Research Notes

Genetic Variation Among Chicken Lines and Mammalian Species in Specific Genes

H. Zhou, W. Liu, and S. J. Lamont[1]

Department of Animal Science, Iowa State University, Ames, Iowa 50011-3150

ABSTRACT Thirteen gene-specific primer sets provided by the U.S. Poultry Genome Coordinators were used to investigate DNA polymorphisms between two highly inbred chicken lines of Leghorn and Fayoumi origin. Nucleotide and predicted amino acid sequences were then compared among these chicken lines and the Genbank sequences of chicken, mouse, and human. The following genes were selected as candidates for immune response or transcription activation: *B2M*, *DAD1*, *IAP1*, *IL2*, *IREB1*, *LAP18*, *MAFL*, *POU1F1*, *RREB1*, *TAD*, *TBP1*, *TCRG*, and *ZOV3*. Total cDNA was obtained from the spleens of Leghorn and Fayoumi lines by reverse transcriptase-polymerase chain reaction (PCR) and was used as a template to PCR-amplify gene-specific products. All primers except *POU1F1* and *TCRG* generated single PCR products of the predicted 325- to 667-bp size, confirming the efficacy of these gene-specific primers in the chicken. Three and seven of the 11 amplified gene fragments yielded line-specific nucleotide polymorphisms between the Leghorn and Fayoumi sequences and between the Leghorn and Genbank chicken sequences respectively. Similarities between inbred Leghorn and mammalian species were 36 to 86% for nucleotides and 25 to 96% for predicted amino acid sequence. The polymorphisms of some gene fragments between the Leghorn and Fayoumi lines will allow for investigation of associations of these genes with immune response and other biological traits.

(*Key words*: inbred chicken line, immune response, candidate gene, nucleotide sequence, amino acid sequence)

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INTRODUCTION

The development of a comparative map has gained high priority in the poultry genome community due to the relatively limited information on genes available in the poultry genome. Burt et al. (1999) constructed comparative maps of chicken with human and mouse, species that share a common ancestor 300 million years ago. In total, 223 genes that define 81 autosomal conserved segments were used in the chicken-human comparison, and 100 were used in the chicken-mouse comparison. The number of chromosome rearrangements (72) for the chicken-human comparison are less than that for mouse-chicken (128) and mouse-human (171). Thus, Burt et al. (1999) concluded that the organization of the human genome is closer to that of the chicken than the mouse. Studies by Palmer and Jones (1986), Burt et al. (1995), and Groenen et al. (2000) suggest that extensive conserved synteny exists between mammalian genomes and the chicken genome. As the human genome project progresses, more of the genes have been identified through the positional candidate gene approach (Collins, 1995). Functional genes have revealed conserved linkage associations among species, and expressed genes are especially informative candidates for conserved synteny mapping. By using anchor loci, syntenic comparisons may provide clues to the location and orientation of orthologous genes (Smith et al., 1997). The vast amount of information collected on the human genome should serve as a resource for agricultural animal species, and the development of a comparative map between the chicken and human genome is a logical course of action (Cheng, 1997).

Genes with known function from other species offer an opportunity to explore the genetic variation of these genes in poultry. Primer pairs for genes of known function were developed by the U.S. Poultry Genome Coordinator using primer optimization software from Genbank sequences. Thirteen gene-specific primers (Table 1) were selected as potential candidates for immune response or gene transcription and were used to define molecular variation between genetically divergent chicken, human, and mouse lines. These genes were chosen because they

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[1]To whom correspondence should be addressed: sjlamont@iastate.edu.

Abbreviation Key: BCM = Baylor College of Medicine; PCR = polymerase chain reaction.

TABLE 1. Description of the 13 gene-specific fragments studied and Genbank accession numbers for the new nucleotide sequences

Locus symbol	Gene name	Product size (bp) Predicted/actual	Accession number Leghorn	Fayoumi
B2M	Beta-2-Microglobulin	325/325	AF221077	AF221076
DAD1	Defender Against Cell Death 1	343/343	AF221078	AF221079
IAP1	Inhibitor of Apoptosis 1	622/622	AF221082	AF221083
IL2	Interleukin 2 Precursor	328/328	AF221080	AF221081
IREB1	IRE-Binding Protein 1	664/664	AF221553	AF221554
LAP18	Leukemia-Associated Phosphoprotein p18	358/358	AF221555	AF221556
MAFL	L-MAF, bZIP Transcription Factor	466/466	AF221557	AF221558
POU1F1	Pou Domain, Class I Transcription Factor 1	122/NP[1]		
RREB1	Ras-Responsive Element Binding Protein 1	613/613	AF221559	AF221560
TAD	Thymocyte Activation and Developmental Protein	546/546	AF221561	AF221562
TBP1	TA-TA Binding Protein 1	667/667	AF221563	AF221564
TCRG	T-cell Receptor Gamma Chain Vg1-Jg2	489/NP[1]		
ZOV3	Ig-Superfamily Protein	577/577	AF221565	AF221566

[1]NP = No polymerase chain reaction product.

have been described in some species to have an important role in antigen identification and processing, molecular interactions and cellular cooperation in the immune response, or to perform a crucial function in regulation of expression and transcription of genes.

Genetic selection, with the aid of proven molecular markers for immune response and disease resistance, may be used to improve health in poultry (Lamont 1998). The highly inbred chicken lines used in this study have been previously defined for specific characteristics of disease resistance. The G-B1 line originated from U.S. commercial layer stock, and the Fayoumi lines were from Egypt (Lamont and Chen, 1992). The G-B1 line is resistant to transient paralysis caused by Marek's disease (Parker and Schierman, 1983) and against the early lethal effects of highly virulent transplantable Marek's disease lymphomas (Schierman, 1984). The Fayoumi lines are the first genetic stocks in which Rous Sarcoma virus resistance on the chorioallantoic membrane was observed (Prince, 1958) and are less susceptible to Marek's disease and tumor development than the Leghorn line (Lakshmanan et al., 1996). The Fayoumi lines are more resistant to *Salmonella enteritidis* colonization than the G-B1 line (Lamont, unpublished data). Identification of sequence polymorphisms between the Fayoumi and G-B1 lines will make it possible to determine associations of these genes or linked loci with immunity and resistance to disease in resource populations generated from these lines, an ongoing effort in our laboratory.

The objectives of the current study were to test the efficacy of the gene-specific primers in chickens, define polymorphisms of these gene fragments between Leghorn and Fayoumi lines, and compare the nucleotide sequences and predicted amino acid sequences among Leghorn, Fayoumi, and Genbank sequences of chicken, human, and mouse.

MATERIALS AND METHODS

Total RNA was isolated from the spleen of chickens (one male and one female per line) of the Leghorn line (G-B1) and Fayoumi lines (M15.2 and M5.1) using the Totally RNA™ Kit,[2] and chicken cDNA were obtained by reverse transcriptase-polymerase chain reaction (PCR) (RETRO script™),[2] using total RNA as a template. These cDNA were then used as templates, and PCR amplifications were carried out in a PTC 100,[3] using 30 cycles with denaturation at 92 C for 45 s, annealing at 50 C for 40 s, and extension at 72 C for 1 min. The first denaturation was performed at 95 C for 5 min, and the last extension was 5 min at 72 C. The CENTRI.SPIN™-40 Spin columns[4] were employed to purify these PCR products. The nucleotide sequences of the purified products were determined by an ABI 377 sequencer.[5] The Baylor College of Medicine (BCM) Search Launcher program was used to compare nucleotide sequences and amino acid sequences among chicken, human, and mouse (Smith et al., 1996). In instances of very low identity or large insertions or deletions on the ends of fragments, those sequences were eliminated from the identity comparison.

RESULTS AND DISCUSSION

Eleven of 13 of these gene-specific primers amplified PCR products under the conditions used, of which most were confirmed by direct sequencing of the PCR product as having high similarity to the predicted gene fragment. This ratio demonstrates the efficacy of these gene-specific primers in the chicken. All primers, except *POU1F1* and *TCRG*, which amplified no PCR product under the recommended conditions, generated single PCR products of the predicted sizes of 325 to 667 bp. Sequences of all individuals within each inbred line were identical. No amplified PCR product was obtained from chicken genomic DNA template for all 13 pairs of primers.

The nucleotide sequences and predicted amino acid sequences among Leghorn, Fayoumi, Genbank chicken

[2]Ambion Inc., Austin, TX 78744.
[3]MJ Research Inc., Watertown, MA 02172.
[4]Princeton Separations, Adelphia, NJ 07710.
[5]Perkin Elmer, Foster City, CA 94404-1128.

data, human, and mouse were compared by using the BCM Search Launcher program (Table 2). The G-B1 Leghorn line was used as a reference line for sequence comparisons, because of its widespread usage in molecular genetics and immune genetics research (Lamont and Chen, 1992; Plotsky et al., 1995). The G-B1 line was previously used as a standard for B blood group alleles by Briles et al. (1982). Sequence polymorphisms were identified for three genes (*IAP1*, *IL2*, and *ZOV3*) between the highly inbred Leghorn and Fayoumi lines and for seven genes (*IAP1*, *IL2*, *MAFL*, *RREB1*, *TAD*, *TBP1*, and *ZOV3*) between the Leghorn and Genbank chicken sequences. Similarities of the individual gene fragments between inbred Leghorn and mammalian species in nucleotide sequences were 34 to 86% and in predicted amino acid sequences were 25 to 96%. The amplified fragments in this study represent only a part of these genes; thus, they might not have included all polymorphic sites, and the genetic variation with these chicken lines might be underestimated.

In comparison of the nucleotide sequences of the 10 genes that were available across all three species, chicken vs. human identity is 62.7%, whereas chicken vs. mouse is 62.8%. For amino acid sequence comparisons, chicken vs. human is 63.2%, and chicken vs. mouse is 62.6%. Thus, identities of nucleotide and amino acid sequences between chicken and human were generally close with those between chicken and mouse, in the 10 genes examined. For the four genes with chicken-mammalian amino acid identities above 75%, the nucleotide sequence identities were slightly lower (average 76%) than amino acid (average 86%). The reverse was observed for genes of low mammalian-chicken identity, in which amino acid identity was lower than that of nucleotides.

Among all sequence comparisons, identities of nucleotide and amino acid sequence were lowest (25 to 36%) between chicken *IL-2* and human/mouse *IL-2*. This finding is in agreement with work by several other laboratories. Kaiser and Mariani (1999) reported low identity between chicken and human *IL-2* amino acid sequence. Sundick and Gill-Dixon (1997) reported a cloned chicken lymphokine that had 24 and 25% amino acid identity to bovine IL-2 and IL-15, respectively. In the present study, sequence identities between chicken *IL-2* and human/mouse *IL-2* were slightly higher than those between chicken *IL-2* and human/mouse *IL-15* (data not shown). The genomic organization of chicken *IL-2* is similar to that of mammalian *IL-2*, not *IL-15* genes (Kaiser and Mariani, 1999). Choi et al. (1999) identified a cDNA encoding chicken *IL-15* that had 46% amino acid identity with bovine IL-15. Also, Tirunagaru et al. (2000) have recently identified a clone showing a significant match to mammalian IL-15 from a chicken liver cDNA library, which suggests that the sequence identified as chicken *IL-2* is truly *IL-2* or another related cytokine and not *IL-15*.

The *ZOV3* gene primers were developed from a cDNA of a chicken cDNA library, whose sequence had partial similarity to two different immunoglobulin superfamily

TABLE 2. Nucleotide (DNA) and predicted amino acid sequence identity percentage and number of amino acid (aa) replacements of Fayoumi, Genbank chicken, human, and mouse compared to chicken reference line Leghorn (G-B1)[1]

	Fayoumi			Database chicken			Human			Mouse			
	DNA	Amino acid	Replacement	DNA	Amino acid	Replacement	DNA	Amino acid	Length (aa)	Replacement	DNA	Amino acid	Length (aa)	Replacement
B2M	100	100	0	100	100	0	52	46	102	54	56	48	102	53
DAD1	100	100	0	100	100	0	73	81	106	19	73	79	106	22
IAP1	99	99	3	99	99	2	73	70	180	52	69	65	183	60
IL2	98	98	4	99	99	3	36	27	77	41	34	25	118	55
IREB1	100	100	0	100	100	0	77	83	210	32	76	83	210	32
LAP18	100	100	0	100	100	0	86	96	108	4	85	96	108	3
MAFL	100	100	0	99	99	1	69	67	92	30	53	57	172	31
RREB1	100	100	0	99	99	2	59	56	195	62	. . .	. . .	. . .	. . .
TAD	100	100	0	99	99	1	51	44	133	69	47	39	160	91
TBP1	100	100	0	99	99	2	69	82	242	7	74	88	220	9
ZOV3	98	98	3	99	99	1	41	36	119	73	61	46	176	93

[1]Note. Percentage identities were determined using BCM Search Launcher program. GenBank accession numbers for sequences and amino acids, respectively for gene *B2M* are:AB021288, P01884 (human), Y00441, P07151 (mouse); for gene *DAD1* are: D15057, P46966 (human), MMU83628, 149285 (mouse); for gene *IAP1* are: L49432, AAC41943.1 (human), U88908, O08863 (mouse); for gene *IL2* are: X01586, P01585 (human), K02292, P04351 (mouse); for *IREB1* gene are: Z11559, P21399 (human), X61147, P28271 (mouse); for gene *LAP18* are: J04991, P16949 (human), J04979, P13668 (mouse); for gene *MAFL* are: M95925, P54845 (human), L36435, P54841 (mouse); for gene *RREB1* are: D49835, BAA23165.1 (human); for *TAD* gene are: AF001622, AAC80267 (human), AF001104, AAC80266 (mouse); for *TBP1* gene are: M55654, P20226 (human), D01034, P29037 (mouse); for gene *ZOV3* are: X64364, P35613 (human), J03535, P21995 (mouse).

proteins: mouse GP-70 and chicken HT7 (Saitoh et al., 1993). Later, a similarity with human basigin was noted (Guo et al., 1998). Comparisons presented in Table 2 for *ZOV3* are, therefore, with different genes in each species, emphasizing the complexity of comparisons with the superfamily of immunoglobulin-like genes. No homologous mouse *RREB1* gene was identified in the database search (Table 2).

There are one to four replacement substitutions between the reference G-B1 Leghorn and Fayoumi or database chicken sequence in 7 of the 11 studied genes. These polymorphisms can be utilized in identification of functionally different polymorphisms. The number of replacement substitutions between chicken and human or mouse range from 3 to 91 and were inversely proportional to the identities, in general, except for three genes (*IL-2*, *TBP1*, and *RREB1*) for which there were large internal deletion or insertion occurrences between chicken and human/mouse sequences (Table 2).

Less than half of the studied genes are currently mapped (*B2M*, *DAD1*, *IREB1*, *ZOV3*) (Suzuki et al., 1999; Saitoh et al., 1993; Wang et al., 1997), and so the identification of single nucleotide polymorphisms (SNP) between these chicken lines offers an opportunity to linkage map the remaining polymorphic fragments in chickens. Also, sequence polymorphisms will make it possible to investigate control of biological traits by these genes in resource populations.

The Leghorn line (G-B1) and Fayoumi lines included in this study have been previously genetically characterized by microsatellite markers (Zhou and Lamont, 1999), DNA fingerprinting, and randomly amplified polymorphic DNA (Plotsky et al., 1995). Studies using these genomic screening techniques, which were not based on gene-specific sequences, estimated a very large genetic distance between these two lines. MHC Class I genes were also investigated by restriction fragment length polymorphism in these chicken lines (Chen and Lamont, 1992). Although gene sequences generally exhibit less polymorphism than anonymous markers, they allow utilization of gene sequence information from other species for comparative genetics study. Thus, the efficacy of use of the readily available gene-specific primers from the U.S. Poultry Genome Coordinator to examine gene sequences in poultry will allow rapid accumulation of structural and functional genomic information in the chicken.

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