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Short Communication
Identification of the Immunoglobulin Light Chain Alleles in White Leghorn Line $15I_5 \times 7_1$

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Abstract

Background and Objective: Our long-term studies of B-cell development in the bursa of Fabricius now involves the understanding of repertoire development by immunoglobulin gene conversion. This study was conducted to identify the Ig-light chain alleles in the $15I_5$ and 71 inbred White Leghorn line used in the cross. Given that the pseudogene variable-region donors differ among the three common alleles of the immunoglobulin light chain and that immunoglobulin-conversion occurs intrachromosomally, the correct identification of pseudogene donors involved in gene conversion events requires the identification of the immunoglobulin light chain allele in the inbred White Leghorn lines $15I_5$ and 7_1 used to generate the F1 cross. **Materials and Methods:** The 2,333 bp germline immunoglobulin light chain gene was PCR amplified from genomic DNA of line $15I_5$ and line 7_1 and ligated into a plasmid vector. The variable-region gene segment was isolated from the plasmid vector by restriction endonuclease digestion and evaluated by nucleotide sequencing. **Results:** The nucleotide sequence alignments with the three common alleles of the immunoglobulin light chain showed that the variable-region gene segment sequence from line $15I_5$ was identical to immunoglobulin light chain allele S3 and the line 7_1 sequence was identical to the G4 light chain allele. **Conclusion:** This information will be critical for future studies to evaluate immunoglobulin gene conversion in the $15I_5 \times 7_1$ F1 cross, given the nucleotide sequence variation in the pseudogene variable-region donors between the S3 and G4 immunoglobulin light chain alleles.

Key words: B-cells, Bursa of Fabricius, chickens, immunoglobulin light chain alleles, white leghorn line $15I_5 \times 7_1$

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The bursa of Fabricius guides the development of B-cell antigen specificities in the chicken[1]. In chicken B-cells, the heavy and light chain immunoglobulin (Ig) gene is repertoire is expanded with sequences from pseudogene variable (V)-region gene segments[2,3]. This process is described as Ig-gene conversion[4].

Our long-term plan is to define the microenvironmental signals responsible for Ig-gene conversion in the bursa[5]. This work will require monitoring the onset and duration of Ig-gene conversion during the embryonic period. The Ig-light chain locus, being more compact than the Ig-heavy chain locus is therefore used in Ig-gene conversion analysis[4]. Moreover, the pseudogene V-region gene segments have been characterized for the three common alleles of the Ig-light chain gene: Alleles CB, G4 and S3[3,6]. Methods to determine Ig-gene conversion events entail PCR amplification of the rearranged V-region gene segment and nucleotide sequencing. Nucleotide sequence data from B-cells is then compared with sequences of pseudogene V-region gene segments from the appropriate light chain allele to identify a possible pseudogene donor(s)[7]. This is critical in that the pseudogene V-region gene segments of the CB, G4 and S3 alleles differ from one another in nucleotide sequence[6].

Our bursal microenvironmental studies[8] have been conducted with the F1 cross of the $15I_5$ and 7_1 highly inbred White Leghorn lines which was developed at the USDA/ARS Avian Disease and Oncology Laboratory. The $15I_5 \times 7_1$ line is well characterized and has been used in avian immunology research for decades[9]. However, Ig-light chain alleles of line $15I_5 \times 7_1$ are unknown at present. The objective of this project was to identify the Ig-light chain alleles in the $15I_5$ and 7_1 inbred White Leghorn lines that were used to generate the cross.

MATERIALS AND METHODS

Experimental animals: The highly inbred White Leghorn chicken line 7_1 (USDA-ARS, ADOL, East Lansing, MI USA) were used at 8 weeks posthatch. The chickens were killed with carbon dioxide and the bursa removed and placed in RPMI-1640 medium. Developing B-cells were isolated from the bursas as described by Nuthalapati *et al.*[5] and genomic DNA prepared with a QIAGEN DNA extraction kit (QIAGEN, USA). Genomic DNA from inbred White Leghorn chicken line $15I_5$ erythrocytes was a generous gift from Dr. Henry D. Hunt, USDA-ARS, ADOL, East Lansing, USA. This project was reviewed by the Mississippi State University Animal Care and Use Committee.

Polymerase chain reaction amplification and nucleotide sequencing: Genomic DNA from lines $15I_5$ and 7_1 was amplified with published primers for the Ig-light chain gene:

- **Forward primer V_{L2}:** 5′-TGGGCTCCTCTCCTCCTGGC-3′ corresponding to nucleotides 473-493 in the leader exon[10]
- **Reverse primer J_{L3}:** 5′-GAGTCGCTGACCTCGTCTCG-3′ modified from Benatar and Ratcliffe[11] to encompass nucleotides 2786-2805 located 3′ of the J-region gene segment[10]

A Hybaid Omn-E thermocycler (Scientific Consultants Inc, Baton Rouge, LA, USA) was used with the program: 94°C, 2 min, 30 cycles of 94°C, 30 sec, 66°C, 30 sec and 72°C, 2 min, and extension at 72°C, 10 min.

The 2,333 bp germline Ig-light chain gene was ligated into the pCR2.1-TOPO vector (ThermoFisher, USA). The V-region gene segment was digested from the 2,333 bp PCR product with enzymes EcoRI and PvuII and isolated by agarose gel electrophoresis. The 525 bp restriction fragment containing the V-region gene segment was then ligated into the pCR2.1-TOPO vector that had been treated with EcoRI and EcoRV and ligation reaction products were transformed into *E. coli* TOP10F' (ThermoFisher, USA). A minimum of 3 recombinant *E. coli* colonies were selected from the line $15I_5$ PCR experiment and line 7_1 PCR experiment for nucleotide sequencing.

Sequence data analysis: The published nucleotide sequences from the Ig-light chain alleles G4, S3 and CB[3,6] were compared with the nucleotide sequences from lines $15I_5$ and 7_1 using the Clustal X alignment software[12].

RESULTS AND DISCUSSION

To evaluate Ig-gene conversion events, the non-germline nucleotide substitutions in the nucleotide sequences of PCR amplified clones are compared with pseudogene V-region gene segment sequences from the CB, G4 and S3 Ig-light chain alleles[3,6] to identify a possible donor(s). To facilitate studies into Ig-gene conversion in developing B-cells from line $15I_5 \times 7_1$, the germline V-region gene segment of the Ig-light chain gene was PCR amplified from genomic DNA from line $15I_5$ and line 7_1 and completely sequenced. The nucleotide sequences were compared with the germline V-region gene sequences of the CB, G4 and S3 Ig-light chain alleles to identify the Ig-light chain allele of line $15I_5$ and line 7_1 (Fig. 1). The germline V-region gene segment sequence from line 7_1 is identical to the G4 allele and only differs from the CB allele

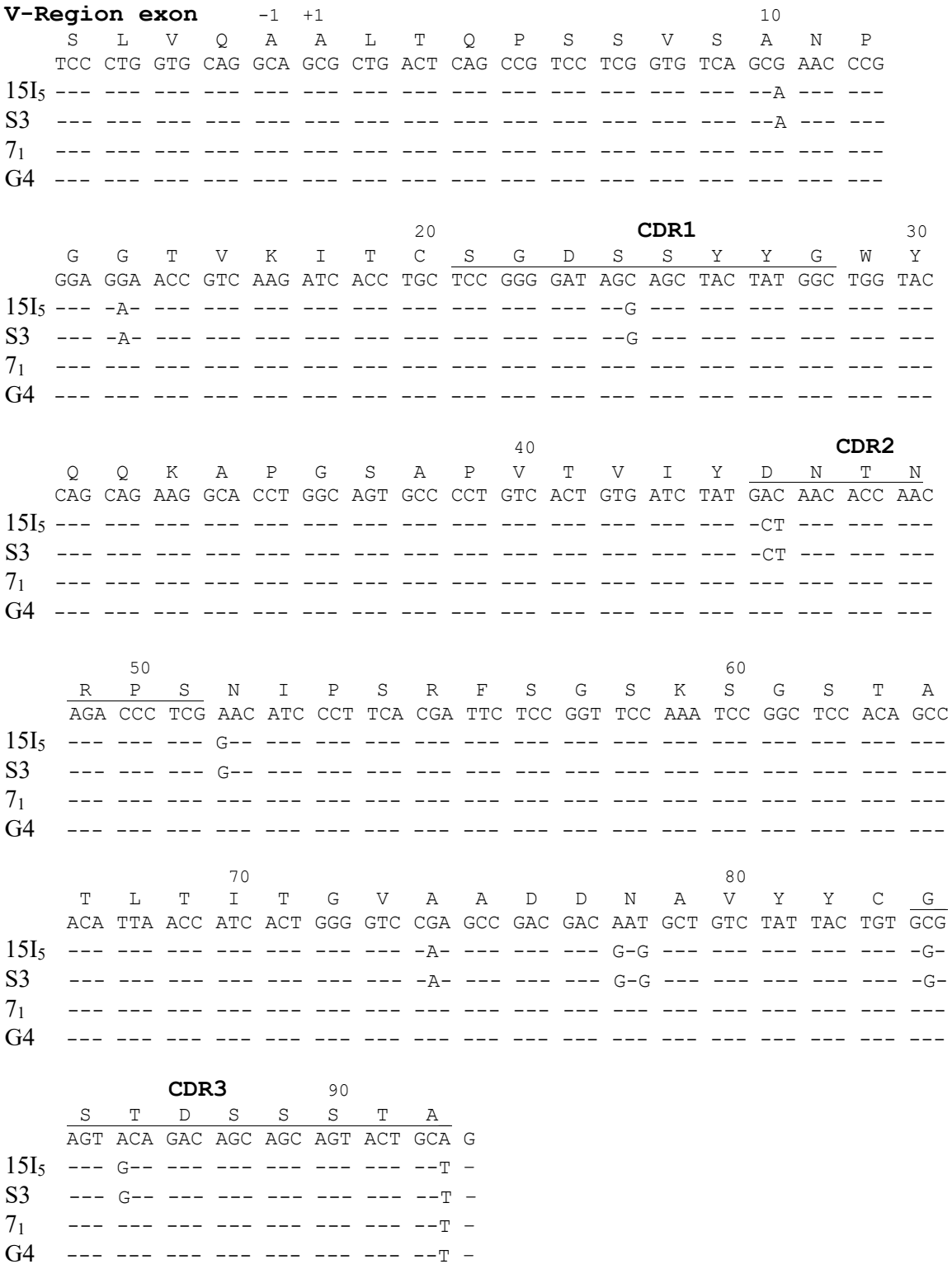

Fig. 1: Alignment of the nucleotide sequence of the germline V-region gene segment from lines $15I_5$ and 7_1 with the V-region gene segment sequences from the S3 and G4 alleles[6].

The V-region gene segment sequence from the CB allele is used as the template sequence[3]. The codons are numbered according to Wu and Kabat[13]. The amino acids specified by the codons are listed in single letter code. The overlines show the locations of the three complementarity-determining regions (CDR)

sequence at codon position 92. However, the germline V-region gene segment sequence from line $15I_5$ is identical to the S3 allele, sharing polymorphic nucleotides at codon positions 10, 14, 24, 45, 52, 74, 78, 84 and 86 (Fig. 1). We concluded that line 7_1 has the G4 Ig-light chain allele and that line $15I_5$ has the S3 Ig-light chain allele.

The goal of this short-term project was to identify the Ig-light chain alleles in the $15I_5 \times 7_1$ line. This information is needed for our plans to study Ig-gene conversion in bursal B-cells from the $15I_5 \times 7_1$ line. Given that pseudogene V-region gene segments of the CB, S3 and G4 Ig-light chain alleles are polymorphic[6], matching the pseudogene donor sequences from the correct rearranged Ig-light chain allele is critical for accurate identification of Ig-gene conversion events.

CONCLUSION

This project identified the S3 and G4 alleles of the Ig-light chain in White Leghorn line $15I_5 \times 7_1$. This knowledge will aid in the identification of Ig-gene conversion events during bursal B-cell development.

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