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Assignment of *CCR7* gene to chicken chromosome 27 by radiation hybrid panel mapping*

TIAN Yong^{1,2}, LU Li-zhi², FU Yan¹, TAO Zheng-rong², SHEN Jun-da²,
 WANG De-qian², YUAN Ai-ping², YIN Zhao-zheng^{†‡1}

(¹Laboratory of Animal Genetics, College of Animal Sciences, Zhejiang University, Hangzhou 310029, China)

(²Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China)

[†]E-mail: yzhzh@zju.edu.cn

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Abstract: The protein encoded by CC chemokine receptor 7 (*CCR7*) is a member of the G protein-coupled receptor family. This receptor was identified as a gene induced by the Epstein-Barr virus (EBV), and is thought to be a mediator of EBV effects on B lymphocytes. This receptor is expressed in various lymphoid tissues and activates B and T lymphocytes. It has been shown to control the migration of memory T cells to inflamed tissues, as well as stimulate dendritic cell maturation. To map the *CCR7* gene in chicken chromosome, a 6000 rads chicken-hamster radiation hybrid panel (ChickRH6) was used. PCR of samples from ChickRH6 revealed that the location of *CCR7* gene is linked to the marker *SEQ0347* (6 cR away) with LOD score of 16.6 and that the marker *SEQ0347* is located on chromosome 27 at 27 cR of RH (radiation hybrid) map. We compared the corresponding human mRNA sequence with the predicted coding sequence of chicken *CCR7* gene, and found that the assembled contig shared a high percentage of similarity with that of the human gene.

Key words: Radiation hybrid panel, Mapping, *CCR7* gene, Chicken
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INTRODUCTION

Chemokine receptors are members of the family of seven transmembrane-spanning (7 TMS) proteins that signal through heterotrimeric G proteins upon ligand binding. At a total length of 340 to 370 amino acids, they are composed of seven hydrophobic transmembrane domains with an extracellular N-terminal segment and a cytoplasmic C-terminal tail containing structural motifs which are critical for ligand dependent signalling, desensitization, and receptor trafficking. Chemokine receptor nomenclature is simply based on the chemokine group (CC, CXC, C or CX3C) to which its ligand(s) belong (Rollins, 1997; Murphy *et al.*, 2000), and the protein

encoded by CC chemokine receptor 7 (*CCR7*) belongs to the CC group.

The gene for *CCR7* is on human chromosome 17q12-21.2 (Schweickart *et al.*, 1994). The open reading frame is on two exons separated by an intron in the N-terminal domain, and predicts a polypeptide of 378 amino acids. The chemokine (C-C motif) ligands 19 (CCL19/ECL) and 21 (CCL21/SLC) have been reported to be specific ligands of this receptor (Rossi *et al.*, 1997; Yoshida *et al.*, 1997; 1998). Consistent with the chemotactic action of CCL19/MIP-3 β and CCL21/SLC on lymphocytes and DCs (dendritic cells), *CCR7* expression can be demonstrated on T cells and on mature dendritic cells (Sallusto *et al.*, 1998; Willmann *et al.*, 1998; Jang *et al.*, 2006). Important functions of *CCR7* have been shown to control the migration of memory T cells to inflamed tissues and stimulate dendritic cell maturation (Dieu *et al.*, 1998; Sallusto and Lanzavecchia, 2000; Lipp

[‡] Corresponding author

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and Muller, 2003). In the CNS (central nervous system), microglia express CCR7 under specific inflammatory conditions (Dijkstra *et al.*, 2006). CCR7 activates two independent signaling modules, one involving G(i)-dependent activation of MAPK members ERK1/2, JNK and p38 and a hierarchy of MAPK family members and another involving Rho/Pyk2/cofilin, which control chemotaxis and the migratory speed of dendritic cells, respectively (Iijima *et al.*, 2005; Riolf-Blanco *et al.*, 2005; Okada and Cyster, 2007). It has been demonstrated that CCR7 is endocytosed into early endosomes containing transferrin receptor upon CCL19 binding, but less upon CCL21 triggering (Otero *et al.*, 2006). In spite of our knowledge of CCR7 in human, its functional and hereditary mechanism in chicken is not fully understood. In the present study, precise localization of *CCR7* gene was first performed by RH (radiation hybrid) mapping in chicken. We also compared the predicted coding region of chicken *CCR7* with that of the human gene.

MATERIALS AND METHODS

Primers design, PCR conditions and sequencing

The PCR primers for the chicken *CCR7* sequence were designed using Oligo 6.0 software (MBI Cascade Co., USA) supplied from Integrated Technologies Inc. (Coralville, IA, USA). Based on consensus sequences from human (GenBank accession No. NC_000017) and mouse (GenBank accession No. NC_000077), primers were designed to amplify chicken *CCR7* genomic sequence (Table 1).

The PCR was performed in a 25 µl reaction mixture containing 0.8 U Taq DNA polymerase (Takara, Dalian, China), 1×PCR buffer, 1.5 mmol/L MgCl₂, 200 µmol/L of each dNTP, 0.5 µmol/L of each primer and approximately 50 ng of genomic

DNA. The thermal cycling profile was 95 °C for 5 min, 35 cycles of 94 °C for 45 s, 57 °C for 45 s and 72 °C for 1 min, with final extension of 72 °C for 10 min. The amplified product was analyzed by electrophoresis on 1.5% agarose gel in 0.5×TBE buffer and sequenced in 3730 DNA analyzer (Applied Biosystems, CA, USA). A 749 bp nucleotide sequence was obtained. We have deposited the sequence in GenBank (GenBank accession No. EF092460). Identity of the PCR product was confirmed by sequence analysis. Using previously described sequence, new primers of chicken *CCR7* gene were designed for RH mapping (Table 1). In all cases, the primers designed for RH mapping only amplified chicken genomic DNA, but not hamster controls.

Radiation hybrid mapping

Chromosomal localization was performed with the INRA chicken radiation hybrid panel (ChickRH6) containing 90 hamster-chicken hybrid cell lines (Morisson *et al.*, 2002). The PCR was performed in 10 µl reactions containing 30 ng of genomic DNA, 0.3 U Taq DNA polymerase (Takara, Dalian, China), 1×PCR buffer, 1.5 mmol/L MgCl₂, 200 µmol/L of each dNTP, 0.5 µmol/L of each primer. The thermal cycling profile was 95 °C for 5 min, 35 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min, with final extension of 72 °C for 10 min. The amplified product was 517 bp and separated on 1.5% agarose gel in 0.5×TBE buffer. The gene was typed in duplicate with the chicken ChickRH6 panel, and the results are scored as present (1) and absent (0), or ambiguous (2) as directed (Morisson *et al.*, 2002). Two-point results were calculated and multipoint radiation hybrid maps (see APPENDIX A) were built with ChickRH server (<http://chickrh.toulouse.inra.fr/>). Linkage groups were assigned using a minimum LOD score threshold of 2.0. The RH mapping data are summarized in Table 2.

Table 1 Primers used for isolating and mapping *CCR7* gene in chicken

Gene	GenBank accession No. (chicken/human)	Sequence similarity to human (%) ^a	Primer sequences (5'→3')	Size (bp)
<i>CCR7</i>	EF092460/NM_001838	76	PF ^b : GAGATGATGTGTGAGAAGAAGG	749
			PR ^{bc} : CTGGTGTGTTGAAGACTGAG	
			PF ^c : GGTGTTTTGGAGTATTTCCT	517

^aIdentity scores after comparison of predicted coding region with the human sequence; ^bPrimers used for sequencing; ^cPrimers used for mapping

Table 2 Chromosome assignment of *CCR7* gene

Gene	Chicken RH mapping result					
	RF ^d (%)	Closest marker	Chromosome	RH map (cR) ^e	Distance (cR) ^f	LOD score
<i>CCR7</i>	22	<i>SEQ0347</i>	27	27	6	16.6

^dRetention frequency; ^eChromosome localization of the closest marker on the RH map (RH map units); ^fDistance between the *CCR7* gene and the closest marker (RH map units) with two-point analysis

RESULTS AND DISCUSSION

The radiation hybrid panel showed the following distribution of positive and negative amplifications within the 90 clones: 00000 00000 00000 00010 00010 00001 01001 00000 01000 00100 10000 01111 00000 10010 00200 01100 01101 10000 for *CCR7*. A retention frequency (RF) of 22% was calculated based on twenty chicken-specific PCR bands, and this RF value is higher than the mean RF of 21.9% observed in other studies reported in this panel (Morisson *et al.*, 2002). Two-point LOD scores showed that *CCR7* is linked to *SEQ0347* (6 cR away) with an LOD score of 16.6. The marker *SEQ0347* is located on chromosome 27 at 27 cR of RH map (Table 2). Comparing the human mRNA sequence with the predicted coding sequence of chicken *CCR7* gene using bl2seq software provided by NCBI server (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi>), we found that the assembled contig shared a high percentage of similarity with that of the human gene. The results revealed that the expected chicken gene sequence was isolated.

In conclusion, our present results revealed that the chicken *CCR7* gene is located on chromosome 27 at 6 cR of the marker *SEQ0347* (LOD score 16.6). This study suggests a new method for *CCR7* gene mapping in chicken.

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APPENDIX A

Fig.A1 The location of CCR7 gene in chicken chromosome GGA27