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QTL for traits related to humoral immune response estimated from data of a porcine F2 resource population

K. Wimmers,* E. Murani,* K. Schellander† & S. Ponsuksili‡

Summary

This study aimed to map quantitative trait loci (QTL) for traits related to humoral innate immune defence. Therefore, haemolytic complement activity in the alternative and the classical pathway, serum concentration of C3c and of haptoglobin (HP) were measured in blood samples obtained from F2 piglets ($n = 457$) of a porcine F2 resource population before and after *Mycoplasma hyopneumoniae*, Aujeszky's disease virus (Suid herpesvirus I, SuHVI) and porcine reproductive and respiratory syndrome virus (PRRSV) vaccination at 6, 14 and 16 weeks of age. Animals were genotyped at 88 autosomal markers. QTL analysis was performed under the line cross and the half sib. Phenotypic data were adjusted for systematic effects by mixed models with and without repeated measures statement. In total, 46 and 21 estimated QTL positions were detected with genome-wide significance at the 0.05 and 0.01 level, respectively. The proximal region of SSC2 (orthologous to HSA11 0–70 Mb), the distal region of SSC4 (HSA1 95–155 Mb), and the intermediate region of SSC16 (HSA5 0–73 Mb and 150–174 Mb) showed a clustering of estimated QTL positions for complement activity based on the different models. A common genetic background, i.e. a single true QTL, might underlie these QTL positions for related traits. In addition, QTL for antibody titres were detected on SSC1, 2, 6 and 7. With regard to number and magnitude of their impact, QTL for humoral innate immune traits behave like those for other quantitative traits. Discovery of such QTL facilitates the identification of candidate genes for disease resistance and immune competence that are applicable in selective breeding and further research towards improving therapeutic and prophylactic measures.

* Research Institute for the Biology of Farm Animals (FBN), Research Unit 'Molecular Biology', Dummerstorf, Germany,
† Institute of Animal Science, University of Bonn, Bonn, Germany,
‡ Research Institute for the Biology of Farm Animals (FBN), Research Group 'Functional Genomics', Dummerstorf, Germany

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Correspondence: K. Wimmers, Research Institute for the Biology of Farm Animals (FBN), Research Unit 'Molecular Biology', 18196 Dummerstorf, Germany. Tel: +49 398 2086 8700; Fax: +49 38 2086 8702;
E-mail: wimmers@fbn-dummerstorf.de

Introduction

Variation of susceptibility to infectious diseases has successfully been used to identify loci modulating response to specific diseases (Staeheli *et al*., 1986; Vögeli *et al*., 1999; Peng *et al*., 2007; Uthe *et al*., 2007). However, since major health problems in intensive as well as animal welfare friendly production systems arise from multifactorial diseases, there is a need for animals with optimal endogenous protection against many pathogens including intra or extracellular microorganisms. Selection experiments and surveys of parameters of humoral and cellular immunity in mice, cattle, chicken and pig show partly unfavourable correlations between some immune traits implying that selection for a balanced immune response is necessary (Biozzi *et al*., 1979; Biozzi, 1982; van der Zijpp *et al*., 1983; van der Zijpp, 1983; Buschmann *et al*., 1985; Harmon *et al*., 1985; Edfors-Lilja *et al*., 1994; Reber *et al*., 2006). The selection of pigs for high and low immune responsiveness, using an index of five immune parameters of acquired and innate humoral and cellular mechanisms, showed improvement of protection and of responsiveness to numerous pathogens and of growth performance (Mallard *et al*., 1992, 1998). Identification of genes or markers linked to genes for disease resistance and immune competence facilitates the genetic improvement of animal health.

First attempts to identify quantitative trait loci (QTL) for immune responsiveness were in F2 mice, derived from progenies divergently selected for high and low antibody response, 'Biozzi-mice' (Puel *et al*., 1995, 1998). From this QTL study it was estimated that 10 loci are involved in the genetic control of the trait, each explaining some 10% of the phenotypic variation (Puel *et al*., 1995, 1998). In pigs, QTL for cellular and humoral immune response (leucocyte counts, phagocytosis, mitogen-induced proliferation, IL-2 production, interferon-alpha production, antibody response) were identified by Edfors-Lilja and colleagues (Edfors-Lilja *et al*., 1998, 2000; Edfors-Lilja, 1999; Wattrang *et al*., 2005). QTL for haematological traits including white blood cell counts and traits related to erythrocyte counts before and after *Sarcocystis miescheriana* infection were detected that are relevant to immune capacity as well as QTL for loci controlling defence against this parasitic infection (Reiner *et al*., 2007a,b, 2008).

Liver largely contributes to the overall metabolic status of the organism. All nutrients ingested pass the liver but also many pathogens. Consequently, the liver shows particular immunological functions that are increasingly recognized (Li & Diehl, 2003). These functions cover locally regulated immune response mechanisms that are adapted to the requirement of efficient elimination of pathogenic microorganisms and of tolerization of the large number of antigens derived from the gastrointestinal tract (Knolle & Gerken, 2000). The liver has one of the largest resident populations of macrophages (Kupffer cells), natural killer (NK) cells, and natural killer T cells (NKT), all of which are key components of the innate immune system (Li & Diehl, 2003). The liver is also the major site of expression of complement factors and acute phase proteins (APP), components of acute systemic response to injury and inflammation. The complement system is a highly regulated and complex set of interacting proteins in blood plasma and on cell surfaces, capable of direct killing of microorganisms and of modulating phagocytosis, inflammation, humoral and cellular immune response as well as graft rejection. It is activated, either in the presence (classical pathway), or in absence of specific antibodies (alternative and lectin pathway). The acute phase reaction (APR) is an early physiological systemic reaction to local or systemic disturbances such as infection, injury or immunological disorders. APR involves release of proinflammatory cytokines that alters synthesis of liver-derived serum proteins, APP. In the pig, in the course of an inflammatory state, APR is marked by a very pronounce response of the major acute phase protein (MAP) but also of C-reactive protein (CRP) and haptoglobin (HP) (Lampreave *et al.*, 1994; Eckersall *et al.*, 1996; Miller *et al.*, 2008).

We have previously shown association of complement genes and other immune relevant genes with complement activity and acute phase response (Wimmers *et al.*, 2003, 2004; Kumar *et al.*, 2004; Phatsara *et al.*, 2007). Here we report for the first time a QTL analysis for complement activity and acute phase response, i.e. hepatic mechanisms of innate humoral immune defence.

Materials and methods

Animals

A three-generation porcine F2 resource population (DUMI) was examined that is based on reciprocal cross of Duroc and Berlin Miniature Pig (Hardge *et al.*, 1999). F2 animals (n = 474, comprising 21 full-sib families) born from 11 F1 sows mated to three F1 boars with at maximum five parities per sow were reared at the Frankenforst research farm, Institute of Animal Breeding and Genetics, University of Bonn. According to veterinary regulations sows were vaccinated against Aujeszky's disease (AD) in the pig production units. Only samples of animals that showed no clinical signs of any disease were included in the experiment (n = 457). Corresponding to the routine vaccination program of herds kept at the Frankenforst research farm, F2 animals of the experimental population received a *Mycoplasma hyopneumoniae* (MH) vaccination at 7 days of life. Following, the F2 animals were immunized with MH once again (Stellamune Mycoplasma, Pfizer, Karlsruhe, Germany; a killed vaccine containing inactivated MH), and against Aujeszky's disease virus (ADV) (Porcilis, Begonia Diluvac, Intervet, Tönisvorst, Germany; a freeze-dried live attenuated vaccine containing ADV strain Begonia) and porcine reproductive and respiratory syndrome virus (PRRSV) (Ingelvac PRRS MLV, Boehringer Ingelheim, Germany; a live vaccine based on the European PRRSV strain that is assumed to provoke a less intense immune response than the US PRRSV) vaccines at 6, 14 and 16 weeks of age, respectively. Blood samples were taken immediately prior to immunization (day 0) and at day 4 and 10 after MH and ADV vaccination, but only at day 10 after PRRSV vaccination (Table 1).

Phenotypes

Classical (CH50) and alternative (AH50) haemolytic complement activity, C3c and HP APP serum concentrations as well as antibody titres were measured in serum/plasma samples of members of the 21 full-sib families of the F2 population at different time points as indicated in Table 1 (Wimmers *et al.*, 1999, 2003). In brief, for measuring classical complement activity the serum titre was determined that lyses 50% of sensitized sheep red blood cells (CH50 U mL^{-1}), but for alternative complement activity natural rabbit red blood cells were used (AH50 U mL^{-1}). C3c and HP serum concentrations were measured by immunonephelometry using a Behring Nephelometer System and antihuman-antisera (Dade Behring GmbH, Marburg, Germany). Results are given as concentration of C3c or HP (mg mL^{-1}). ELISA assays were used that nearly exclusively detect immunoglobulin G subclass antibodies. In particular, antibody response to MH and PRRSV vaccine was determined by ELISA using DAKO *Mycoplasma hyopneumoniae* ELISA kit (Dako Diagnostik GmbH, Hamburg, Germany) and the Idexx HerdCheck PRRS ELISA (Idexx GmbH, Wörrstadt, Germany), respectively; antibody titre against ADV vaccine was measured with an ELISA established in house using Aujeszky vaccine as an antigen (Wimmers *et al.*, 2004).

Markers and QTL analysis

Animals of the DUMI population were genotyped at 88 loci covering the porcine autosomes with mean interval size of 30.7 cM. The set of markers includes 72 microsatellites and 16 biallelic markers. Linkage analysis was performed using the program CRI MAP, version 2.4 (Green *et al.*, 1990). The order of markers and the genetic distances between them are given in Table 2.

The QTL analysis was carried out using QTLexpress (Seaton *et al.*, 2002; QTLexpress [http://qtl.cap.ed.ac.uk/]) by interval mapping based on least square regression analysis developed for three generation F2 populations and half-sib families (Haley *et al.*, 1994; Knott *et al.*,

Table 1. Means and standard errors of haemolytic complement activity in the alternative and classical pathway (U mL^{-1}), C3c and HP serum concentration (mg mL^{-1}), and antibody titres observed before (day 0) and after (day 4, 10) *Mycoplasma hyopneumoniae* (MH) , Aujeszky's disease virus (ADV) and porcine reproductive and respiratory syndrome virus (PRRSV) immunizations

Vaccination	MH			ADV			PRRSV	
Sampling time	Day 0	Day 4	Day 10	Day 0	Day 4	Day 10	Day 0	Day 10
In vitro haemolytic complement activity in alternative pathway (AH50)								
Trait	AH50$_{MH0}$	AH50$_{MH4}$	AH50$_{MH10}$	AH50$_{ADV0}$	AH50$_{ADV4}$	AH50$_{ADV10}$	AH50$_{PRRS0}$	AH50$_{PRRS10}$
Mean ± SE	53.64 ± 1.62^{ab}	53.22 ± 1.58^{a}	55.05 ± 1.37^{ab}	56.86 ± 1.62^{b}	62.31 ± 1.74^{c}	68.46 ± 2.62^{d}	69.75 ± 2.31^{d}	69.71 ± 2.03^{d}
n	304	323	325	346	339	328	323	339
In vitro haemolytic complement activity in classical pathway (CH50)								
Trait	CH50$_{MH0}$	CH50$_{MH4}$	CH50$_{MH10}$	CH50$_{ADV0}$	CH50$_{ADV4}$	CH50$_{ADV10}$	CH50$_{PRRS0}$	CH50$_{PRRS10}$
mean ± SE	40.70 ± 1.38^{a}	48.23 ± 1.64^{b}	47.97 ± 1.59^{b}	59.08 ± 1.92^{c}	64.91 ± 1.83^{f}	59.79 ± 2.08^{cd}	62.53 ± 1.91^{e}	60.99 ± 2.15^{de}
n	279	306	334	359	380	353	345	358
C3c serum concentration, *in vivo* complement activation/acute phase response (C3c)								
Trait	C3c$_{MH0}$	C3c$_{MH4}$	C3c$_{MH10}$	C3c$_{ADV0}$	C3c$_{ADV4}$	C3c$_{ADV10}$	C3c$_{PRRS0}$	C3c$_{PRRS10}$
Mean ± SE	0.162 ± 0.004^{a}	0.169 ± 0.003^{b}	0.115 ± 0.003^{ac}	0.193 ± 0.004^{cd}	0.198 ± 0.004^{de}	0.124 ± 0.004^{dcd}	0.118 ± 0.002^{c}	0.124 ± 0.003^{be}
n	363	361	139	389	389	189	182	182
HP serum concentration, acute phase response (HP)								
Trait	HP$_{MH0}$	HP$_{MH4}$	HP$_{MH10}$	HP$_{ADV0}$	HP$_{ADV4}$	HP$_{ADV10}$	HP$_{PRRS0}$	HP$_{PRRS10}$
Mean ± SE	0.388 ± 0.017^{a}	0.547 ± 0.017^{be}	0.367 ± 0.018^{a}	0.344 ± 0.016^{c}	0.812 ± 0.018^{f}	0.587 ± 0.019^{e}	0.479 ± 0.020^{d}	0.533 ± 0.021^{b}
n	195	194	155	189	189	189	182	182
Antibody titres								
Trait	ABMH0		AB$_{MH10}$	AB$_{ADV0}$		AB$_{ADV10}$		AB$_{PRRS10}$
Mean ± SE	0.417 ± 0.027^{a}		0.570 ± 0.038^{b}	0.423 ± 0.011^{a}		0.637 ± 0.018^{b}		0.679 ± 0.050
n	109		109	290		290		85

Different superscripts indicate significant ($P < 0.05$) differences between means at different time points of measurement.

Table 2. Markers used in the quantitative trait loci analysis and genetic map as established for the DUMI resource population (sex average, Kosambi cM)

Chromosome	Markers and genetic distances[a] [cM]								
SSC1	*SW1515* (16.4)	33.0	*SW1851*	33.4	*S0155*	8.3	*RLN*[d]	61.6	*SW1301* (140.5)
SSC2	*SW2443* (0)	42.7	*FTH1*[d]	2.2	*SW240*	23.0	*STS2*[d]	13.8	*C3*[d]
				18.3	*SW1564*	14.2	*BHMT*[d]	13.5	*S0226* (74.8)[b]
SSC3	*SW72* (17.8)	5.8	*S0164*	25.7	*SW2570*	33.2	*S0002* (102.2)		
SSC4	*S0227* (4.1)	47.2	*S0001*	14.3	*STS3*[d]	4.6	*CRH*[d]	1.3	*STS1*[d]
				1.5	*STS4*[d]	28.3	*S0214*	36.6	*S0097* (120.0)
SSC5	*SW1482* (8.4)	59.3	*SW1134*	4.0	*IGF1*	34.6	*SW378* (102.9)		
SSC6	*S0035* (7.3)[c]	24.1	*HP*[d]	39.4	*S0087*	1.4	*SW1067*	9.7	*SW193*
		5.8	*S0300*	5.9	*TGFB1*[d]	26.9	*S0220*	35.0	*LEP*[d]
						8.5	*S0059*	16.7	*S0003* (102.0)[c]
SSC7	*S0025* (3.7)	24.4	*S0064*	23.5	*DQB*[d]	23.5	*BF*[d]	15.5	*S0102*
				15.8	*SW175*	36.2	*S0115*	3.2	*S0101* (134.9)
SSC8	*SW2410* (0)	79.5	*S0086*	24.7	*S0144*	23.8	*SW61* (112.3)		
SSC9	*SW21* (11.1)	26.3	*SW911*	33.1	*SW54*	17.1	*S0109*	32.9	*S0295* (96.5)
SSC10	*SW830* (0)	77.7	*S0070*	49,3	*ITIH2*[d]	36.1	*SW2067* (124.1)		
SSC11	*SW2008* (14.1)	32.0	*S0071*	32.0	*S0386*	34.0	*SW703* (76.2)		
SSC12	*S0143* (6.6)	49.6	*SW874*	42.6	*SW605* (108.3)				
SSC13	*S0219* (1.6)	4.0	*SW344*	36.6	*SW398* (79.3)				
SSC14	*SW857* (7.4)	53.4	*S0007*	3.4	*VCL*[d]	37.8	*SWC27* (111.5)		
SSC15	*S0355* (1.3)	35.3	*SW1111*	48.5	*SW936*	33.5	*SW1119* (107.4)		
SSC16	*S0111* (0)	51.2	*S0026*	42.4	*S0061* (92.6)				
SSC17	*SW335* (0)	34.6	*SW840*	35.1	*SW2431* (94.0)				
SSC18	*SW1023* (5.0)	23.9	*SW787*	43.0	*SWR414* (57.6)				

[a] Numbers in parentheses at the first and last marker are relative positions of these in the USDA-MARC v2 linkage map; [b] S0226 not covered by USDA-MARC v2, but SW14, which is closely linked to S0226 (PigMaP v1.5); [c] S0035 at 0 cM and S0003 at 144.5 cM in the International Workshop 1 SSC6 integrated map with a total length of 166.0 cM; [d] biallelic markers; gene symbols are official symbols according to HUGO gene nomenclature committee; RLN, relaxin; FTH1, ferritin, polypeptide1; STS, sequence tag sites (Murani *et al.*, 2006); C3, complement component 3; BHMT, betaine homocysteine methyltransferase; CRH, corticotrophin-releasing hormone; IGF1, insulin-like growth factor 1; HP, haptoglobin; TGFB1, transforming growth factor beta 1; LEP, leptin; DQB, MHC class II DQ-beta chain; BF, complement factor B; ITIH2, interalpha globulin inhibitor H2; VCL, vinculin.

1996). Therefore, phenotypic data were adjusted for systematic effects by analysis of variance performed with the procedure 'Mixed' of the SAS software package (SAS System for Windows, Release 8.02; SAS Institute, Cary, NC, USA). For AH50, CH50, C3c and HP concentration, models with and without the repeated measures statement (1.order autoregressive R-matrix) were applied, while for antibody titres the repeated statement was omitted. Models were fitted in order to identify other significant effects apart from the QTL effects by stepwise elimination of non-significant effects. The repeated measures model applied in the analysis of variance revealed significant effects of sire, dam, parity and time point on all four trait complexes, CH50, AH50, C3c and HP. Treatment, i.e. vaccination or non-vaccination, showed a significant effect on CH50 and C3c but did not on AH50. Sire, dam, parity and treatment had also significant effects on antibody titres. For analysis under the line cross (LC) model additive genetic effects were estimated at 1 cM intervals as half of the difference of the trait value between homozygous carriers of the Duroc and the Miniature Pig alleles, i.e. positive values of additive genetic effects point to a higher trait value for homozygous carriers of the Duroc allele. Dominance effects are estimated as the difference between the trait value of heterozygous individuals and the mean trait value observed for homozygous animals. Subsequently, adjusted phenotypic values were regressed onto the additive and dominance coefficients in intervals of 1 cM. For half-sib analysis, which does not make assumptions on the relative frequencies of the QTL alleles in the founder populations, the probability for the occurrence of a paternal allele was estimated in intervals of 1 cM. The probabilities of inheritance of distinct paternal gametic phases were regressed onto allele substitution effects at the putative QTL.

Significance thresholds at the 5% and 1% level were determined empirically by permutation for individual chromosomes and traits (Churchill & Doerge, 1994). Chromosome-wide 1% and 5% significance thresholds became genome-wide significance thresholds after Bonferroni correction for 18 autosomes of the haploid porcine genome.

Results

Line cross and half-sib analysis of traits related to complement activity, acute phase response and antibody response measured at different time points before and after vaccinations revealed in total 49 and 22 estimates of most likely positions of QTL with genome-wide significance at $P = 0.05$ and $P = 0.01$ level, respectively (Table 3).

QTL for haemolytic complement activity in the alternative pathway prior and after the various vaccinations were found on SSC2, 3, 4, 7, 11 and 17. The QTL explain between 3% and 6% of phenotypic variation. For the

proximal region of SSC2, four neighbouring QTL positions for AH50 after ADV vaccination were estimated using either the line cross or the half-sib model and either repeated measures analysis or distinct trait analysis (Fig. 1). Additive genetic effects were negative as were the additive genetic effects of the QTL estimated for AH50 after ADV and MH vaccination on SSC7, 11 and 17, indicating that Berlin Miniature Pig QTL alleles lead to higher values.

In the distal region of SSC4 QTL for haemolytic complement activity in the classical pathway at different time points along the vaccination program were detected using the half-sib model (Fig. 1). Further QTL for CH50 were

Table 3. Location and estimated effects (means ± standard error) of quantitative trait loci for traits related to humoral immune response estimated from data of the DUMI F2 resource population

	Trait[a]	Models[b]	SSC	Marker interval	Position cM[c]	*F*-value	Additive genetic effect[d]	Dominance effect[e]	Var[f]
Haemolytic complement activity in the alternative pathway	AH50_ADV10	LC, dt	2	SW2443 — FTH1	29	11.7*	–23.4 ± 6.9		3.4
	AH50_ADV10-0	HS, dt	2	SW2443 — FTH1	35	11.5*			5.2
	AH50_ADV10	LC, rm	2	SW2443 — FTH1	48	7.7*	–27.2 ± 7.5	–29.2 ± 23.6	4.3
	AH50_PRRS10	LC, rm	3	Close to S0164	76	17.2**	11.9 ± 2.9		4.7
	AH50_PRRS0	LC, rm	4	S0214 — S0097	155	17.4**	18.9 ± 4.5		4.9
	AH50_MH10	LC, rm	7	S0064 — DQB	47	14.4*	–10.8 ± 2.9		4.1
	AH50_ADV10	HS, rm	11	SW2008 — S0071	16	6.1*			5.2
	AH50_ADV10	LC, rm	11	SW2008 — S0071	25	17.0**	–19.3 ± 4.7		4.7
	AH50_MH4	LC, rm	17	Close to SW840	51	10.3**	–8.1 ± 2.6	11.5 ± 3.5	5.9
Haemolytic complement activity in the classical pathway	CH50_ADV10-0	HS, dt	2	C3 — SW1564	132	7.4*			6.2
	CH50_MH10-4	LC, dt	2	SW1564 — S0226	157	7.6*	–6.5 ± 3.7	–22.9 ± 6.8	5.6
	CH50_ADV10-4	HS, dt	4	S0214 — S0097	164	10.6**			9.5
	CH50_PRRS10	HS, rm	4	S0214 — S0097	165	6.4*			5.0
	CH50_PRRS10	HS, dt	4	S0214 — S0097	166	6.4*			5.2
	CH50_ADV10	HS, rm	4	S0214 — S0097	173	6.5**			5.1
	CH50_ADV10	HS, dt	4	S0214 — S0097	175	7.3**			6.0
	CH50_mean	HS, dt	4	S0214 — S0097	176	7.1**			4.8
	CH50_ADV10	HS, rm	6	TGFB1 — S0220	135	7.0*			5.5
	CH50_ADV10-0	HS, dt	6	TGFB1 — S0220	136	7.2*			6.8
	CH50_ADV0	HS, dt	6	S0059 — S0003	205	4.4*			3.6
C3c serum concentration	C3c_PRRS10	HS, rm	1	S0155 — SW1301	135	7.0*			11.8
	C3c_PRRS0	LC, rm	2	SW2443 — FTH1	39	10.4**	0.038 ± 0.009	0.066 ± 0.023	11.7
	C3c_PRRS10	LC, rm	2	SW2443 — FTH1	43	9.4*	0.036 ± 0.009	0.051 ± 0.020	1.6
	C3c_PRRS10	LC, dt	2	SW2443 — FTH1	50	8.3*	0.033 ± 0.008	0.040 ± 0.017	9.4
	C3c_ADV10-4	HS, dt	2	SW1564 — S0226	173	11.2*			18.6
	C3c_MH10	LC, rm	3	S0164 — SW2570	84	8.4*	–0.001 ± 0.006	0.043 ± 0.010	12.4
	C3c_PRRS0	HS, rm	4	S0214 — S0097	157	6.8*			11.6
	C3c_PRRS10	LC, rm	5	Close to SW1482	0	9.7**	–0.015 ± 0.006	–0.039 ± 0.010	1.8
	C3c_PRRS10	HS, dt	5	Close to SW1482	0	6.4*			11.1
	C3c_PRRS10	HS, rm	5	SW1482 — SW1134	16	6.1*			1.5
	C3c_MH10	HS, rm	5	Close to SW378	191	5.9**			13.5
	C3c_MH10	LC, rm	6	S0220 — S0059	161	11.3**	0.009 ± 0.005	0.038 ± 0.009	16.8
	C3c_ADV4	LC, rm	7	DQB — BF	61	9.9*	–0.002 ± 0.005	0.033 ± 0.007	4.8
	C3c_ADV0	LC, dt	7	S0115 — S0101	195	15.6*	0.018 ± 0.005		4.0
	C3c_MH10	HS, dt	8	Close to SW61	186	7.2*			16.5
	C3c_ADV10-0	LC, dt	10	Close to SW2067	244	9.0*	–0.054 ± 0.019	0.647 ± 0.230	1.4
	C3c_ADV10-4	LC, dt	10	Close to SW2067	244	9.0*	–0.042 ± 0.019	0.743 ± 0.227	1.3
	C3c_MH4	HS, rm	12	Close to SW605	128	9.9**			7.8
	C3c_MH10	HS, rm	12	Close to SW605	128	6.0*			13.7
	C3c_ADV0	LC, rm	14	SW857 — S0007	32	8.0*	–0.005 ± 0.007	0.065 ± 0.016	3.9
	C3c_MH10	HS, dt	14	Close to S0007	76	10.9**			25.2
	C3c_MH10	LC, rm	15	SW1111 — SW936	65	10.1**	–0.028 ± 0.012	0.081 ± 0.018	14.9
	C3c_MH4	HS, dt	16	S0111 — S0026	36	5.7*			4.8
	C3c_PRRS0	HS, dt	16	S0111 — S0026	64	12.3**			21.2
	C3c_MH0	HS, rm	16	Close to S0026	76	8.3**			6.5
	C3c_MH4	HS, rm	16	Close to S0026	76	10.5**			8.3
	C3c_MH10	HS, rm	16	Close to S0026	76	6.7*			15.2
	C3c_PRRS10	HS, rm	16	Close to S0026	76	9.6**			16.4
	C3c_PRRS0	HS, rm	16	S0026 — S0061	80	7.5*			12.8
	C3c_MH10	HS, dt	16	S0026 — S0061	99	8.0*			18.3
	C3c_ADV0	LC, rm	18	SW787 — SWR414	39	11.4*	0.019 ± 0.006		2.8

Table 3. *Continued*

	Trait[a]	Models[b]	SSC	Marker interval	Position cM[c]	*F*-value	Additive genetic effect[d]	Dominance effect[e]	Var[f]
HP serum concentration	HP_ADV0	LC, dt	3	SW72 — S0164	34	7.9*	−0.033 ± 0.047	0.541 ± 0.136	9.7
	HP_MH4	LC, rm	5	SW113 — IGF1	96	8.7*	0.041 ± 0.03	−0.190 ± 0.046	9.8
	HP_MH10-4	HS, dt	6	S0220 — S0059	188	6.8*			14.6
	HP_ADV4	HS, dt	8	SW2410 — S0086	37	7.6**			13.4
	HP_PRRS10-0	LC, dt	9	S0109 — S0295	110	9.5**	0.045 ± 0.041	−0.356 ± 0.084	12.4
	HP_PRRS0	LC, dt	9	Close to S0295	138	9.5*	−0.031 ± 0.027	0.150 ± 0.036	12.2
	HP_MH10	HS, dt	10	Close to SW2067	244	5.8*			12
	HP_ADV10-4	HS, dt	11	S0071 — S0386	70	5.5*			9.8
	HP_MH10-4	HS, dt	12	S0143 — SW875	22	7.6*			16.2
	HP_MH0	LC, rm	12	S0143 — SW875	28	8.5*	0.176 ± 0.062	0.613 ± 0.186	9.4
	HP_MH10	LC, rm	12	S0143 — SW875	36	8.8*	0.123 ± 0.065	0.666 ± 0.169	11.9
	HP_PRRS0	HS, rm	14	SW857 — S0007	54	5.9*			1.7
	HP_mean	HS, dt	14	S0007 — VCL	89	6.0*			10
	HP_MH10	LC, dt	14	VCL — SWC27	155	13.1*	−0.122 ± 0.034		9.5
	HP_MH4	HS, dt	17	SW335 — SW840	7	7.5*			12.8
	HP_MH0	LC, rm	17	SW840 — SW2431	89	7.3*	−0.147 ± 0.040	−0.060 ± 0.055	8.1
Antibody titres	AB_PRRS10	HS, dt	1	Close to SW1851	44	15.0**			16.3
	AB_ADV0	LC, dt	2	Close to SW1564	149	8.3*	−0.034 ± 0.025	0.168 ± 0.044	5.1
	AB_ADV0	LC, dt	6	TGFB1 — S0220	141	8.0*	−0.059 ± 0.017	−0.118 ± 0.042	5.0
	AB_PRRS10	HS, dt	7	SW175 — S0115	133	11.6*			12.7

[a] Trait: AH50 and CH50, haemolytic complement activity in the alternative and classical pathways; C3c and HP, C3c and haptoglobin serum concentrations; AB, antibody titres; _time point of measurement either just before (0) or 4 or 10 days after MH (*Mycoplasma*), ADV (*Aujeszky*) or PRRS vaccination, or mean of eight measurements, or differences between respective measurements, respectively; [b] models: LC, line cross model or HS, half-sib model; data adjustment: rm, repeated measure or dt, distinct trait refers to analyses of variance either with or without repeated measures statement; [c] position of genetic map established for the DUMI resource population in Haldane cM; [d] positive values of additive genetic imply higher trait values forced by the Duroc allele; [e] dominance effects were omitted if they were not significant and QTL were subsequently estimated based on additive genetic effects only; [f] the fraction of phenotypic variance in the F2 explained by a QTL, calculated as the proportion of residual variance of the statistical models with and without the QTL effect.
* Significant at the 5% genome-wise level; ** significant at the 1% genome-wise level.

on SSC2 and SSC6, all of which were identified with the half-sib model except the QTL for difference in CH50 after MH vaccination.

Most autosomes, except SSC9, 11, 13 and 17 were found to exhibit loci affecting C3c serum concentration. The proximal region of SSC2, where QTL for AH50 were detected, also revealed QTL for C3c (Fig. 1). However, in contrast to observations for the AH50-QTL, additive genetic effects of the QTL for C3c indicated the allele forcing higher traits values coming from the Duroc breed. Using the different statistical models to adjust the phenotypes for systematic effects, a number of QTL positions were estimated on SSC16, all by the half-sib model (Fig. 1).

Also QTL for HP serum level were found on most autosomes. In the proximal region of SSC12 three QTL were detected by the half-sib and the line cross model, with the later showing positive additive genetic effects indicating higher phenotypic values caused by the Duroc alleles of the QTL.

Likely positions of QTL for antibody titres against PRRSV were detected by half-sib analysis on SSC1 and 7. These QTL explain more than 10% of the phenotypic variation. On SSC2 and 6 there were QTL for antibody titres against ADV, with 5% effect that were obtained by F2 analysis. Negative additive genetic effects indicated that the QTL alleles originated from Berlin Miniature Pig associate with high antibody titres.

Discussion

A QTL linkage analysis was performed to identify genomic regions harbouring genes that affect complement activity, acute phase response and antibody titres after modulation of the immune system by bacterial and viral immunizations. For the first time QTL for haemolytic complement activity and acute phase response (C3c, HP) were detected here. Furthermore, QTL for antibody titres were reported here on SSC1, 2, 6 and 7 — supporting and supplementing previous results obtained in the pig (Edfors-Lilja *et al.*, 1998, 2000; Edfors-Lilja, 1999; Wattrang *et al.*, 2005).

The half-sib and the line cross model revealed a similar number of estimated QTL positions. The line cross model, assuming that different QTL alleles are fixed in founder populations, is very powerful when this assumption corresponds to the true state of nature of the QTL and it is quite robust to limited deviations from this ideal situation, even though it tends to underestimate QTL effects in such situations (Alfonso & Haley, 1998). The half-sib model is more general with no assumption about the number and frequency of QTL alleles in founder populations and probably more realistic for many QTL. In experimental crossbred populations based on divergent founder breeds as well as in commercial pig populations and their crosses

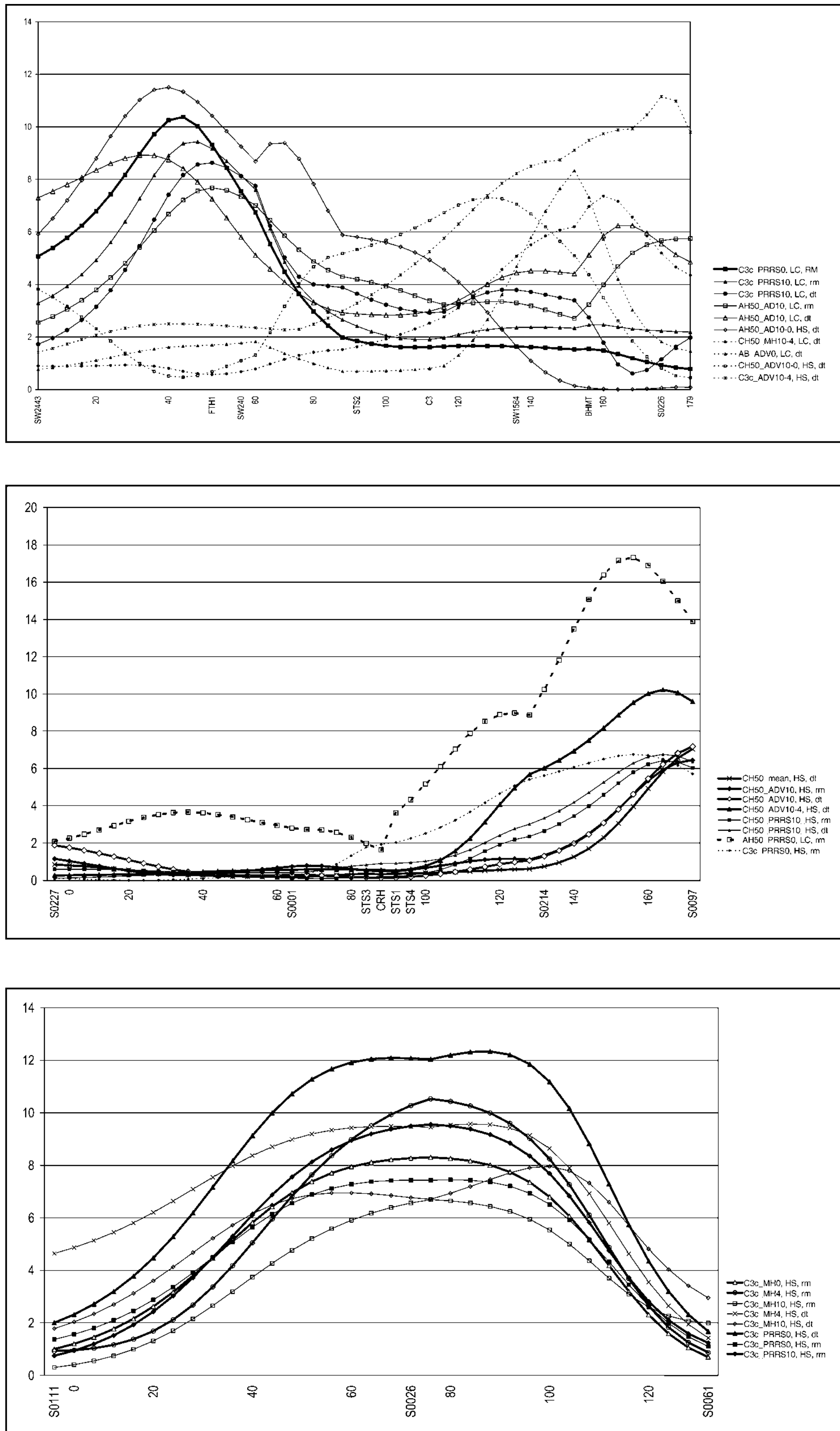

Figure 1. *F*-value plots indicating the existence of quantitative trait loci for traits related to humoral immune response with genome-wide significance at $P < 0.05$ and $P < 0.01$ (curves in bold) on SSC2, SSC4, and SSC16 estimated from data of the DUMI F2 resource population. Positions of markers are indicated at the *x*-axis, *F*-values are at the *y*-axis.

QTL for production traits were detected using both models, indicating that there is still a considerable amount of genetic variation even at loci affecting production traits that have been selected for in commercial lines (Bidanel *et al*., 2001; de Koning *et al*., 2001). The present study showed that also loci affecting immunological traits like haemolytic complement activity, acute phase and antibody response segregate within and between the founder populations of the experimental F2 population, which is based on a commercial breed and a closed experimental breed. In particular, in the prominent QTL regions on SSC4 for CH50 and SSC16 for C3c, QTL effects were found under the haft-sib model, suggesting that the responsible QTL are segregating in both founder populations, whereas QTL in the proximal region of SSC2 for AH50 (except for AH50_ADV10-0) and C3c were detected by the line cross model, suggesting that the underlying QTL are close to fixation in the founder populations.

It was shown that vaccination leads to an acute phase response measurable by an increased serum level of HP in pigs (Asai *et al*., 1999; Rekitt *et al*., 2001). Increment of the C3c serum concentration, as observed in this experiment, is part of the acute phase response (Kushner *et al*., 1982; Volanakis, 1995). At the same time it reflects *in vivo* complement activation, while haemolytic complement activity describes the *in vitro* capacity of the complement system (Storm *et al*., 1992). Also haemolytic complement activity is elevated after immunizations. Lipopolysaccharides (LPS) of various *Mycoplasma* species were shown to interact with C1 and to induce an antibody-independent activation of the complement system via the classical pathway (Bredt *et al*., 1977; Rosendal, 1984). Also viruses were shown to activate the complement cascade independent from existing antibodies via both the classical and the alternative pathway. Alphaherpesviridae, in particular Epstein–Barr viruses, activate both pathways of the complement (Mayes *et al*., 1984; Mold *et al*., 1988). The glycoprotein C (gC) of *HSV-1* and -2, bovine herpesvirus type 1, porcine herpesvirus 1 (ADV; Suid herpesvirus I (SuHVI) or pseudorabies virus (PrV), and equine herpesvirus types 1 and 4 interact with C3b (Friedman *et al*., 2000). Thus the increment of complement activity in blood samples obtained after vaccinations might be at least partly due to specific interaction between the bacterial and viral vaccination strains and complement components. Of course, complement activation also depends on other signals of the immune system like interleukins. The typical time course of the acute phase response and the specific interactions between vaccines and the complement system imply that the measures of complement activity and acute phase response at different time points after various immunizations are functionally distinct traits, as are antibody titres against the different vaccines at different time points. However, measures of the parameters, CH50, AH50, C3c and HP at different time points of the same animals are not fully independent and can be regarded as repeated measures. Accordingly, in order to evaluate QTL effects on these traits also a repeated measures mixed model analysis was conducted using first order autoregressive covariance structure (heterogeneous variances) that best fit the data, where covariances of temporally adjacent measures are higher than the covariances of distant measures. Autoregressive correlations between two adjacent measures were $r = 0.3$, 0.6, 0.5 and 0.4 for AH50, CH50, C3c- and HP-serum concentration, respectively. Consequently, in this study, traits were statistically treated as distinct traits and repeated measures in order to account for the facts (i) that on one hand, there are facts suggesting the traits being functionally independent, and (ii) on the other, traits are measures redundantly taken from the same animals.

Towards improved global defence capability against pathogens, selection for parameters of different immune functions has successfully been applied in experimental porcine populations (Mallard *et al*., 1992). In the pig, genome-wide significant QTL for cellular and humoral immune traits were shown to segregate on chromosomes 1, 4, 5 and 6 in an experimental cross of wild boar and Yorkshire (Edfors-Lilja *et al*., 1998, 2000; Edfors-Lilja, 1999). Further QTL affecting the resistance/susceptibility to pseudorabies virus in pigs in terms of expression of neurological symptoms and body temperature were mapped to chromosomes 9, 5 and 6, and 2, 4, 8, 10, 11 and 16, respectively (Reiner *et al*., 2002). Significant QTL for antibody titres against *Escherichia coli* antigens were detected on SSC 5 and 6, putative QTL were on SSC 1, 2, 4, 7 and 16 (Edfors-Lilja *et al*., 1998). QTL on SSC1, 2 and 6 fit those detected here for antibody titres against PRRS and AD. A number of QTL for HP serum concentration were found, however, not close to the position of *HP* on SSC6, indicating that variation at HP has no major impact on its own concentration (Ponsuksili *et al*., 2002; Wimmers *et al*., 2003).

Three genomic regions showed a clustering of QTL for complement activity as revealed by either the line cross or the half-sib model, and either analysis of distinct traits or repeated measures. These QTL may share common genetic background. The proximal region of SSC2 contains QTL that affect complement activity in the alternative pathway. The same region exhibits also QTL effects on C3c serum concentration. In the distal region of SSC4 effects on haemolytic complement activity were evident using different statistical models. QTL for C3c serum concentration segregating in the resource population were found on the intermediate region of SSC16 (Fig. 1). All QTL effects on SSC16 were from the half-sib model, indicating that the QTL were not fixed in the founder populations of the DUMI.

In order to facilitate addressing positional candidate genes we aimed to gain advantage of the existing genome maps, especially the human porcine comparative map, and also of currently available bioinformatics tools, i.e. the Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics resources (Dennis *et al*., 2003; Meyers *et al*., 2005). In particular for the three regions of clustered QTL positions, we assigned generously the human genomic regions that are orthologous

to the QTL regions identified here. In particular, for the SSC2 QTL region HSA11 from 0 to 70 Mb, for SSC4 the interval from 95 to 155 Mb of HSA 1, and for SSC16 the human chromosome 5 from 0 to 73 Mb and 150–174 Mb were regarded as origins of positional candidate genes. Lists comprising of 1233, 629 and 560 loci, respectively, were obtained from the NCBI Map viewer (build 36.2) and were subsequently submitted to the DAVID functional annotation tool while focusing on Gene Ontology biological pathway terms covering genes of immunological relevance. For these QTL regions 42, 25 and 25 genes were functionally annotated to the term 'response to stimulus' (GO 0050896) and subterms (immune response GO 0006955, defence response GO 0006952). For some of these genes functional association to complement activity and/or acute phase response is known; these are exemplarily mentioned here.

CD59 was assigned to SSC2 (SSC 2p17-p14, Garrido *et al*., 1998). CD59 is a potent inhibitor of the complement membrane attack complex (MAC) action. It acts by binding to the C8 and/or C9 components of the assembling MAC, thereby preventing incorporation of the multiple copies of C9 required for complete formation of the osmolytic pore (Davies *et al*., 1989). Due to its action on the terminal sequence of the complement cascade, which is common to all modes of complement activation, *CD59* is a candidate gene for haemolytic complement activity. *SERPING1* (serpin peptidase inhibitor, clade g (c1 inhibitor), member 1) encodes a highly glycosylated plasma protein involved in the regulation of the complement cascade. It forms a proteolytically inactive stoichiometric complex with the C1r or C1s proteases and by this inhibits activated C1r and C1s of the first complement component and thus regulates complement activation (Davis, 2004). Thus *SERPING1* is a functional candidate gene for haemolytic complement activity and C3c serum concentration.

C reactive protein (CRP) is an APP gene located on SSC4q21 (Pinton *et al*., 2000; Chomdej *et al*., 2004). Binding of CRP at microbial polysaccharides activates the haemolytic complement activity in the classical pathway and at the same time reduces formation of the MAC via the alternative pathway and the formation of C3b via the lectin pathway (Mold *et al*., 1999). In particular CRP interacts with C1q and a number of amino acid positions of CRP have been recognized that affected binding to CRP (Bang *et al*., 2005). Another candidate gene for the QTL for haemolytic complement activity on SSC4 is *IL6R*, IL6 receptor, with its ligand IL6 being one of the interleukins regulating acute phase response and complement activity. IL6 responsive elements were detected in the promoter of the human and porcine *C3* (Fong *et al*., 1990; Vik *et al*., 1991; Wimmers *et al*., 2003).

The genes encoding *C6*, *C7* and *C9* of the terminal lytic sequence of the complement cascade map to SSC16 (Do *et al*., 2006). As components of the membrane attack complex they may directly affect haemolytic complement activity. The C5b-9 complex inhibits the formation of classical and alternative pathway C3 convertases (Bhakdi *et al*., 1988). Due to this feedback mechanism, the impact of the components of the C5b-9 complex on the C3c serum concentration, that reflects *in vivo* complement activation, might be expected, i.e. *C6*, *C7* and *C9* are positional functional candidate genes for the QTL detected on SSC16.

Other complement genes that map close to QTL detected here are *C5*, and *C8G*, that map to the q-arm of SSC1 and are associated with complement activity (Ponsuksili *et al*., 2001; Kumar *et al*., 2004; Do *et al*., 2006). *C3* was assigned to SSC2 and shows association with haemolytic complement activity (Wimmers *et al*., 2001, 2003). *C1qA*, encoding a factor within the classical complement cascade, was assigned to SSC6q22–q23 and consequently is a candidate gene for both haemolytic complement activity and C3c serum concentration (Jorgensen *et al*., 1997). *C8A* and *C8B* map to SSC6 (Do *et al*., 2006) close to the QTL for haemolytic complement activity, C3c-serum concentration and antibody titre. As components of the membrane attack complex both might have effects on haemolytic complement activity. *MBL1* and *MBL2* were assigned to chromosome 14 and were shown to affect complement activity (Phatsara *et al*., 2007). Furthermore, the MHC class II covers a number of complement factors like *C2*, *C4* and *BF*, that map to SSC7. Within the MHC region a number of other functional candidate genes are known that modulate acquired and innate immune response.

This study suggests that there is considerable genetic variation of loci affecting innate humoral immune response in commercial pig breeds as well as in the closed experimental breed. In terms of number and magnitude of the effects, QTL for immune traits behave like those for other quantitative traits. Taking into account the facts that data were redundantly treated (i) under the line cross and half-sib model (ii) and as repeated measures and single traits obtained at different time points and that (iii) thus different estimated most likely QTL positions represent common genetic effects, particularly three genomic regions largely contribute to the variation in complement activity. The proximal region of SSC2 (orthologous to HSA11 0–70 Mb) contains QTL for complement activity in the alternative pathway and for C3c serum concentration. In the distal region of SSC4 (HSA1 95–155 Mb) harbours QTL for haemolytic complement activity and the intermediate region of SSC16 (HSA5 0–73 Mb and 150–174 Mb) harbours QTL for C3c serum concentration.

The discovery of the QTL regions facilitates identifying candidate genes for disease resistance and immune competence. The integration of this approach with function-driven genomics study will provide more statistical and functional evidence for the role of any functional positional candidate gene identified. Anyhow, it is still a major task to identify causative genes and polymorphisms. Of particular interest is the relationship between improved (optimized) immune functions and the incidence of infectious diseases. Moreover, one of the future challenges still is delineating the interrelationship between different immune mechanisms and other traits, including traits related to growth, adaptability and metabolic status, which largely depend on liver functions. In this regard the

pig can serve as a valuable model to gain basic knowledge and for any attempts to modulate pharmaceutically immune functions (Thorgersen *et al.*, 2006).

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