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

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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 maker *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 hydrid) 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-3β 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; Willimann *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

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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; Riol-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 $MgCl_2$, 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 $MgCl_2$, 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)
CCR7	EF092460/NM_001838	76	PF[b]: GAGATGATGTGTGAGAAGAAGG	749
			PR[bc]: CTGGTGTTGTTGAAGACTGAG	
			PF[c]: GGTGTTTTGGAGTATTTGCCT	517

[a]Identity scores after comparison of predicted coding region with the human sequence; [b]Primers used for sequencing; [c]Primers 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
CCR7	22	*SEQ0347*	27	27	6	16.6

[d]Retention frequency; [e]Chromosome localization of the closest marker on the RH map (RH map units); [f]Distance 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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References

Dieu, M.C., Vanbervliet, B., Vicari, A., Bridon, J.M., Oldham, E., Ait-Yahia, S., Briere, F., Zlotnik, A., Lebecque, S., Caux, C., 1998. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J. Exp. Med.*, **188**(2):373-386. [doi:10.1084/jem.188.2.373]

Dijkstra, I.M., de Haas, A.H., Brouwer, N., Boddeke, H.W., Biber, K., 2006. Challenge with innate and protein antigens induces CCR7 expression by microglia in vitro and in vivo. *Glia*, **54**(8):861-872. [doi:10.1002/glia.20426]

Iijima, N., Yanagawa, Y., Clingan, J.M., Onoe, K., 2005. CCR7-mediated c-Jun N-terminal kinase activation regulates cell migration in mature dendritic cells. *Int. Immunol.*, **17**(9):1201-1212. [doi:10.1093/intimm/dxh297]

Jang, M.H., Sougawa, N., Tanaka, T., Hirata, T., Hiroi, T., Tohya, K., Guo, Z., Umemoto, E., Ebisuno, Y., Yang, B.G., *et al*., 2006. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. *J. Immunol.*, **176**(2):803-810.

Lipp, M., Muller, G., 2003. Shaping up adaptive immunity: the impact of CCR7 and CXCR5 on lymphocyte trafficking. *Verh. Dtsch. Ges. Pathol.*, **87**:90-101.

Morisson, M., Lemiere, A., Bosc, S., Galan, M., Plisson-Petit, F., Pinton, P., Delcros, C., Feve, K., Pitel, F., Fillon, V., *et al*., 2002. ChickRH6: a chicken whole-genome radiation hybrid panel. *Genet. Sel. Evol.*, **34**(4):521-533. [doi:10.1051/gse:2002021]

Murphy, P.M., Baggiolini, M., Charo, I.F., Hebert, C.A., Horuk, R., Matsushima, K., Miller, L.H., Oppenheim, J.J., Power, C.A., 2000. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol. Rev.*, **52**(1):145-176.

Okada, T., Cyster, J.G., 2007. CC chemokine receptor 7 contributes to Gi-dependent T cell motility in the lymph node. *J. Immunol.*, **178**(5):2973-2978.

Otero, C., Groettrup, M., Legler, D.F., 2006. Opposite fate of endocytosed CCR7 and its ligands: recycling versus degradation. *J. Immunol.*, **177**(4):2314-2323.

Riol-Blanco, L., Sanchez-Sanchez, N., Torres, A., Tejedor, A., Narumiya, S., Corbi, A.L., Sanchez-Mateos, P., Rodriguez-Fernandez, J.L., 2005. The chemokine receptor CCR7 activates in dendritic cells two signaling modules that independently regulate chemotaxis and migratory speed. *J. Immunol.*, **174**(7):4070-4080.

Rollins, B.J., 1997. Chemokines. *Blood*, **90**(3):909-928.

Rossi, D.L., Vicari, A.P., Franz-Bacon, K., McClanahan, T.K., Zlotnik, A., 1997. Identification through bioinformatics of two new macrophage proinflammatory human chemokines: MIP-3alpha and MIP-3beta. *J. Immunol.*, **158**(3):1033-1036.

Sallusto, F., Lanzavecchia, A., 2000. Understanding dendritic cell and T-lymphocyte traffic through the analysis of

chemokine receptor expression. *Immunol. Rev.*, **177**(1): 134-140. [doi:10.1034/j.1600-065X.2000.17717.x]

Sallusto, F., Schaerli, P., Loetscher, P., Schaniel, C., Lenig, D., Mackay, C.R., Qin, S., Lanzavecchia, A., 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol.*, **28**(9): 2760-2769. [doi:10.1002/(SICI)1521-4141(199809)28:09<2760::AID-IMMU2760>3.0.CO;2-N]

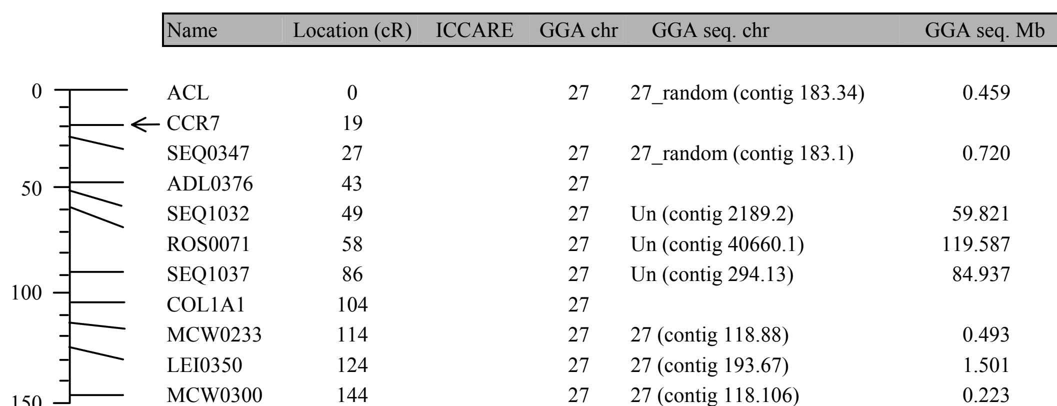
Schweickart, V.L., Raport, C.J., Godiska, R., Byers, M.G., Eddy, R.L.Jr, Shows, T.B., Gray, P.W., 1994. Cloning of human and mouse EBI1, a lymphoid-specific G-protein-coupled receptor encoded on human chromosome 17q12-q21.2. *Genomics*, **23**(3):643-650. [doi:10.1006/geno.1994.1553]

Willimann, K., Legler, D.F., Loetscher, M., Roos, R.S., Delgado, M.B., Clark-Lewis, I., Baggiolini, M., Moser, B., 1998. The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7. *Eur. J. Immunol.*, **28**(6): 2025-2034. [doi:10.1002/(SICI)1521-4141(199806)28:06<2025::AID-IMMU2025>3.0.CO;2-C]

Yoshida, R., Imai, T., Hieshima, K., Kusuda, J., Baba, M., Kitaura, M., Nishimura, M., Kakizaki, M., Nomiyama, H., Yoshie, O., 1997. Molecular cloning of a novel human CC chemokine EBI1-ligand chemokine that is a specific functional ligand for EBI1, CCR7. *J. Biol. Chem.*, **272**(21):13803-13809. [doi:10.1074/jbc.272.21.13803]

Yoshida, R., Nagira, M., Kitaura, M., Imagawa, N., Imai, T., Yoshie, O., 1998. Secondary lymphoid-tissue chemokine is a functional ligand for the CC chemokine receptor CCR7. *J. Biol. Chem.*, **273**(12):7118-7122. [doi:10.1074/jbc.273.12.7118]

APPENDIX A:



Name	Location (cR)	ICCARE	GGA chr	GGA seq. chr	GGA seq. Mb
ACL	0		27	27_random (contig 183.34)	0.459
CCR7	19				
SEQ0347	27		27	27_random (contig 183.1)	0.720
ADL0376	43		27		
SEQ1032	49		27	Un (contig 2189.2)	59.821
ROS0071	58		27	Un (contig 40660.1)	119.587
SEQ1037	86		27	Un (contig 294.13)	84.937
COL1A1	104		27		
MCW0233	114		27	27 (contig 118.88)	0.493
LEI0350	124		27	27 (contig 193.67)	1.501
MCW0300	144		27	27 (contig 118.106)	0.223

Fig.A Supplementary material indicates the location of *CCR7* gene in chicken chromosome GGA27