Developmental and Comparative Immunology 89 (2018) 66–72

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/devcompimm

A comprehensive analysis of the genomic organization, expression and phylogeny of immunoglobulin light chain genes in pigeon (Columba livia)

Tian Huang[a], Zheya Sheng[b], Xiaoxing Guan[c], Linyun Guo[a], Gengsheng Cao[a,*]

[a] Henan Engineering Laboratory for Mammary Bioreactor, School of Life Science, Henan University, Kaifeng, 475004, PR China
[b] Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, 430070, PR China
[c] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, 100193, PR China

ARTICLE INFO

Keywords:
Pigeon
Immunoglobulin light chain gene
Evolution

ABSTRACT

Previous studies on immunoglobulin light chain (IgL) genes in avian species are limited to Galloanseres, and few studies have investigated IgL genes in Neoaves, which includes most living birds. Based on published genome data, we demonstrate that the pigeon (*Columba livia*) IgL locus spans approximately 24 kb of DNA and contains twenty Vλ segments located upstream of a single pair of Jλ-Cλ. Among the identified Vλ gene segments, four segments are structurally intact and all four segments are able to recombine with Jλ. Moreover, the four functional Vλ segments are preferentially utilized in VλJλ recombination. Phylogenetic analysis suggests that the presence of the four functional Vλ segments in pigeon was likely generated by gene duplication that occurred after the divergence of pigeon and other birds. Our study provides insight into IgL gene evolution and evolutionary diversity of Ig genes in birds.

1. Introduction

Diversity is essential for ensuring that antibodies can recognize and prevent various pathogens. Immunoglobulin light chains (IgL) substantially contribute to antibody diversity by combining with heavy chains (Nemazee, 2006b). The diversity of light chains in mammals is generated primarily by a somatic recombination process in which the gene encoding the variable region of IgL is assembled from multiple germline variable (V) and joining (J) gene segments (Bassing et al., 2002). This process is mediated by a recombination signal sequence (RSS) possessing both a conserved heptamer and a conserved nonamer motif, and the junctional diversity produced by the imprecise joining of the coding end is introduced during this process (Bassing et al., 2002; Ramsden et al., 1994). Light chains also play a relatively important role in the development of B cells. B cell selection is initiated upon light chain gene expression in early immature B cells, and autoreactive B cells can be rescued from elimination through a secondary rearrangement of light chain genes (Gay et al., 2011; Nemazee, 2006a; Rajewsky, 1996).

IgL genes are present in all jawed vertebrates; however, the isotypes and genomic organization of IgL genes differ among species. Mammals express two IgL isotypes, i.e., κ and λ, which are encoded by different loci. In the κ locus, multiple Vκ gene segments and a few Jκ gene segments are located upstream of a single Cκ gene, whereas the Igλ locus contains numerous functional Vλ gene segments followed by Jλ-Cλ repeats (Bengten et al., 2000). In contrast to mammals, four IgL isotypes, including κ, λ, σ and σ-cart, have been identified in elasmobranchs (Criscitiello and Flajnik, 2007). Furthermore, in contrast to the mammalian κ and λ loci, which are organized in a translocon configuration, the IgL genes in cartilaginous fish are arranged in clusters of (VL-JL-CL)n, and germline joined VL and JL genes are found in some elasmobranch species (Edholm et al., 2011; Fleurant et al., 2004; Hohman et al., 1993; Rast et al., 1994). The clustered organization of IgL genes has also been described in several teleost species, and three IgL isotypes, i.e., κ (L1/G and L3/F), λ and σ (L2), have been revealed in this group (Hikima et al., 2011). Furthermore, similar to cartilaginous fish, the number of IgL loci for each isotype in teleost differs among species (Bao et al., 2010; Edholm et al., 2009; Ghaffari and Lobb, 1993; Timmusk et al., 2000). Starting with amphibians, vertebrates developed a translocon-type of IgL gene arrangement, and most tetrapods express fewer IgL isotypes. Similar to teleost, three IgL isotypes, i.e., κ (originally termed ρ), σ and type III (lambda like), have been identified in amphibians (Das et al., 2008; Qin et al., 2008). Reptiles and mammals express the following two types of light chains: κ and λ (Sun et al.,

* Corresponding author. Henan Engineering Laboratory for Mammary Bioreactor, School of Life Science, Henan University, Kaifeng,475004, PR China.
E-mail address: gscao@henu.edu.cn (G. Cao).

https://doi.org/10.1016/j.dci.2018.08.006
Received 22 July 2018; Accepted 6 August 2018
Available online 07 August 2018
0145-305X/

2013).

While amphibians, reptiles and mammals possess more than one IgL isotype, birds exclusively express only one type of IgL, which is orthologous to the λ chain based on phylogenetic analyses (Bao et al., 2012; Das et al., 2010; Huang et al., 2012, 2016; Magor et al., 1994; Sanders and Travis, 1975). In addition to the reduced number of IgL isotypes, the genomic organization of the λ chain in birds differs from that in other tetrapods. The IgL locus in chicken contains a single set of functional Vλ, Jλ and Cλ genes, and 25 pseudogenes (ΨVλs) located upstream of the single functional Vλ gene (Reynaud et al., 1987). Therefore, VJ recombination makes little contribution to the generation of variable region diversity, and further diversification of rearranged IgL genes in chicken mainly depends on gene conversion (GCV) occurring in the embryonic bursa of Fabricius. GCV is an intrachromosomal homologous recombination event during which the sequences of the rearranged functional VJ are partially replaced by upstream ΨVλs (Arakawa and Buerstedde, 2004; Carlson et al., 1990; McCormack et al., 1991).

A similar genomic organization of the Igλ locus has also been observed in zebra finch and turkey (Bao et al., 2012; Das et al., 2010). However, a recent study revealed that nine functional Vλ and 79 ΨVλ genes upstream of the single Jλ-Cλ block exist in the IgL locus in Pekin Duck (Guan et al., 2016). Compared with chicken and other birds, the presence of nine functional Vλ gene segments in the duck increases VJ recombination diversity, and the presence of more ΨVλ genes may increase the sequence donors in gene conversion. The extant avian lineage is the most species-rich class of tetrapods and exhibits extremely diverse morphologies and rates of diversification (Zhang et al., 2014). As mentioned above, the IgL genes have mainly been investigated in species that belong to Galloanseres, and VL genes in these species exhibit differences. *Columba livia* (pigeon) belongs to Neoaves, and current knowledge regarding the IgL genes in this clade is limited (Jarvis et al., 2014). Here, we present a comprehensive analysis of pigeon IgL genes based on the published genome. Interestingly, four functional Vλ gene segments and a single Jλ-Cλ block were identified in the pigeon IgL locus. Moreover, the four functional Vλ gene segments were all able to rearrange with the Jλ segment. The expression and phylogeny of IgL genes in pigeon were also fully analyzed.

2. Materials and methods

2.1. Sample collection, DNA and RNA extraction, and reverse transcription

Pigeons (*C. livia*) aged ~1 y were purchased from a local pet market. The genomic DNA was extracted from the spleen tissue following a routine phenol-chloroform protocol. The total RNA from the spleen and intestinal tissues was isolated using a TRNzol kit (TIANGEN Biotech, China). The reverse transcription reactions were conducted using M-MLV reverse transcriptase according to the manufacturer's instructions (Promega). All animal studies and procedures were approved by the Animal Care and Use Committee of Henan University.

2.2. Annotation of pigeon IgL genes

The pigeon IgL gene sequences were retrieved from the complete pigeon (*C. livia*) genome from the National Center for Biotechnology Information (NCBI). Based on sequences derived from chicken and other birds, a BLAST approach was used to search for candidate genome scaffolds. Potentially functional, open reading frames (ORFs) and pseudo-V segments (ΨV) were identified according to functionality of IMGT (Lefranc, 1998). The adjacent RSSs of VL and JL genes were analyzed using the online program fuzznuc (http://embossgui.sourceforge.net/demo/fuzznuc.html). The VL region gene domain (framework regions or CDRs) was classified using the ImMunoGeneTics (IMGT) numbering system (Lefranc et al., 2003).

2.3. Analysis of VλJλ recombined fragments

Four potentially functional Vλ gene segments were identified in the pigeon IgL locus. The sense and antisense primers (LL-F: 5′-ATGGCCT GGCTCCCTCTCCTC- 3'; Jλ-R: 5′-TGAAATCGTCAACTGGGTCC-3′) were designed based on the conserved sequence of leader peptide of the four potentially functional Vλ gene segments and the single Jλ gene, respectively. The primers were used for the PCR amplification of recombination fragments of VλJλ using high-fidelity enzyme KOD-Plus- (Toyobo) and spleen genomic DNA as templates. The resulting products were cloned into pMD-19T vector (Takara) and sequenced.

2.4. Cloning of the expressed pigeon VL genes

The expressed pigeon VL genes were amplified using primers (LL-F: 5′-ATGGCCTGGCTCCCTCTCCTC-3'; Cλ-R1: 5′-CACATCGCGAGGGTAG AAGTT-3′) designed based on the conserved sequence of leader peptide of the four potentially functional Vλ and Cλ genes, respectively. The PCR products were cloned and sequenced. Then, the CDR3 length and amino acid usage of the cloned VL genes were calculated and compared with those of mouse, human, chicken (data from the IMGT and NCBI) and duck (data from our lab).

2.5. Quantitative RT-PCR

Total RNA was isolated from different tissues using an RNeasy Mini Kit (Qiagen), and RNA quantity and quality were assessed with a Nanodrop 2000 (Thermo Fisher Scientific). The first-strand cDNA was synthesized using 1 μg RNA and a QuantiTect Reverse Transcription Kit (Qiagen). The quantitative RT-PCR (qRT-PCR) reactions were performed using LightCycler 480 SYBR Green I Master (Roche) under the following cycling conditions: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 62 °C for 15 s, and 72 °C for 10 s. Each sample was run in triplicate. The pigeon EIF2A gene was chosen as an internal control. The following primers were used: Cλ (Cλ-F1: 5′-TGTTCCCACCATCCT CTGAG-3'; Cλ-R2: 5′-ACGCCGTCATGATGG TGTT-3′) and EIF2A (EIF2A-F: 5′-ACGAAGGTTGCTGTGTATGTT-3'; EIF2A-R: 5′-CTTGTTCC AGAGCATTGTCAC-3′).

2.6. Sequence alignment and phylogenetic analysis

The DNA and protein sequence editing, alignments and comparisons were performed using DNAStar software. Multiple sequence alignments were performed using the Clustal*W* program. Phylogenetic trees were constructed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) and viewed in Treeview (Page, 1996). The accession numbers of the used VL gene sequences (http://www.ncbi.nlm.nih.gov) are as follows: λ genes: *X. laevis* V1 (AAL40100); *X. laevis* V2 (AAL40101); *X. laevis* type V3 (AAL40102); lizard V1 (XP_008115579); lizard V2 (XP_008115579); lizard V3 (XP_008115579); alligator V10, V28, and V74 (KU535866); chicken (BAB71862); ostrich (AF41943); zebra finch (ACH44209); turkey (AFA52542); goose (KT253945); duck V1, V5, V6, V9, V14, V18, V19, V23, and V29 (KU361591); κ genes: *X. laevis* (AAH68859); alligator V24, V62, and V47 (KU535867); lizard V1 (ACB45836), V2 (ACB45824), and V3 (ACB45819); σ genes: *X. laevis* (NP_001087883); zebrafish (AAG31698); trout (AAB41310); fugu (XP_01161959); nurse shark (ABO64187); skate (AES92997); σ-cart genes: nurse shark (AAV34678); skate (AAA59381); horn shark (CAA33376); dogfish (AES93000). The accession numbers of the used VL regions of human and mouse (http://www.imgt.org/IMGTrepertoire/Proteins/) are as follows: human Vλ1 (Z73653), Vλ2 (X97462) and Vλ3 (X57862); mouse Vλ1 (J00590), Vλ2 (J00599) and Vλ3 (M34597); human Vκ1 (M64858), Vκ2 (X72814) and Vκ3 (X02725); mouse Vκ1 (AJ231200), Vκ2 (AJ132683) and Vκ3 (K02162). The accession numbers of the used IgL constant regions (http://www.ncbi.nlm.nih.gov) are as follows: λ genes: chicken (AAA48862); duck