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ASSIGNMENT FOR GENES ENCODING THE TERMINAL COMPLEMENT COMPONENTS TO PORCINE CHROMOSOME

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Abstract: One of the major goals of the porcine genome projects is building a physical map. To assign the porcine genes encoding the complement components C6, C7, C8 and C9 to porcine chromosomes, we used a porcine 7000Rad Radiation Hybrid panel (IMpRH) containing 118 clones provided by INRA-University of Minnesota. It resulted in assignment of the porcine C6, C7 and C9 genes to chromosome 16q1.4, the porcine C8A and C8B genes to chromosome 6q3.1-q3.5 as well as the porcine C8G gene lonely to chromosome 1q2.13.

Key words: IMpRH mapping, C6, C7, C8A, C8B, C8G, C9, pig

Introduction

The assignment of genes using radiation hybrid (RH) panels is an efficient way to map genes and markers as well as to integrate the linkage and cytogenetic maps of a species (*Hawken et al., 1999; Yerle et al., 1998*). RH mapping enhances linkage map reliability because of unambiguous determination of marker order and provides a powerful tool for fine mapping (*Yerle et al., 1998*). Moreover, radiation hybrid allows gene assignments without the detection of genetic polymorphism as needed for linkage mapping. Genes encoding the complement factors of the terminal lytic sequence of the complement cascades are considered as candidate genes for immune traits and disease resistance. We aimed at mapping the porcine genes C6, C7, C8A, C8B, C8G, and C9 to porcine chromosomes by using IMpRH tool.

Materials and methods

Preparation of genomic DNA from tail or ear samples was performed by standard procedures involving Proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. Therefore, the samples were cut into small pieces of 2-3 mm, weighed about 0.1g, and then placed in 2 ml tube containing 700 µl of digestion buffer. To lyse the cells and digest proteins, 35 µl of Proteinase K solution (20 mg/ml) was added and the samples were then incubated at 55°C overnight with mixing. Addition of an equal volume of phenol-chloroform (1:1 v/v, 700 µl for each) was done thereafter. The two phases were mixed until they formed a homogenous emulsion and separated by centrifuging at 5000 rpm for 3 min at 4°C. The aqueous phase was collected in fresh tubes. Phenol-chloroform extraction was repeated and always followed by a chloroform extraction. One-tenths volume of sodium acetate (3 M, pH 6.0) and an equal volume of isopropanol (700 µl) were put in. The samples were shaken gently until precipitation of DNA. The DNA pellet was, then, washed three times with 1 ml ethanol (70%) and dried at room temperature. Finally, the DNA was resuspended in 1× TE buffer and stored at 4°C for further analysis.

In order to perform physical mapping of the candidate genes the INRA-University of Minnesota porcine 7000Rad Radiation Hybrid panel (IMpRH) containing 118 pig/ hamster DNA hybrid clones (*Hawken et al. 1999, Yerle et al. 1998)* was employed. Based on accession number sequence DQ333199, NM_214282, 5´flanking region of clone XX-1C1, DQ333201, DQ333202 and DQ333198, specific primer pairs, which allowed amplifying DNA fragments of 159 to 707 bp for mapping C6, C7, C8A, C8B, C8G and C9 were derived. Amplicon design, primer sequences and PCR conditions are summarized in table 1. Prior to mapping, PCR conditions were optimized so that specific amplification of porcine DNA but not DNA of the hamster parental lines or amplification of fragments unambiguosly distinguishable between the two species was achieved. The expected porcine DNA fragments were, then, sequenced to verify their identity.

PCR reaction was performed in a total 15 µl reaction mixture containing 25 ng of hybrid DNA, 100 µM of each dNTP, 0.1 µM of each primer, 1×supplied PCR buffer containing 1.5 mM $MgCl_2$ and 1 U Taq polymerase. PCR reactions were prepared for 118 DNA templates of the IMpRH panel (positive), a hamster DNA template (negative control), a blank-template without DNA (negative control) and a porcine genomic DNA (positive control). The PCR products were

amplified using standardized thermal profile as follows: 4 min of initial denaturation at 94°C, 40 cycles at 94°C for 30 sec, at the annealing temperature 58°C or 60°C (depending on the specific primer pairs shown in Table 1) for 30 sec, at 72°C for 1 min and a final extension at 72°C for 5 min. For mapping of the C7 gene a modified, touch-down thermal profile was employed: 94°C for 4 min, followed by 9 cycles at 94°C for 30 sec, annealing temperature from 60-51°C (-1°C per cycle) for 30 sec, 72°C for 1 min, followed by 40 cycles of 94°C for 30 sec with annealing temperature at 50°C for 30 sec, at 72°C for 1 min, ending with an extension step at 72°C for 5 min.

The entire PCR reactions were separated on 3% agarose gel stained with ethidium bromide and amplification products were independently scored as present (1), absent (0), or ambiguous (?). The results translated into vector format for submitting to IMpRH mapping tool. The PCR-screening of the IMpRH panel was performed twice. For ambiguous data, the PCR was repeated to minimize genotyping errors and the remaining discrepancies scored as ambiguous (Figure 1). Two-point linkage analysis was done using the IMpRH mapping tool available at the IMpRH Web Server (http://imprh.toulouse.inra.fr).

Linkage mapping of candidate genes was performed using CRIMAP version 2.4 *(Green et al. 1990).* Therefore, genotype information of the respective complement genes and of microsatellite markers previously obtained of the DUMI resource population was used (*Wimmers et al., 2002*).

Results and discussion

In this study, to determine the location and order of certain candidate genes, we used the IMpRH panel, which allows assigning unambiguously the six loci to porcine chromosomes from pig/hamster genomic DNA amplification products converted into vector sequences.

Two-point RH analyses were used for the identification of linkage groups using LOD score threshold of 5.0. With all information for markers, including vectors summarized in table 3, it was demonstrated that both porcine C8A and C8B loci were physically assigned to the same chromosome 6q3.1-q3.5 whereas the porcine C6, C7 and C9 genes were closely linked on the same q-arm of chromosome 16 (16q1.4). C8G was mapped to chromosomes 1q2.13. Moreover, the porcine C6, C7, C8A, C8B, C8G and C9 significantly linked to markers S0077, SW1069, SW322, SSC10D08 and SW403, respectively (Figure 2). All LOD scores are greater than 6. These were in a strong agreement with genetic linkage and chromosomal localization studies previously established that the porcine C8A and

C9 are located on chromosome 6q3.1-q3.3 *(Nakajima et al. 1998)* and 16q1.4 *(Thomsen et al. 1998)* using the porcine bacterial artificial chromosome (BAC) clone as a hybridization probe and fluorescence *in situ* hybridization, respectively. Genetic linkages using two-point analysis from CIRMAP 2.4 *(Green et al. 1990)* were confirmed between C7 and C6 with rec. fracs. = 0.02, LOD = 50.45, between C9 and C6 with rec. fracs = 0.06, LOD = 31.50, between C9 and C7 with rec. fracs = 0.06, LOD = 35.84, between C8B and C8A with rec. fracs = 0.05, LOD = 65.72.

Table 1. Oligonucleotide primers used for radiation hybrid mapping

Primer name (Length, bp)	Primer sequence (genome localization, exon)	Tm (°C)
C6 (159)	up 5´-3´: ttcctttttgcaaggatcaga (nt. 1437-1457, 10) down 3´-5´: tcaatcacagcaggattttcc (nt. 1575-1595, 10)	58
C7 (196)	up 5´-3´: agttatcagttgttggttgttca (nt. 739-761, 8) down 3´-5´: ctcctcctaaggacccagac (nt. 915-931, 8)	50 (*)
C8A (707)	up 5´-3´: tgcttctggaggtgttcattt (clone xx-1c1) down 3´-5´: cggttcaccttctcctgtatg (clone xx-1c1)	60
C8B (160)	up 5´-3´: gaaacaagagaagcagcatgg (nt. 1302-1322, 9) down 3´-5´: ttaattttgatgatgtctgggttg (nt. 1438-1461, 9)	60
C8G (295)	up 5´-3´: cctcttgacgctgctcct (nt. 75-92, 1) down 3´-5´: gagccacgtgcagtgaagt (nt. 268-286, 2)	58
C9 (511)	up 5´-3´: ggagcattgagacctttgga (nt. 283-302, 3) down 3´-5´: gccagctcagactcttccac (nt. 528-547, 4)	60

(*) A touch down PCR program was used to amplified a fragment of 196 bp under following thermal cycling conditions: 94°C for 4 min, followed by 9 cycles at 94°C for 30 sec, annealing temperature step-downs every 1 cycle of 1°C (from 60-51°C) for 30 sec, at 72°C for 1 min, then followed by 40 cycles of 94°C for 30 sec, at 50°C for 30 sec, at 72°C for 1 min, and ending with an extension step at 72°C for 5 min.

Table 2. Chromosomal assignments of the genes in difference species

Gene	Mapping localization							
	B. taurus	*D. rerio*	*G. gallus*	*H. sapiens*	*M. mulatta*	*M. musculus*	*R. norvegicus*	*P. troglodytes*
C6	20	21	-	5p13	6	15A1	2q16	5
C7	20	21	Z	5p13	-	15A1	2q16	5
C8A	3	-	-	1p32	1	4C6	5q34	1
C8B	3	-	8	1p32	1	4C6	5q34	1
C8G	-	24	-	9q34.3	-	2A3	3p13	-
C9	1	5	-	5p14-p12	6	15A1	2q16	-

Genetic mapping correspondingly revealed linkage of the porcine C6 and C7 to C9 and AGXT2 that have previously been assigned to the q-arm of chromosome 16 (*Ponsuksili et al. 2001, Thomsen et al. 1998, Wintero et al. 1998*) while on chromosome 6q3.1-q3.5, C8B links closed to the porcine C1q and C8A that have been reported by *Jorgensen et al. (1997)* and *Nakajima et al. (1998),* respectively. This is in agreement with the most recent human-porcine comparative map *(Meyers et al. 2005).*

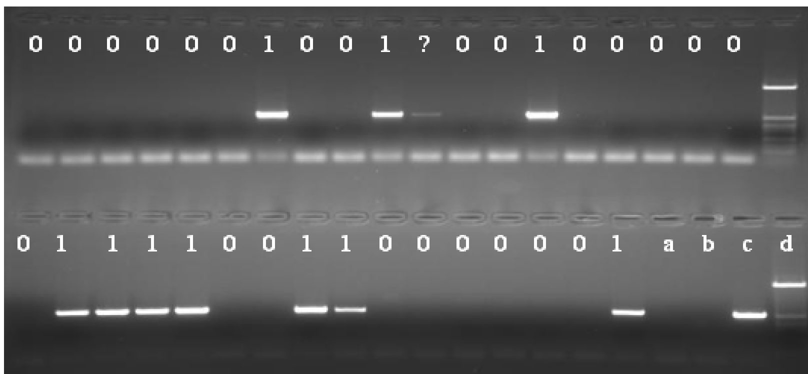

Figure 1. A representative pattern for PCR result of 118 DNA clones of the IMpRH. The symbols (0), (?), and (1) used for absent, ambigous and present results, respectively. The letters a, b, c, and d show a hamster sample (negative control), a blank sample without DNA (negative control), a pig DNA (positive control) and a marker to estimate length of DNA fragments.

The mapping results also fit the current pig-mouse and pig-rat comparative maps as accessible via the MGI webpage (http://www.informatics.jax.org/searches/homology_form.shtml). Further, the location of distinct C8 loci on chromosomes supports genetic evidence that C8 contains three separate genes encoding different proteins C8A, C8B and C8G.

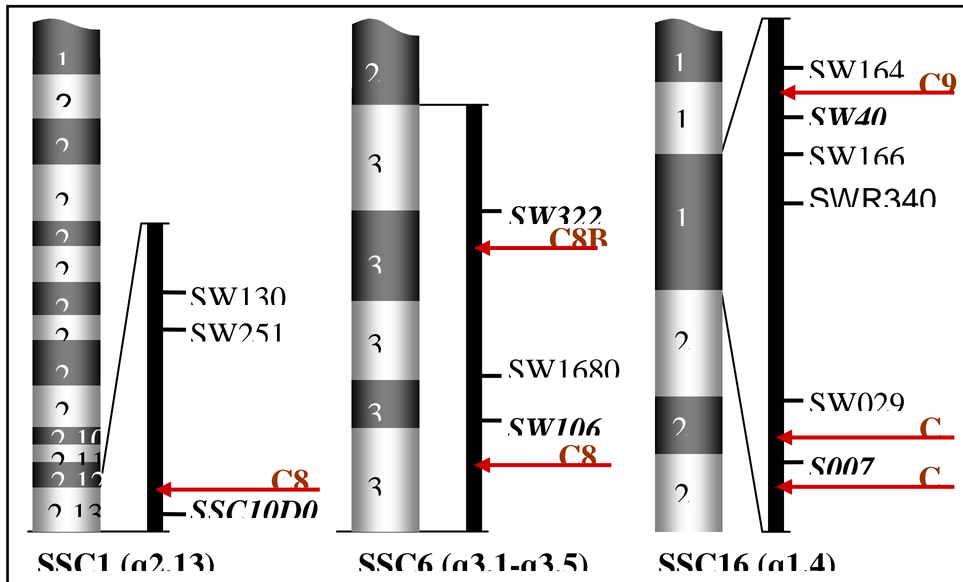

Figure 2. Position of the candidate genes on porcine chromosomes. Linkage is closed to markers in bold and italic

Table 3. Summary of RH mapping results of the candidate genes

Gene	Result in vector	LOD score, distance in cR, retention fraction (%), SSC
C6	000010000000100110000111100011100100100011001101 00 0001101001001101101101101110000000011100000000010000 1000010111111111111100	18.13, 21, 42, 16q1.4
C7	1101100000001000100000011100010?0010010001100100101 0000000000001100101101101110000000011100000000010000 1000010111111111111011	16.65, 23, 38, 16q1.4
C8A	0010000101011000000001110000010000000000000000100010 0000000000000100001101000000000101101000110000000 1 0010110000000000010001	7.00, 56, 22, 6q3.1-q3.5
C8B	0010000101011000000011110000010000000001000000100010 000000000000010000110001000000010110100011000000001 0010110000000000010001	8.18, 50, 24, 6q3.1-q3.5
C8G	0000000000001000011000000000000000000000001000010000 0001000000011000000000000000000000011000000100110000 0000001010000100101 0	8.26, 44, 15, 1q2.13
C9	1101100000001000100000000100010001100100010000000100 0000001000000100010110110101000000001000000000011000 1000110111111111000101	14.45, 27, 31, 16q1.4

Looking on the genetic maps of other species, C6 and C7 are mapped to the same chromosome while C8A and C8B components are always found on the same chromosome. Additionally, C9 complement component could be assigned to the same chromosome carrying C6 and C7 in all species addressed here except in *B. taurus* and *D. rerio*. Especially, C8G gene is always located in a different chromosome (Table 2).

In summary, the study revealed four new assignments and confirmed previous results.

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Zadatak za gene koji kodiraju terminalne komplementarne komponente hromozoma svinja

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Rezime

Jedan od glavnih ciljeva projekata koji se bave genomom svinje je stvaranje fizičke mape. Da biste dodelili gene svinja koji kodiraju komplementarne komponente C6, C7, C8 i C9 hromozomu svinje, koristili smo 7000Rad Radiation Hybrid panel (IMpRH) koji sadrži 118 klonova koje je obezbedio INRA-Univerzitet Minesota. To je rezultiralo u dodeli C6, C7 i C9 geni svinje hromozomu 16q1.4, C8A i C8B gena hromozomu 6q3.1-q3.5, kao i gena C8G hromozomu 1q2.13.

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