain differences between the secondary structure of this protein in geese and chickens [8]. These results may aid in clarifying the tertiary structures of goose IFNGR1 and IFNGR2. Differences in secondary structures between IFNGR1 and IFNGR2 may result in subtle changes of the higher order structures and endow them with different functions. Additionally, the minimal divergence of IFNGR between geese and ducks further indicated the conservation of goose IFNGR1 and IFNGR2 during the evolution of waterfowl. The structural and evolutionary approaches to studying immune genes such

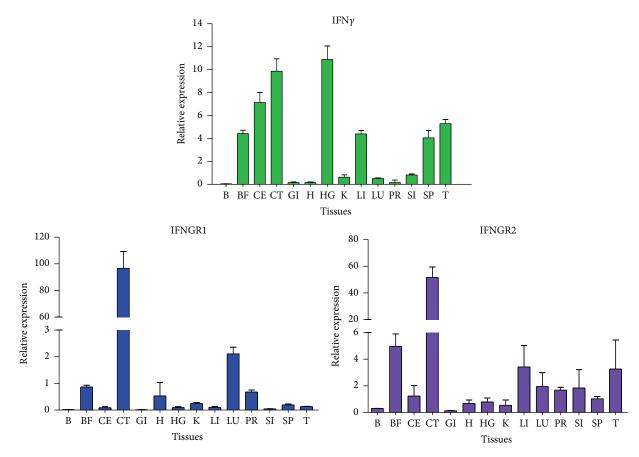


FIGURE 7: Tissue distribution of IFN γ , IFNGR1, and IFNGR2 in goslings. Tissues of three goslings (1 week of age) were collected, and mRNA levels of IFN γ , IFNGR1, and IFNGR2 (normalized to β -actin) were quantified by RT-qPCR. Data are represented as the mean ± SEM (n = 3). Cecal tonsil: CT, liver: Li, lung: Lu, kidney: K, harderian gland: HG, brain: B, bursa of Fabricius: BF, cecum: CE, heart: H, small intestine: SI, spleen: Sp, thymus: T, gizzard: Gi, and proventriculus: Pr.

as IFNGR will also help us to unravel interspecies similarities and differences in host defense.

Analysis of the tissue distribution of IFNy, IFNGR1, and IFNGR2 in goslings showed that these genes were constitutively and widely expressed in different tissues. Notably, the results showed that expression patterns of IFNy, IFNGR1, and IFNGR2 were not completely the same in different periods of development. IFNy was widely detected in various samples, but the level of IFNy in the brain of goslings was lowest. Similarly, IFNGR1 and IFNGR2 were found at relatively low levels in the brain. The main reason for these observations may be that the brain does not participate in the IFNmediated immune response or lacks immune cells. Chickens infected with infectious bursal disease virus have shown extensive viral replication in the bursa and cecal tonsils with an associated accumulation of T cells [20]. In this study, both IFNGR1 and IFNGR2 were readily detected in the cecal tonsil of goslings. A possible explanation for this phenomenon is that abundant lymphocytes accumulate in the cecal tonsil, which is responsible for the intestinal antiviral immune response. The abundant expression of goose IFNGR1 and IFNGR2 in the cecal tonsil may contribute to the strong intestinal mucosal immunity. Notably, as shown in Figure 7,

IFNGR1 and IFNGR2 levels in the lung were also relatively higher than those in the kidney and heart, which may be attributed to alveolar macrophages as being the predominant cells in the lung. As a result, the lungs can secrete a large number of bioactive cytokines, which subsequently participate in the mucosal immune defense. In addition, IFN γ and IFNGR2 were observed to be widely expressed in the immune-related tissues including the bursa of Fabricius, cecum, spleen, and thymus, while IFNGR1 was extensively expressed in the bursa of Fabricius and cecal tonsil. The similar tissue distribution of goose IFN γ and its cognate receptors suggested that these cytokines are immune-associated factors. To some extent, the induction of the IFN γ immune response may be reasonably connected with its associated receptors due to the similar tissue-specific expression patterns.

In order to explore the expression patterns during goose developmental period, we detected levels of IFN γ , IFNGR1, and IFNGR2 in goose embryos and adult geese. In the spleen, the expression of IFN γ increased from embryos to gosling during the early developmental period, which is consistent with prior observations of chicken IFN γ [21]. The decrease of IFN γ was observed in adult geese. One of the possible reasons for the reduction of IFN γ may be the functional

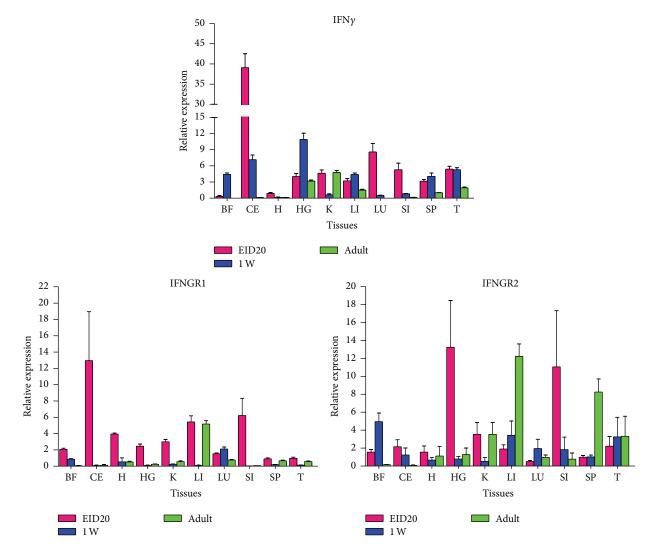


FIGURE 8: Age-related mRNA expression analysis of goose IFN γ and IFNGRs. Comparative mRNA sequence analysis of goose IFN γ and its receptors in certain tissues of embryos at EID20, goslings (1 week of age), and adult geese (3 months of age). β -actin was amplified as an internal control. Data are represented as the mean \pm SEM (n = 3). Spleen: Sp, thymus: T, bursa of Fabricius: BF, harderian gland: HG, small intestine: SI, heart: H, liver: Li, lung: Lu, kidney: K, and brain: B.

degeneration of the spleen in adult geese. The expression of IFNGR2 also showed a downward trend in the spleen, while that of IFNGR1 did not, which differed from expression patterns of these genes in the harderian gland. These genes were expressed in an organ-specific manner, which was similar to the concept of tissue-specific innate immune gene expression profiles [21-24]. As both IFNGR1 and IFNGR2 are potentially TM proteins, their expression patterns remained stable in most tissues. Finally, the defective production of IFN γ may be compensated by the high expression of IFNGR2 in the adult period of development, thus keeping a certain balance of the effectiveness of IFN γ in the host defense system. However, in the cecum and small intestine, IFN γ , IFNGR1, and IFNGR2 were detected at extremely high levels during the embryonic stage, but they declined gradually during goose development. Previous studies had demonstrated that IFNy directly affected the barrier function

in model intestinal epithelial monolayers [25]. Receptors for IFNy have been reported on the surface of epithelial cells [26] and endothelial cells [27]. Thus, observing high expression levels of goose IFNy and its associated receptors in the cecum and small intestine in this study was reasonable. The results also indicated that the IFN γ immune system may be established during the embryonic stage. Furthermore, under unstimulated conditions, no specific correlation between the expression patterns of IFNy, IFNGR1, and IFNGR2 in the same tissue was observed. Intriguingly, low expression of IFNGR1 in goslings may have been compensated by IFNy and IFNGR2. These differences in the expression patterns of IFN γ and its receptors in geese to some extent may have been simply caused by the functional compensation of these molecules in different organs. Furthermore, R848 could significantly upregulate IFN γ , but it did not influence the expression of IFNGR1 and IFNGR2 by 10 h after stimulation.

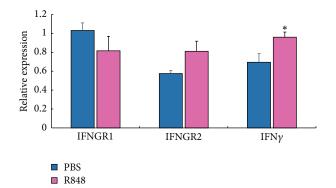


FIGURE 9: Effect of R848 on transcriptional levels of goose IFN γ and IFNGRs. The relative mRNA levels of IFNGR1, IFNGR2, and IFN γ at 10 h after stimulation of goose MNCs with R848. Each mRNA expression value was normalized by β -actin. Data are presented as the mean \pm SEM (n = 4), and differences between agonist-treated cells and mock-treated cells were analyzed by the two-tailed *t*-test. *P < 0.05.

Altogether, these findings will expand our knowledge of IFNGR-mediated immune responses in waterfowl.

5. Conclusion

In summary, we have identified and characterized IFN γ receptors in geese for the first time, providing new insights into these immune molecules in this species. Goose IFN γ and its receptors were found to be transcribed primarily in immune-related tissues, but the overall age-related expression of goose IFN γ , IFNGR1, and IFNGR2 did not appear to be directly correlated. Furthermore, R848 could significantly induce IFN γ but not IFNGR1 or IFNGR2. Nevertheless, much work is still needed to clarify the interaction between goose IFN γ and IFNGR1 or IFNGR2, which will contribute to a better understanding of the antiviral defense system of aquatic birds.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Hao Zhou and Shun Chen contributed equally as co-first authors of this work.

Acknowledgments

This work was funded by grants from the National Natural Science Foundation of China (31201891), The Ph.D. Programs Foundation of Ministry of Education of China (20125103120012), the Sichuan Provincial Cultivation Program for Leaders of Disciplines in Science (2012JQ0040), the Major Project of Education Department in Sichuan Province (12ZA107), the Innovative Research Team Program in Education Department of Sichuan Province (nos. 12TD005, 2013TD0015), the National Science and Technology Support Program (2015BAD12B05), the National Special Fund for Agro-Scientific Research in the Public Interest (201003012), and China Agricultural Research System (CARS-43-8).

References

- K. Schroder, P. J. Hertzog, T. Ravasi, and D. A. Hume, "Interferon-γ: an overview of signals, mechanisms and functions," *Journal of Leukocyte Biology*, vol. 75, no. 2, pp. 163–189, 2004.
- [2] B. Saha, S. Jyothi Prasanna, B. Chandrasekar, and D. Nandi, "Gene modulation and immunoregulatory roles of Interferony," *Cytokine*, vol. 50, no. 1, pp. 1–14, 2010.
- [3] E. A. Bach, M. Aguet, and R. D. Schreiber, "The IFNgamma receptor: a paradigm for cytokine receptor signaling," *Annual Review of Immunology*, vol. 15, pp. 563–591, 1997.
- [4] J. E. Darnell Jr., I. M. Kerr, and G. R. Stark, "Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins," *Science*, vol. 264, no. 5164, pp. 1415–1421, 1994.
- [5] M. A. Farrar and R. D. Schreiber, "The molecular cell biology of interferon-γ and its receptor," *Annual Review of Immunology*, vol. 11, pp. 571–611, 1993.
- [6] C. A. Bonjardim, P. C. P. Ferreira, and E. G. Kroon, "Interferons: signaling, antiviral and viral evasion," *Immunology Letters*, vol. 122, no. 1, pp. 1–11, 2009.
- [7] X. Han, T. Chen, and M. Wang, "Molecular cloning and characterization of chicken interferon-γ receptor α-chain," *Journal of Interferon and Cytokine Research*, vol. 28, no. 7, pp. 445–453, 2008.
- [8] C.-L. Han, W. Zhang, H.-T. Dong, X. Han, and M. Wang, "A novel gene of β chain of the IFN-γ receptor of Huiyang chicken: cloning, distribution, and CD assay," *Journal of Interferon and Cytokine Research*, vol. 26, no. 7, pp. 441–448, 2006.
- [9] A. J. Karpala, A. Bagnaud-Baule, K. E. Goossens, J. W. Lowenthal, and A. G. D. Bean, "Ontogeny of the interferon system in chickens," *Journal of Reproductive Immunology*, vol. 94, no. 2, pp. 169–174, 2012.
- [10] E. Bar-Shira, D. Sklan, and A. Friedman, "Establishment of immune competence in the avian GALT during the immediate post-hatch period," *Developmental and Comparative Immunol*ogy, vol. 27, no. 2, pp. 147–157, 2003.
- [11] H. Zhou, S. Chen, M. Wang, and A. Cheng, "Interferons and their receptors in birds: a comparison of gene structure, phylogenetic analysis, and cross modulation," *International Journal of Molecular Sciences*, vol. 15, no. 11, pp. 21045–21068, 2014.
- [12] B. Muhire, D. P. Martin, J. K. Brown et al., "A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus Mastrevirus (family Geminiviridae)," *Archives of Virology*, vol. 158, no. 6, pp. 1411–1424, 2013.
- [13] K. Tamura, J. Dudley, M. Nei, and S. Kumar, "MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0," *Molecular Biology and Evolution*, vol. 24, no. 8, pp. 1596– 1599, 2007.
- [14] U. Boehm, T. Klamp, M. Groot, and J. C. Howard, "Cellular responses to interferon-gamma," *Annual Review of Immunol*ogy, vol. 15, pp. 749–795, 1997.

- [15] S. Pestka, C. D. Krause, and M. R. Walter, "Interferons, interferon-like cytokines, and their receptors," *Immunological Reviews*, vol. 202, no. 1, pp. 8–32, 2004.
- [16] L. A. Lieberman and C. A. Hunter, "Regulatory pathways involved in the infection-induced production of IFN-γ by NK cells," *Microbes and Infection*, vol. 4, no. 15, pp. 1531–1538, 2002.
- [17] J. R. Schoenborn and C. B. Wilson, "Regulation of interferonγ during innate and adaptive immune responses," *Advances in Immunology*, vol. 96, pp. 41–101, 2007.
- [18] D. M. Frucht, T. Fukao, C. Bogdan, H. Schindler, J. J. O'Shea, and S. Koyasu, "IFN-γ production by antigen-presenting cells: mechanisms emerge," *Trends in Immunology*, vol. 22, no. 10, pp. 556–560, 2001.
- [19] R. Shtrichman and C. E. Samuel, "The role of gamma interferon in antimicrobial immunity," *Current Opinion in Microbiology*, vol. 4, no. 3, pp. 251–259, 2001.
- [20] N. Tanimura and J. M. Sharma, "Appearance of T cells in the bursa of Fabricius and cecal tonsils during the acute phase of infectious bursal disease virus infection in chickens," *Avian Diseases*, vol. 41, no. 3, pp. 638–645, 1997.
- [21] M. F. Abdul-Careem, D. B. Hunter, M. D. Lambourne, J. Barta, and S. Sharif, "Ontogeny of cytokine gene expression in the chicken spleen," *Poultry Science*, vol. 86, no. 7, pp. 1351–1355, 2007.
- [22] A. Lammers, W. H. Wieland, L. Kruijt et al., "Successive immunoglobulin and cytokine expression in the small intestine of juvenile chicken," *Developmental & Comparative Immunology*, vol. 34, no. 12, pp. 1254–1262, 2010.
- [23] S. S. Reemers, D. van Leenen, M. J. Groot Koerkamp et al., "Early host responses to avian influenza A virus are prolonged and enhanced at transcriptional level depending on maturation of the immune system," *Molecular Immunology*, vol. 47, no. 9, pp. 1675–1685, 2010.
- [24] D. Schokker, A. J. W. Hoekman, M. A. Smits, and J. M. J. Rebel, "Gene expression patterns associated with chicken jejunal development," *Developmental and Comparative Immunology*, vol. 33, no. 11, pp. 1156–1164, 2009.
- [25] J. L. Madara and J. Stafford, "Interferon-γ directly affects barrier function of cultured intestinal epithelial monolayers," *The Journal of Clinical Investigation*, vol. 83, no. 2, pp. 724–727, 1989.
- [26] U. Ucer, H. Bartsch, P. Scheurich, and K. Pfizenmaier, "Biological effects of γ -interferon on human tumor cells: quantity and affinity of cell membrane receptors for γ -IFN in relation to growth inhibition and induction of HLA-DR expression," *International Journal of Cancer*, vol. 36, no. 1, pp. 103–108, 1985.
- [27] A. H. Stoplen, E. C. Guinan, W. Fiers, and J. S. Pober, "Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers," *The American Journal of Pathology*, vol. 123, no. 1, pp. 16–24, 1986.



BioMed Research International

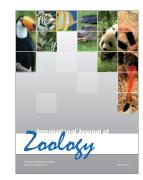








International Journal of Genomics











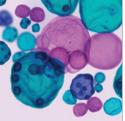
The Scientific World Journal



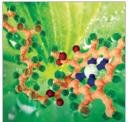
Genetics Research International



Anatomy Research International



International Journal of Microbiology



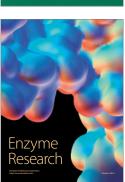
Biochemistry Research International





Journal of Marine Biology







International Journal of Evolutionary Biology



Molecular Biology International