

Association analysis of aquaporin 7 (AQP7) gene variants with semen quality and fertility in bulls

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Abstract: Aquaporin 7 (AQP7) gene as a candidate Antifreeze gene was investigated and associated with fresh and frozen semen quality traits in 45 Simmental and Charolais bulls. PCR-SSCP, PCR-RFLP, and sequencing method were employed to detect the single nucleotide polymorphisms (SNPs) of the AQP7 gene. Two mutations, A264G and G371C, were identified. The allele frequencies were A (0.97, 0.96) to B (0.03, 0.04) and C (0.97, 0.91) to D (0.03, 0.09) in Simmental and Charolais bulls, respectively. A264G substitution was significantly associated with acrosome integrity ratio ($P < 0.01$) and motility ($P < 0.05$) in frozen semen. Meanwhile, G371C substitution was significantly associated with acrosome integrity ratio ($P < 0.05$), percentages of viable sperm ($P < 0.05$), and motility ($P < 0.01$) in frozen semen. The results prove that AQP7 gene SNPs are useful for marker-assisted selection in early seed selection in the bull industry.

Key words: AQP7 gene, single nucleotide polymorphisms, SNPs, association analysis, Simmental bulls, Charolais bulls

Aquaporin 7 (AQP7) gene is a member of aquaglyceroporins that function in the rapid transport of glycerol and water (1-3). Immunohistochemical analysis demonstrated that the AQP7 protein exists in elongated spermatids, testicular spermatozoa, and residual bodies (4). The AQP7 gene is expressed at the tail of spermatids and spermatozoa in the human testis. The AQP7 protein is also detected at the middle piece and the anterior tail portion of ejaculated sperm (5). The AQP7 expression patterns between infertile and fertile ejaculated human sperms, as well as the

motility rates between AQP7 positive or negative sperms, are different, proving their roles in male fertility (6). The ontogeny and distribution of AQP7 in rat testis are associated with testis development and spermatogenesis (4). The present study aimed to identify the single nucleotide polymorphisms (SNPs) in the AQP7 gene and to associate these polymorphisms with semen quality and fertility in 2 pure bull groups. The results can help identify some useful markers that can be applied in early seed selection by MAS in the bull industry.

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A total of 45 bulls were investigated, including 34 Simmental and 11 Charolais bulls. All bulls were bred at the Institute of High-quality Cattle, Changchun, Jilin, Northeast China. The average age of the bulls was 37.5 ± 1.3 months. The data collection of semen quality accurately followed the previous work of our laboratory (7).

DNA samples were extracted from whole blood based on salt-chloroform extraction (8). The primers of 221 bp of 5'-UTR and 199 bp of exon 2 were designed according to AQP7 gene-genome sequence (chr 8: 79,256,000-79,294,000) as shown in Table 1. The amplification of the DNA samples was carried out in a final 25 μ L volume containing 50 ng of DNA template, 0.2 μ M of each primer, 0.2 mM dNTP, 2.5 mM MgCl₂, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The amplification conditions for both primers were as follows: denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min. The selected PCR samples were subjected to SSCP according to previous instructions (9). The PCR products were separated in 30% acrylamide gel (acrylamidebis-acrylamide, 29:1 Sigma) and stained by silver, whereas those with different SSCP strips were sequenced. Two mutations, A264G and G371C (NM_001076378.1), were identified. Enzyme analysis of Primer 5 software indicated that 2 SNPs can both be identified by *Taa*I-PCR-RFLP. Then 4 μ L of the PCR products was digested by 10 U *Taa*I (MBI, Vilnius, Lithuania) at 65 °C for 12 h and then subjected to 2% agarose gel electrophoresis. The *Taa*I-PCR-RFLP patterns of the bovine AQP7 gene are shown in Figure 1.

In the bovine AQP7 gene, 2 RFLP strip patterns were found at A264G (AA, AB) and G371C (CC, CD). Bioinformatics analysis shows that A264G substitution was 2 nucleotides before the ATG

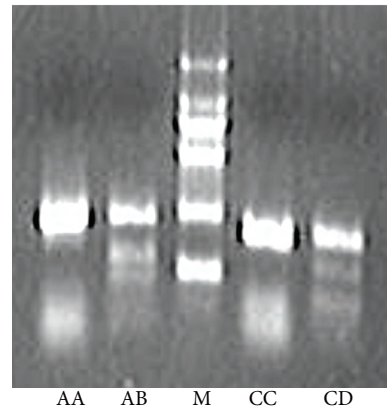


Figure 1. *Taa*I-PCR-RFLP patterns of the AQP7 gene A264G and G371C. M = marker DL2000 (ladder 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp, 100 bp); genotypes: AA = 221 bp; AB = 221 bp, 129 bp and 92 bp; CC = 199 bp; CD = 199 bp, 129 bp and 70 bp.

starting point, and G371C substitution caused the variation in amino acids from Glu (G-base) to Gln (C-base), which is the 36th amino acid of the bovine AQP7 protein. The allele frequencies were A (0.97, 0.96) to B (0.03, 0.04) and C (0.97, 0.91) to D (0.03, 0.09) in Simmental and Charolais bulls, respectively, according to the method of Cerit et al. (10). In the 2 SNPs, the other homozygous genotypes BB and DD cannot both be found.

The linear model of SAS software (SAS Institute Inc., Cary, NC, USA) was employed to associate the A264G and G371C SNPs with 10 semen quality traits according to our previous study (7). The results in Table 2 indicate that A264G substitution was significantly associated with acrosome integrity ratio ($P < 0.01$) and motility ($P < 0.05$) in frozen semen. Meanwhile, G371C substitution was significantly associated with acrosome integrity ratio ($P < 0.05$), percentages of viable sperm ($P < 0.05$), and motility ($P < 0.01$) in frozen semen.

Table 1. Primers for PCR-RFLP analysis of the bovine AQP7 gene.

Primer Pairs	Forward primer (5'→3')	Reverse primer (5'→3')
A264G	TGAGGTCTGATGGGAATGAGG	CTGCCATGAGGGAGGAGCTA
G371C	TCACCTGATCTCATTCTGCC	AGTCTGCTCACCCCTGTACCC

Table 2. Associations between 2 SNPs and 10 phenotypic traits in Simmental and Charolais.

SNP	Genotype	N	Traits									
			Ejaculation volume	Semen density	Fresh acrosome integrity ratio	Fresh sperm deformity ratio	Fresh percentages of viable sperm	Fresh motility	Frozen acrosome integrity ratio	Frozen sperm deformity ratio	Frozen percentages of viable sperm	Frozen motility
A264G	AA	42	6.85 ± 0.56	6.645 ± 0.40	0.91 ± 0.01	0.07 ± 0.02	0.84 ± 0.02	67.48 ± 1.20	0.88 ± 0.01 A	0.18 ± 0.02	0.42 ± 0.01	0.62 ± 0.02a
	AB	3	4.99 ± 1.78	7.48 ± 1.25	0.93 ± 0.04	0.10 ± 0.06	0.79 ± 0.05	60.87 ± 3.74	0.77 ± 0.04 B	0.25 ± 0.05	0.41 ± 0.03	0.50 ± 0.05 b
G371C	CC	41	6.79 ± 0.44	6.89 ± 0.31	0.91 ± 0.01	0.07 ± 0.02	0.86 ± 0.01	69.07 ± 0.95	0.86 ± 0.01a	0.17 ± 0.02	0.44 ± 0.01 a	0.64 ± 0.01 A
	CD	4	5.75 ± 1.39	6.20 ± 0.99	0.91 ± 0.03	0.10 ± 0.06	0.83 ± 0.04	66.88 ± 3.05	0.79 ± 0.03 b	0.23 ± 0.06	0.38 ± 0.03 b	0.54 ± 0.04 B

A, B: least squares means marked by the different letters differ significantly ($P < 0.01$); a,b: least squares means marked by different letters differ significantly ($P < 0.05$)

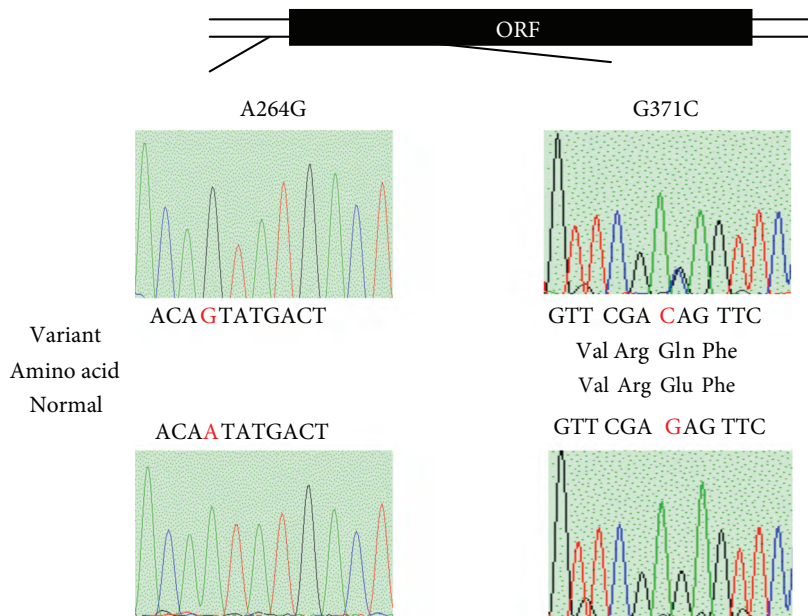


Figure 2. Sequence and amino acids variations at positions 264(A264G) and 371(G371C) of the *AQP7* gene.

To date, no data have been reported on the polymorphisms on the *AQP7* gene and its correlation with semen quality in bulls. Several papers have reported *AQP7* functions in male fertility and rat testis development and spermatogenesis (6), but there has been no report associating the bovine *AQP7* gene with semen quality traits to date. In the present study, 2 SNPs were found in the bovine *AQP7* gene. A264G substitution was 2 nucleotides before the ATG starting point, indicating that point mutations may impact gene transcription (11). Here, A264G substitution was supposed to change the GC content of the translation start region and influence

the ribosome to combine the ATG point and to translate the *AQP7* gene mRNA, finally decreasing *AQP7* protein expression. However, further validation is needed. G371C substitution caused E36Q substitution while they were both hydrophilic polar amino acids and did not change the water channel structure, indicating that the amino acids in the *AQP7* gene were relatively conserved. In this study, neither of the other homozygous genotypes BB and DD could be found. This can be attributed to artificial insemination or natural selection, leading to less utility of the poor semen. The precise reason for such condition must be examined in further studies.

In conclusion, the bovine *AQP7* gene A264G and G371C SNPs were first found in this work, and these 2 SNPs were associated with 10 semen quality traits. The results show that the suggested bovine *AQP7* gene is a useful maker for early seed selection by MAS in the bull industry. Further research on this area can mainly focus on the validation of these SNPs on MAS in larger populations.

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