Association of *MAP4K4 gene* single nucleotide polymorphism with mastitis and milk traits in Chinese Holstein cattle

Dinesh Bhattarai, Xing Chen, Zia ur Rehman, Xingjie Hao, Farman Ullah, Rahim Dad, Hira Sajjad Talpur, Ishwari Kadariya, Lu Cui, MingxiaFan and Shujun Zhang

Supplementary Material

This supplementary file comprises four Supplementary Tables, S1 to S4

Table S1 Primer sequences and information on cow *MAP4K4* gene

Serial No.	Region	Primers	Tm	Product Length
1	5'upstream-1F	F:GCGGAGACATCACTTTGCT	57.80	658
	5'upstream-1R	R:GTTTTGGGAATAAGGTGGC		
2	5'upstream-2F	CACTGTGCCACCTTATTCCCAA	56.70	592
	5'upstream-2R	CCCAGTTCTTGGCAAAGGTCA		
3	5'upstream-3F	GCTGCTGTGACCTTTGCCA	60.30	759
	5'upstream-3R	TCTCCTCCCGTTCTGTTGG		
4	5'upstream-4F	GTGTCTTGTCTCCCTCCAGC	56.40	103
	5'upstream-4R	TATCCAGTCTTCCTTTAGCG		
5	Exon 1F	TCCTCCCGTTCTGTTGGTTG	55.80	585
	Exon 1R	ATTTAAGGCGGAGGAGGTTC		
6	Exon 2F	ACTGTTCACTGGCAGCATC	59.20	399
	Exon 2R	CCAGAGCCAAGTCACCAT		
7	Exon 3F	GTGTCTGCGTCCTGTTGTG	60.00	457
	Exon 3R	GCACTGAACTGACTTGGGAC		
8	Exon 4F	TTTCCATCACCCTCTTCA	62.50	525
	Exon 4R	CAAATCCAGGTCATACATC		
9	Exon 5F	GTCCTCCTCGTGTTTTGCT	64.20	489
	Exon 5R	TGAAACACCAACACATTATTT		
10	Exon6F	CTTCGTTCCTGTTTTATCC	51.60	420
	Exon 6R	GACGGCGGTGCTTCTTAC		

11	Exon7F	CCCCTGAACCAAGCATCCCA	57.50	643
	Exon 7R	TTGTAGCTTGTGACTCAGTCCT		
12	Exon 8F	TGTCCGTGCGATTTTCCAGG	60.00	868
	Exon 8R	GGGCGGGGCAGGTCCGTAAA		
13	Exon 9F	TGGAAATAGTTGGCTGGTGG	61.40	588
	Exon 9R	GCAATCTTTCAGGCATCATACT		
14	Exon 10F	ATCCTGGCTGATTTGCTTTG	62.50	583
	Exon 10R	CCCTTATCACCGAAAATCCA		
15	Exon 11,12F	TCGCTGTCCTGTGCTGTCTG	60.00	495
	Exon 11,12R	AGAACCTTGGCTTTCCCTAC		
16	Exon 13F	GTCCTAAGCCGCCCCGTTTG	60.00	494
	Exon 13R	TGAGAAGTTCCTCTGCCATT		
17	Exon 14F	GCTTCACGCCAGCACCAT	60.00	420
	Exon 14R	TTGTAACGCAATCATCCT		
18	Exon 15F	TGAGGCAGTGGAATGGGTGA	60.80	419
	Exon 15R	AGTGGGTGCCATCATCTCCT		
19	Exon 16F	ATCTGTTTGTGGGCTAATGTTC	63.40	416
	Exon 16R	GGGATGGAGGGCTGACTATG		
20	Exon 17F	GGGGTTCTCAGGAAGGGTCA	60.00	545
	Exon17R	TTAGATGCCCACTCACCAAG		
21	Exon 18F	GGCTCCACTTCACAATACC	62.50	533
	Exon 18R	TCTGACTCTGTGCAACCC		
22	Exon19F	CTGTCTCCCCTCCTTTTGGT	54.80	426
	Exon 19R	GTGGATGAGCAAGTGGGTTA		

23	Exon 20F	GTCAGTAGGGTCTTCGGCATT	60.00	476
	Exon 20R	TGGCAAGTCTGGAGGGCAAT		
24	Exon 21,22F	CTTCCTCCTCCTTTACACCTT	52.30	601
	Exon 21,22R	CGGTCCACCCTTGAGAATAA		
25	Exon 23F	CTGTTTGTGGGTGACTTGAGG	59.30	387
	Exon 23R	AAATCCCGCTACGGTTCTGT		
26	Exon 24F	TGAAACCCTTCTGTGCTCTT	50.00	559
	Exon 24R	ACGGCTGAAGTGAACCTGAT		
27	Exon 25F	TTGAGGAAGATGGGGACAGC	60.00	315
	Exon 25R	CGCACTCGTCGTTATTACCT		
28	Exon 26F	CTTATGGTTTTGCCAGGGTG	58.40	371
	Exon26R	GCCCCTGCCCGTGTTAGTGC		
29	Exon27F	AGACACTCTTATTAAGCCCACG	62.50	375
	Exon 27R	CCAACACCTACTGCCAACCA		
30	Exon 28F	GGGAGCAGTTTTGTGGAAGC	62.50	493
	Exon 28R	CCAAGGAAAGGCTTCACAGA		
31	Exon 29 F	CTGGCTCCTTGCTGATGAATG	60.00	649
	Exon 29 R	GAGCCGCAGACAAGGAACAC		
32	3' downstream-1F	TATCCTGCCCAGAACCTTGT	57.50	490
	3'downstream-1R	GACCACCTGTCTGCAAACTCC		
33	3'downstream-2F	GCTCGGAGTTTGCAGACAGG	57.50	484
	3'downstream-2R	TCACCACGGAGGAGGAATAA		
34	3'downstream-3F	GATTATTTGTTAAAGGGTGGCT	57.50	629
	3'downstream-3R	CCCGAGAAGGGAATCACCAG		

S2 List of Enzymes and Primers used for RFLP

SNP ID	SNP Location	Enzyme for RFLP	RFLP primer	Annealing Tm	Restricted Fragments	
SNP1	Exon 13	FauI	CTGCCCAGACCACCTTGT CCTCTGCCATTTCATCAC	59.3	CC	226, 323
					CT	226, 323, 549
					TT	549
SNP2	Exon 14	BtsI	ACGCCAGCACCATTCAGA GTAACGCAATCATCCTCC	56.0	CC	413
					TC	119, 294, 413
					TT	119, 294
SNP3	Exon 18	HaeII	GGCTCCACTTCACAATACC ACAGGCTCGCTCCATCAC	62.5	GG	498, 87, 70
					TG	569, 498, 87,70
					TT	569, 87
SNP4	Exon 18	Eco24I	GAATGGCTGTTTGATGGATGGT CACTCATCCTCCCTAACGCTCAG	64.0	CC	238, 63
					TC	301, 238, 63
					TT	301
SNP5	Intron 18	PpuMI	CTGTGATGGAGCGAGCCTGTC AGGAGCCGTGACCCACAAGC	64.0	AA	232
					GA	232, 179, 59
					GG	173, 59
SNp6	Intron 21	MluI	GGGTAAGTAATACAGCAGCAA CGAGTATTGGTGGGATTGA	59.0	AA	67, 323
					GA	67, 323, 390
					GG	390
SNP 7	Intron 21	PstI	GGGTAAGTAATACAGCAGCAA CGAGTATTGGTGGGATTGA	59.0	CC	99, 291
					TC	99, 291, 390
					TT	390
SNP8	Intron 21	NdeI	GGGTAAGTAATACAGCAGCAA CGAGTATTGGTGGGATTGA	59.0	AA	104, 286

					AG	104, 286, 390
					GG	390
SNP9	Exon 18	Eco47I	AGCTGCGGGCCGTGGAGGAT	64.0	GG	23,98
			TCCACGTCGTCGTCCTCCTC		GA	23,98,121
					AA	121

S3 List of SNPs detected by sequencing in bovine *Map4k4* gene

S.N.	Genomic Region	*Position on UMD_3.1	AA change	Db SNP ID
1	5’ upstream	-1099C>T		rs135284164
2	5’ upstream	-960C>T		rs137163504
3	5’ upstream	-742G>A		rs132667541
4	5’ upstream	-569C>T		rs208338873
5	Exon 13	c.1562C>T	Ser-Phe	rs210779039
6	Exon 14	c.1714T>C	Gly-Gly	rs207521647
7	Exon 18	c.2061T>G	Ala-Ala	rs209280849
8	Exon 18	c.2115A>G	Thr-Thr	rs211626246
9	Exon 18	c.2196T>C	Pro-Pro	rs20834308
10	Exon 22	c.2730C>T	Arg-Arg	rs209508260
11	Exon 23	c.2904C>T	Asn-Asn	rs211639803
12	Exon 29	c.3624C>G	Ser-Ser	rs210713411
13	Intron 10	11:6632572C>T		rs446541471
14	Intron 13	11:6636830G>A		rs210449517
15	Intron 14	11:6637066A>G		rs208894049
16	Intron 18	11:6642504G>A		rs109082993
17	Intron 21	11:6646091G>A		rs211351050
18	Intron 21	11:6646116T>C		rs210900613
19	Intron 21	11:6646308A>G		rs208279998

*Positions with refrence with starting codin ATG as +1

S4 Genotypes, allele frequencies and the significance of deviations from HWE.

SNP ID	Location of SNP in Gene	Position of SNP on BTA11	Allele Substitution	Genotype	Number of Animals	Genotype Frequencies	Chi-square (X^2）	p-Value
SNP1	Exon 13		C>T	CC	91	0.31	0.21	0.88
				CT	144	0.49		
				TT	55	0.14		
					N=290			
SNP2	Exon 14		T>C	TT	82	0.31	1.28	0.25
				TC	108	0.51		
				CC	48	0.18		
					N=272			
SNP3	Exon 18		T>G	TT	124	0.30		
				TG	202	0.49	0.08	0.77
				GG	87	0.20		
					N=413			
SNP4	Exon18		T>C	TT	107	0.29	0.33	0.57
				TC	173	0.48		
				CC	79	0.22		
					N=351			
SNP5	Intron18		G>A	GG	53	0.18	0.02	0.91
				GA	144	0.49		
				AA	95	0.33		
					N=292			
SNP6	Intron21		G>A	GG	129	0.37	0.45	0.51
				GA	159	0.46		
				AA	57	0.16		
					N=345			
SNP7	Intron21		T>C	TT	47	0.18	0.94	0.33

			TC	136	0.52		
			CC	77	0.23		
				N=260			
SNP8	Intron21	A>G	AA	51	0.27	0.39	0.52
			AG	108	0.52		
			GG	48	0.22		
				N=207			
SNP9	Exon18	G>A	GG	4	0.01	37.2	0.00001
			GA	17	0.04		
			AA	396	0.95		
				N=417			