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Original article

Association of the porcine *C3* gene with haemolytic complement activity in the pig

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Abstract – The complement component *C3* plays an essential role in the activated complement system, which is involved in phagocytosis, inflammation and immunoregulation to destroy infectious microorganisms. The *C3* molecule has more implications in the general defence mechanisms. In this study, the porcine *C3* cDNA sequences including 5′- and 3′- flanking regions were determined and the polymorphisms in this gene were identified to carry out an association analysis between *C3* and complement activity traits. Porcine *C3* gene has high homology with human *C3*. Five single nucleotide polymorphisms (SNPs) and one microsatellite were detected in the porcine *C3* gene. Haemolytic complement activity of alternative and classical pathways (ACH, CCP) was measured in 416 F2 animals of a crossbred of Duroc × Berlin Miniature Pig, which were immunized with Mycoplasma, Aujeszky and PRRS vaccines. *C3* markers were found to be significantly associated ($P < 0.05$) with both ACP and CCP. Animals with the more frequent haplotype present in Duroc and other commercial breeds exhibit higher ACP and CCP levels than the animals with haplotype specific to some Berlin Miniature Pigs. The association of *C3* with complement activity reinforces the importance of *C3* as a candidate gene for natural resistance to microorganisms.

porcine *C3* gene / natural resistance / haemolytic complement activity / association / pig

1. INTRODUCTION

The complement cascade defines an important link between the innate and the specific immune system [13]. The complement component *C3* is the key molecule of the three pathways of complement activation (alternative, classical and lectin pathways), which are involved in phagocytosis, inflammation and immunoregulation processes to destroy infectious microorganism [19].

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C3 deficiency in hosts causes increasing susceptibility to bacterial or viral infections [1,4,13,22]. *C3* has more implication in general defence mechanisms. We have previously found polymorphic sites within the porcine *C3* which were used for genetic mapping of the locus [25]. The present study was carried out to investigate the porcine *C3* gene as a candidate gene for complement activity traits. Therefore, the full length cDNA sequence of the porcine *C3* as well as 5′- and 3′- flanking regions were obtained, screened for additional polymorphisms in this gene and, finally, association analysis was conducted between *C3* markers and complement activity traits.

2. MATERIALS AND METHODS

2.1. Animals

Sequence information of the porcine *C3* gene was identified in a DUMI F2-animal of a reciprocal cross of Duroc × Berlin Miniature Pig [9]. Screening for polymorphisms in the porcine *C3* gene was performed in Duroc (n = 6), German Landrace (n = 28), Pietrain (n = 23), DUMI F2 (n = 902) and Thai native (n = 32) pigs. For association study, haemolytic complement activity was recorded in 416 animals of the DUMI-F2 resource population.

2.2. cDNA sequence and 5′ and 3′ flanking regions of the porcine *C3* gene

Total RNA was extracted from pig liver using TRIzol™ Reagent (Life Technologies, Karlsruhe, Germany). Full length porcine *C3* cDNA sequence was determined by the SMART™ RACE cDNA Amplification Kit (Clontech, Heidelberg, Germany). RACE primers and gene specific primers were designed based on partial porcine *C3* sequence information (GenBank accession number AF110278, F14640) (Fig. 1):

RACE: 5′-ctaatacgactcactatagggcaagcagtggtatcaacgcagagt-3′
C3-A2: 5′-ccttctccacgacatcccagatcctacg-3′
C3-B1: 5′-ccaccaagaccatgaatgtg-3′
C3-B2: 5′-tagagcttctggccaggttc-3′
C3-C1: 5′-ggtggtgaccatgtaccacg-3′.

The 5′and 3′ flanking regions of porcine *C3* gene were isolated from a genomic DNA library, which was constructed using Lambda DASH II® (Stratagene, Amsterdam, Netherlands). Two positive clones of 5′ and 3′ flanking region were amplified with primers specific to porcine *C3* (5′-flanking region 5′-tgtgccccttctctgatttg-3′, 3′-flanking region 5′-gctccgatgaagtgcaggt-3′) and T3/T7

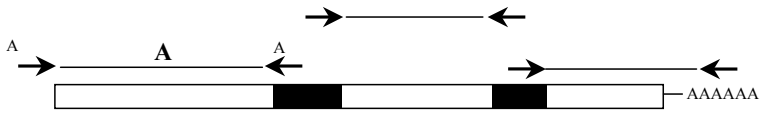

Figure 1. Amplification of the full-length porcine *C3* gene. Three PCR fragments (A, B and C) were amplified with primers based on available porcine *C3* sequence in GenBank, a 580-bp partial cDNA fragment (shaded area 1) (accession No. AF110278) and a 462-bp EST of porcine *C3* (shaded area 2) (accession No. F14640).

standard primers. All the PCR fragments were ligated in pGEM®-T vector (Promega, Mannheim, Germany) and sequenced using the SequiTherm Excel cycle sequencing Kit (Epicentre Technologies, Biozym, Hessisch Oldendrof, Germany) and a LI–COR 4200 automated sequencer (LI–COR Biosciences, Bad Homburg, Germany).

2.3. Polymorphism screening and marker genotyping

To identify polymorphisms in the porcine *C3* gene, oligonucleotide primers were designed revealing 17 overlapping PCR fragments (Tab. I). They were amplified from individual liver cDNA and genomic DNA of six pig breeds. PCR was performed in a 25 μL reaction volume containing 50 ng of liver cDNA, 0.2 μM of each primers, 50 μM each dNTPs, 0.5 U of *Pfu* polymerase (Promega) in 1× *Pfu*-PCR buffer. PCR cycling program was 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 52–60 °C (Tab. I) for 30 s, 72 °C for 1 min and final extension at 72 °C for 5 min. PCR products were gel purified, cloned and sequenced. The nucleotide sequences of each animal were compared to find out polymorphisms in the porcine *C3* gene.

To genotype each polymorphic site in the porcine *C3* gene, PCR-RFLP, allele specific PCR, SSCP and microsatellite analysis were performed. Oligonucleotide primers (Tab. II) were used to amplify PCR fragments from genomic DNA. PCR reactions were carried out in 20 μL volume using *Taq* polymerase (Promega). Amplified PCR fragments with primer C3-I (Tab. II) were mixed with 1:10 with loading buffer (95% formamide, 10 mM NaOH, 0.25% bromophenol blue, 0.25% xylenecyanol), denatured at 95 °C for 5 min, then cooled on ice immediately and separated on 12% polyacryamide gel (49:1 acrylamide/bis-arylamide) at room temperature with a constant 12 W for 6 h in 0.5× TBE. The SSCP-bands were stained by silver staining procedure. Amplicon C3-II was digested with restriction enzyme *Taq*I (Promega). The digested PCR fragments were analysed on 2% agarose gels. Genotyping the SNPs within amplicons C3-III and C3-IV was done as described previously [25]. Fragments with length polymorphism were amplified with primer C3-V (Tab. II) and were identified on 6% SequiGel®XR-denaturating polyacrylamide gels

Table I. Primer sequences used for screening polymorphisms in the porcine *C3* gene.

Name	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)	Annealing temp. (°C)	cDNA (bp)	Genomic-DNA (bp)
C3–5′	GCATCGACTTGAATTCACAG	CCATGATGGAGGGACGGTG	55	–	477
C3–01	TCCTTTCCCTCTGTCCCTTT	GGGAGTCCCGTTTGATGTC	58	577	–
C3–02	TGAGACACCTGAAGGCATTG	GCTGTTCCATCCACACTCTC	58	306	∼ 700
C3–03	TCAACATCATTGCCAGGTTC	GGGTCTTGGTGAAGTGGATC	59	324	–
C3–04	CATCGTGACCTCCCCCTATC	GTGCGCAGGTGGAAGTTAAC	59	393	∼ 1000
C3–05	TCAAGCCAGGGGAGAATCTC	TCTCCAGGGTCATCTGTTGC	56	360	∼ 1500
C3–06	GAAGCAAGACAAGCAGCATC	TGTCCATCCTTTTCTCCATG	56	338	∼ 900
C3–07	GTGCAGCTCATGGAGAAAAG	ATTCATGGTCTTGGTGGAG	56	360	–
C3–08	CTCCACCAAGACCATGAAT	TCTTCAAGGGCACAATG	54	356	–
C3–09	CATTGTGCCCTTGAAGA	GTGCTGTCCAGGTAGTGC	55	396	–
C3–10	CAGTCATCGCTGTGCACTA	GTTCTTGAAGCCACCAATC	55	343	–
C3–11	GATTGGTGGCTTCAAGAAC	CTACCAGCAGAGCCAAG	52	341	–
C3–12	CTTGGCTCTGCTGGTAG	CGTGGTACATGGTCAC	52	338	∼ 1000
C3–13	GTGACCATGTACCACG	CCTCCAGGGTGTGTGAGAC	52	337	∼ 1300
C3–14	GATCTCACACACCCTGGAGG	CACTTCATCGGAGCCTGATT	55	379	–
C3–15	AATCAGGCTCCGATGAAGTG	GGCAACCAAAGACGACCAT	56	270	–
C3–3′	TGGCCAACTTCTCTGAGAAC	TGCAGGCAGATACAGTTGAG	60	–	530

Table II. Nucleotide sequence primers for genotyping the polymorphisms in the porcine *C3* gene.

Amplicon	Primer (5′ to 3′)	Size (bp)	Annealing temp. (°C)
C3-I	F: GCATCGACTTGAATTCACAG	477	55
	R: CCATGATGGAGGGACGGTG		
C3-II	F: CACCCTGATTGCTGCCAATG	383	60
	R: TACCTCAACTTACTGCGGTC		
C3-III	F: TGAGAATGTGGATGGACCAG	384	60
	R: GGACTTGAATGCCCAAGATC		
C3-IV	F1: AAGGATCTGAACCTGGATGTA	454	64
	F2: GGATCTGAACCTGGATGTG	452	
	R: ACCCCGCTAATCTGTGATGC		
C3-V	F: TGGCCAACTTCTCTGAGAAC	204, 207	60
	R: TAGGATGAACCTGAGCTGTG	211	

(Biozym Diagnostik GmbH) by the LI-COR 4200 automated sequencer (LI-COR Biosciences). The fragment size of alleles was analysed by One-Dscan software (Scanalytics, MWG Biotech).

2.4. Haemolytic complement activity phenotypes

Total haemolytic complement activities were determined in 416 F2-animals of the DUMI-resource population produced from 11 F1 sows (2 full-sib groups) mated with 3 F1 boars. The animals were reared in the Frankenforst research farm of the University of Bonn. They were immunized with Mycoplasma, Aujeszky and PRSS vaccines at 6, 14 and 16 weeks of age, respectively. Blood samples were taken immediately before vaccination and on 4 and/or 10 days after vaccination (Fig. 2). Total haemolytic activity of the alternative (ACP) and classical complement pathway (CCP) was determined by a method modified from Liu and Young [15]. Non sensitised rabbit erythrocytes and sensitised sheep erythrocytes were used as target cells, for ACP and CCP respectively. The haemolytic complement activity was expressed as the titre that lysed 50% of erythrocytes (CH50 unit $\cdot$ mL^{-1}).

2.5. Association analysis between *C3* and complement activity traits

Association between *C3* markers and haemolytic complement activity traits was analysed using the REPEATED statement of the SAS® PROC MIXED procedure [14]. The statistical model included *C3* genotype, time of blood

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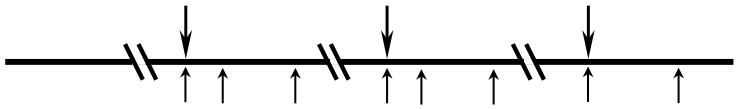

Figure 2. Vaccination programme and time of blood sampling in the DUMI-F2 resource population.

sampling, sire, and interaction of *C3* genotype and time of blood sampling as fixed effects, and dam nested within sire as random effect. A heterogeneous compound-symmetry (CSH) covariance structure was included in the statistical model to analyse fixed effects because it gave the best fit when compared to other covariance structures.

3. RESULTS

3.1. Sequence analysis of the porcine *C3* gene

The complete porcine *C3* cDNA sequence (accession No. AF154933) was determined. The 5127 bp cDNA contains an open reading frame coding for 1661 amino acids, including a 22-amino acids signal peptide, 643 amino acids β-chain, a 4 amino acids linker and 992 amino acids α-chain. The ORF of porcine *C3* shows 80, 78 and 77% homology with human, rat and mouse *C3* nucleotide sequence, respectively [6, 7, 17]. The 63 bp 5′-UTR is a pyrimidine rich region (approximately 80% pyrimidines). A 56 bp of 3′-UTR was sequenced and a polyadenylation signal (AATAAA) was found at position 5074 to 5079 of cDNA sequence (AF154933). The sequence information of the 5′-flanking region (Fig. 3a) contained several features of promoter region. The putative TATA-box (GATAAA) sequence was located at -29 bp of the first nucleotide *C3* sequence (AF154933) and various putative enhancer/transcription factor binding sequences were also found (Tab. III). Comparison of the porcine 5′-flanking sequence with the promoter region of human *C3* sequence (X62904) [10] revealed 74% sequence identity. However, the 3′-flanking region (Fig. 3b) of porcine *C3* had low homology with human *C3* sequence (M63422) [21].

3.2. Polymorphism and allelic frequency of the porcine *C3* gene

Six polymorphic sites were found in the porcine *C3* gene including five SNPs and one microsatellite (Fig. 4a). Two SNPs were found in the 5′-flanking

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-487  GACTCTTGCT GCCAGCATCG ACTTGAATTC ACAGGAAGGG TTTGGTTAAT
-437  AAGTGCATGG CCAAAAGTGG ACTGAGAGCC AGGTGCAGAT ATCGGGGAAG
-387  GGGGGCAGGC ACTATCTGGG GAAGAGAGAA TAATTTTCCT CCACTACAAA
-337  GTGGGTATAG CAGGGACAGG TCCCCATTCA CCAGCCAGCC TTGACCATGA
-287  GAGAGGCCAG GGCAGGAGGC TGTTGTATTC TTGTTGCAGG AGGACGTGCC
-237  CTTAGGTTAT TTTCCCCAGG ACTGTGATGG CCACACAGAT TGAAAAACTT
-187  TGGAAATGAC ATTGAGAAAT CTGGGGCAGC CCCAGGGAGG GGGGAGGCCA
-137  CAGGGAGTGG AGGGCTGGGC TGAAGAGGGG GAAAAGCAGC TGCCA[redacted]
-087  A GGCAGCCT CCAGCAGCCT CTGCTCACTT CCCCCCCCAC CCCCGTCCTT
-037  TCCCTCTGTC CCTTTGTCCC TCCACCGTCC CTCCATCATG ..........
      .......... .......... .......... .......... ..........
      ... G[redacted]                                       ....
      ..........................................................
   1  TGATGCCACT CCCCCACAGT CTACCC[redacted]  A GCTCCAGT TATCTTTCAC
  51  ATTTCCCCCC ACATCTGAAG GTCTTTTTTT TTTTTTTTTT GAAGGTCTTT
 101  GAGAGGGGAA AAGAGGCAGC TGTGATCCCG CCACTGCCTC ACCCACAGCT
 151  CAGGTTCATC CTACTGAAAC TCCACCTGCT TCCCACTTCA TCCCCTCCAG
 201  CAGTTCACCC GGCTTCCTGG CCTCAAGTGC ATGTACCTGC CTTACCTGCA
 251  CCTAGCGGGC AGTACATCTG CAGCTTCCCC TGCATCTCCC CTGCACCCCC
 301  ATCCTTACCC AAATTCTCAT TTACATTCTC ACCTGCATCC TCCCCAGCAG
 351  CTCACTGTCC CTTCACCTGA GCTTTCACCA CCTTGCTTAC CTACCTCCTT
 401  ATTTCATCCT CACCTGAGCT TTTCCCAATA GTCTCAGCTG AATCTTCACC
 451  CTCTCTCACC TGCACACTCA ACTGTATCTG CCTGCATTTT CTGCATCTTC
 501  CCTGCTTCCC CCACCTACAT CTTAATCTGT AAACTTAACT GTATCTCTAC
 551  CAACATTCTT TCCTTTATTC TCACCTTGTC TTTCTACCTG TATCCTTACC
 601  TGTATTTTCT CTGCATCCTC CTCAGGGTGA GGAACTGCAT CTTTGCCTAC
 651  ATCCCTCTCT AGTCTCTTTC CTGTGTTCTC ACCAGCATCC TTAGCTACAT
 701  TCTCACCTAC ATCTTCCCCT GCATCTTTTT TTTGCTTTTT TTTTTTTTGC
 751  TTTTTTTTTG CTTTTTAGGG CCACACCCAC GGCATATGGA GGTTCCCAAG
 801  CTAGGGGGCA AATTGGAGCT GCAGCTGCCA GCCTCACCAC AGCCACATCA
 851  ATGCAGGATC CGAATTCTTC GCCCTATAGT GAGT
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Figure 3. Sequence of the 5′-flanking region upstream from ATG start codon (**a**) and of the 3′-flanking region downstream from TGA codon (**b**) of the porcine *C3* gene (accession No. F154933). The 5′-UTR is indicated in italics and the putative transcription start site is indicated in italics, bold and underlined. A putative TATA box and polyadenylation signal are indicated in bold and underlined in the 5′- and 3′-flanking regions, respectively.

region at position −384 (G > A) and −44 (C > T) nucleotide (nt) upstream ATG start codon. The other three SNPs were located in the coding region at 1905 (C > A), 3882 (G > A) and the nt 204 (T > C) of the intron 13. A microsatellite with $(T)_{14\text{-}21}$-repeat units was found in the 3′-flanking region of *C3* and was located at 71 bp downstream from the TGA stop codon.

The two SNPs of 5′-flanking region were genotyped by SSCP analysis (Fig. 4b). *Taq* I-RFLP was used to detect the variation of the nt 204 in the intron 13 of *C3*-cDNA sequence (amplicon C3-II) and showed either undigested PCR product (384 bp, allele T), and/or digested product (285 bp and 143 bp, allele C) (Fig. 4c). Similarly, restriction enzyme *Hsp*92 I was used to test polymorphism at position 1502 (amplicon C3-III) and revealed either

Table III. Position of transcription factor binding sites in the 5′-flanking region of the porcine *C3* gene.

Factor	Consensus sequence	Position	Homology (%)	Ref.
Enhancer core	GGGGAAAA	−110 to −103	88	[8]
C/EBP	TTGAGAAAT	−176 to −168	100	[23]
IL-6-RE	AGGGGGA	−112 to −106	86	[21]
	TCTGGGG	−168 to −162	100	[21]
	TGAGAAA	−175 to −169	100	[21]
	TGAAAAA	−197 to −191	100	[21]
	TGAGAGA	−290 to −284	100	[21]
	TGGGGAA	−371 to −365	100	[21]
IL-6 RE rev	TTCCCCA	−220 to −226	100	[8]
	TCCTCCA	−351 to −345	100	[8]
IFNγRE	GAAAAACTTTGGAAA	−196 to −192	87	[8]
AP-2	CCCTTAGG	−132 to −139	88	[18]
LF-A1	TGGCCA	−210 to −205	83	[8]
	TGGACT	−420 to −415	83	[8]
LF-B1/HNF-1	GTTAAT	−443 to −438	100	[8]
	GTTATT	−232 to −227	83	[8]
Estrogen RE	TGTCCCTTTGTCC	−31 to −19	85	[21]

digested PCR product (237 bp and 146 bp, allele C) and/or 383 bp (allele A) of undigested product (Fig. 4d). The SNP located at 3882 was genotyped by allele specific PCR technique with two different primer sets (Fig. 4e). Microsatellite analysis in the 3′-flanking region of porcine *C3* revealed three alleles of 211, 207 and 204 bp (amplicon C3-V), and these alleles corresponded to $(T)_{21}$-, $(T)_{17}$-, and $(T)_{14}$-repeat units, respectively (Fig. 4f, allele 204 not shown).

Five of these polymorphic sites (at positions −44, 204 of intron 13, 1509, 3882 and +71) were segregating in the DUMI F2-resource population, and these alleles were coming from some Berlin Miniature Pig of the grandparent generation. All six polymorphic sites were segregating only in Thai native pigs, but no polymorphism was found among the 86 Duroc, German Landrace, Large White and Pietrain pigs. Allelic frequencies of the polymorphic sites in porcine *C3* gene among pig breeds are given in Table IV.

3.3. Association analysis

Means and standard deviation of the haemolytic complement activity of alternative and classical pathways were estimated (Tab. V). Only 3 SNPs

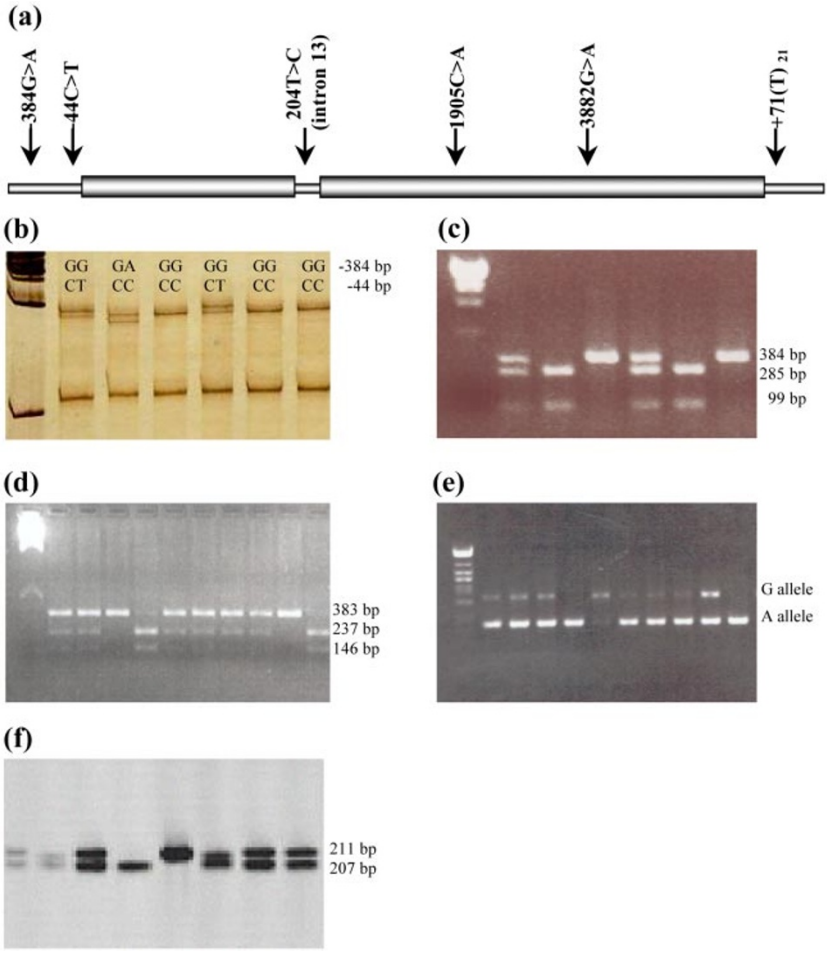

Figure 4. Polymorphism in the porcine *C3* gene including the 5′- and 3′-flanking regions (**a**); SSCP analysis at positions −384 and −44 bp in the 5′-flanking region (**b**); *Taq*I- PCR-RFLP test at position 204 in intron 13 (**c**); *Hsp*92 I-PCR-RFLP test at position 1905 (**d**); allele specific PCR test at position 3882 (**e**); and SSLP analysis at position +71 bp in the 3′-flanking region (**f**).

and one microsatellite (nt 204 of intron 13, 1905, 3882, +71) were genotyped. These four alleles were segregating with two haplotype patterns that can be described [2] as follows: allele *C3*1*, [IVS13+204T; 1905C; 3882G; c.5038+71(T)$_{21}$] and allele *C3*2*, [IVS13+204C; 1905A; 3882A; c.5038+71(T)$_{17}$]. The numbers of pigs for each genotype class were 290, 109 and 17 for *C3*1/C3*1*, *C3*1/C3*2* and *C3*2/C3*2* genotypes respectively. Subsequently, the genotype *C3*2/C3*2* was excluded from the analysis because of its low frequency and unequal distribution over parities and litters.

Table IV. Allele frequencies at the porcine *C3* gene in six genetic groups of pigs.

Position	Allele	Frequency					
		F2-DUMI	Duroc	German Landrace	Large White	Pietrain	Thai native pig
−384	G	1.00	1.00	1.00	1.00	1.00	0.99
−44	G	0.99	1.00	1.00	1.00	1.00	0.97
204 (intron 13)	T	0.75	1.00	1.00	1.00	1.00	0.82
1905	C	0.74	1.00	1.00	1.00	1.00	0.70
3882	G	0.74	1.00	1.00	1.00	1.00	0.82
$+71(T)_{21}$	T_{21}	0.76	1.00	1.00	1.00	0.00	0.71
$+71(T)_{17}$	T_{17}	0.24	0.00	0.00	0.00	0.00	0.12
$+71(T)_{14}$	T_{14}	0.00	0.00	0.00	0.00	0.00	0.17

Table V. Haemolytic complement activities (mean ± SD) of alternative and classical pathways in DUMI resource population.

	Haemolytic complement activity (Unit · mL^{-1})	
Blood sampling	ACH50	CCH50
Time-1	53.64 ± 28.31	40.70 ± 23.13
Time-2	53.22 ± 28.43	48.23 ± 28.81
Time-3	55.05 ± 24.86	47.97 ± 29.19
Time-4	56.86 ± 30.13	59.08 ± 36.46
Time-5	62.31 ± 32.08	64.91 ± 35.71
Time-6	68.46 ± 47.63	59.79 ± 39.10
Time-7	69.75 ± 41.70	62.53 ± 35.58
Time-8	69.71 ± 37.42	60.99 ± 40.89

Table VI. Significance in analysis of variance of alternative (ACH50) and classical (CCH50) complement activity traits.

	Test of fixed effects (*P* value)			
Traits	*C3* marker	Time	*C3* × time	Sire
ACH50	0.0255	< 0.0001	< 0.0001	< 0.0001
CCH50	0.0286	< 0.0001	0.0014	0.0151

Analysis of variance revealed that hemolytic complement activity of both alternative and classical pathways was significantly affected ($P < 0.05$) by *C3* marker, time of blood sampling and their interaction, and by sire effect (Tab. VI). The profiles of the haemolytic complement activities between the different *C3*

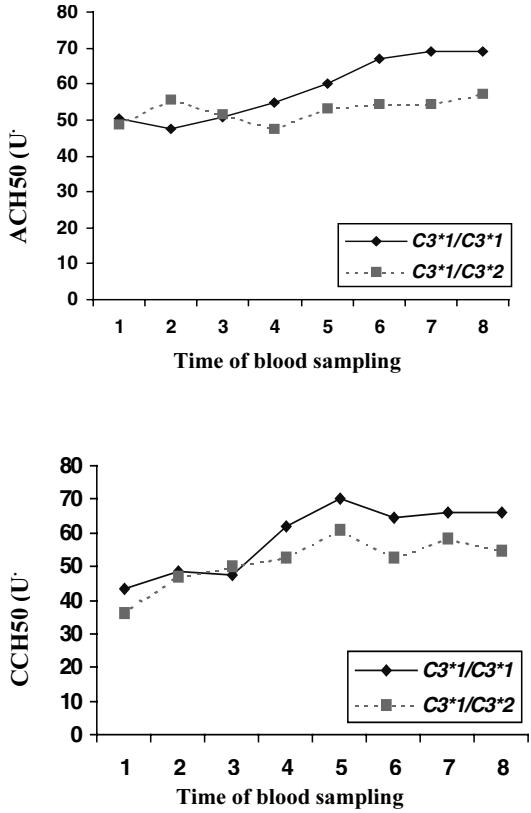

Figure 5. Haemolytic complement activity profiles of alternative (**a**) and classical (**b**) pathways for pigs with *C3*1/C3*1* and *C3*1/C3*2* haplotypes.

genotypes were not parallel over time (Fig. 5). Complement activities in the alternative and classical pathway were 5.8 and 6.9 units $\cdot$ mL^{-1} higher for animals homozygous for the *C3*1* allele than for *C3*1/C3*2* animals.

4. DISCUSSION

Activated complement system plays an important role in killing and neutralization of microorganisms, and *C3* is a key molecule of this system. Therefore, *C3* can be regarded as a candidate gene for complement activities. The 5.1 kb sequence of full-length porcine *C3* gene has a 4983-bp ORF which coded for α- and β-chain of *C3* protein. The porcine *C3* is a conserved gene and has high homology with the other mammalian species *C3* gene. Indeed, the putative promoter region contains one TATA-box and several cis-regulating

elements similar to that of the human *C3* promoter [8,21]. The variation of haemolytic complement activities before and after vaccination within the F2-DUMI resource population was found to be associated with *C3* markers. Although these *C3* markers were silent at the amino acids level, another nucleotide exchange in the 5′-flanking promoter region of the *C3* gene and in linkage disequilibrium with these *C3* variants might be responsible for its function. Animals with the more frequent *C3*1/C3*1* haplotype, present in Duroc and other commercial breeds, exhibited higher complement reactivity than those carrying the allele *C3*2* coming from Berlin Miniature Pigs.

Genetic variation in *C3* affecting the complement activity has been observed in human [3], rabbit [11], and mouse [5]. These reports indicated that the genetic defect of *C3* gene caused low (or lack of) haemolytic complement activity and increased susceptibility to microorganisms. Phenotypic variation of haemolytic complement activity in pigs has been associated with effects due to vaccination, genomic variation at the swine leukocytes antigen locus (*SLA*) [16,20], breed [12], age as well as sire or/and dam [16,24].

Although the complement is a complex system and is controlled by many genes, the present work showed there was an association of *C3* and haemolytic complement activity – a defence mechanism active against many pathogenic agents – which reinforces the importance of *C3* as a candidate gene for natural resistance to microorganisms.

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