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SHORT COMMUNICATION

Single Nucleotide Polymorphisms in the Promoter of *CD4* Gene are Associated with Production and Mastitis Traits in Dairy Cattle

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The present study aimed at investigating the effects of single nucleotide polymorphisms (SNPs) in the promoter of *CD4* gene with mastitis and production traits in dairy cattle. Blood and milk samples were collected from 201 lactating dairy cattle maintained at government dairy farms in the Khyber Pakhtunkhwa, Pakistan. SNPs were identification in the pool of 30 randomly selected DNA samples followed by genotyping of these SNPs in all samples and statistical analysis by the general linear model of SAS (9.4). Two SNPs (SNP1 104010804G>A and SNP2 104010868T>C) were found associated significantly with the annual milk yield and the frequency of mastitis ($P<0.05$). The AA genotype of SNP1 and the TT genotype of SNP2 were significantly associated with milk yield and frequency of mastitis compared to other genotypes ($P<0.05$). Thus, the genotypes significantly associated with mastitis and production traits could be used as significant molecular marker in dairy cattle breeding programs.

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INTRODUCTION

Mastitis is an inflammatory disease of lactating animals. It primarily results from the invasion and duplication of vast variety of micro organisms and is associated with economic, animal health and welfare concern (Hogeveen *et al*., 2011). Thus, developing better models using marker assisted selection is need of the hour to develop improved resistance to mastitis in dairy cattle. Candidate gene approach is a widely proposed strategy that is based upon improving the host genetics through marker assisted selection, which looks for a single nucleotide polymorphisms (SNPs) in the genes that are related to mastitis.

CD4 gene plays an essential role in a variety of inflammatory conditions in many species (Wang *et al*., 2013). As mastitis is an inflammatory condition, therefore, *CD4* gene has received considerable attention as a candidate gene against mastitis resistance. Therefore, the present study was designed to identify the effect of SNPs in the promoter region of *CD4* gene with the mastitis indicator traits i.e. somatic cell count (SCC), somatic cell score (SCS), frequency of mastitis (FOM) and production traits like annual milk yield (AMY), fat percentage (F%), protein percentage (P%) and lactose percentage (L%).

MATERIALS AND METHODS

Sample collection: Blood and milk samples were collected from 201 cows of different cattle breeds including native Achai (29), Holstein-Friesian (104), Jersey (42) and cross breeds (HF x J=26) from four different dairy farms in Khyber Pakhtunkhwa, Pakistan. Primary data were obtained from the records of the animals available in the dairy farms and secondary data were generated from the primary data in MS Excel.

Milk composition and somatic cell count: Composition of milk (P%, F% and L%) was analyzed using milk analyzer (Ekomilk, BulTeh 2000, Bulgaria). SCC was calculated through direct microscopy using protocol of Usman *et al.* (2017).

Primers designing, DNA extraction, PCR and genotyping: The following primers were designed for the 2kb promoter region of *CD4* gene using the NCBI reference sequences by the online software (primer 3).

CD4 2kb P1 F 5`-CAAGAACAGGTGCCTAAGAG-3`,
CD4 2kb P1 R 5`-CTGGGACCTTCATGACCTGG-3`,
CD4 2kb P2 F 5`-TGGCAAATTGGTAGGAACTG-3`,
CD4 2kb P2 F 5`-CCACTCCCCAAGACTTCGCT-3`,
CD4 2kb P3 F 5`-CACCCAAGAACTTGTGAGAC-3`,
CD4 2kb P3 R 5`-AGGTCTGGATACACAAGCTG-3`.

The genomic DNA extraction and gene fragments amplification through PCR were performed through procedure as mentioned by Usman *et al.* (2017). The PCR products were sent to (Tsingke biological technology, Beijing, China) for genotyping using the Snapshot technique.

Statistical analysis: Association analysis of the SNPs with mastitis indicator traits and production traits were performed through Duncan`s multiple range test, using the General Linear Model procedure in SAS program (SAS v. 9.1.3) according to the following formula mentioned by Usman *et al*. (2014).

$$Y_{ijklmno} = \mu + SNP_i + B_j + P_k + H_l + Ym + Sn + e_o$$

Values of P<0.05 were considered as significant.

RESULTS AND DISCUSSION

Genotyping, allele and genotype frequencies and hardy–weinberg equilibrium (HWE): In the 2kb promoter region of *CD4* gene, two SNPs (104010804G>A and 104010868T>C) were revealed and genotyped in the total of 201 samples. Information of the SNPs, allele and genotype frequencies and HWE (χ^2 test) are summarized in Table 1. The SNPs were highly polymorphic and the χ^2 test showed that genotype frequency of SNP 2 was not in line with HWE (P<0.05).

Effect of SNPs on mastitis indicator and production traits: The results revealed that both SNPs were significantly associated with AMY and with FOM (P<0.05) (Table 2). Cows with AA genotype were associated with higher AMY, SCC, SCS, F%, L% and P% and lower FOM than the genotypes AG and GG in SNP1 (P<0.05). Similarly, in SNP2, cows with TT genotype had significantly higher AMY, FOM and the other parameters whereas, the production parameters i.e. L% and P% and SCS were lower compared to the CT and CC genotypes (P<0.05). Previous studies reported the significant association of SNPs in *CD4* gene with various inflammatory conditions in different species. Significant effect of SNP in *CD4* gene with SCC in Chinese Holstein cows was reported by He *et al.* (2011). A recent study reported a novel SNP (T104010752) significantly associated with SCC of clinical mastitis cows in bovine *CD4* gene (Usman *et al.*, 2017).

Effect of combined SNPs on mastitis indicator and production traits: Among nine combined genotypes pairs, the highly prevalent three were selected for statistical analysis. These genotypes were found significantly associated with AMY and FOM (P<0.05). AACC genotypes were associated with highest AMY and lowest FOM values compared to the other genotypes (Table 2). These results showed that both the SNPs individually as well as in combination type (AACC) were significantly associated with higher production and lower mastitis indicator traits. The significant association of these SNPs with production traits and with mastitis traits showed that these SNPs can play potentially important role in mastitis resistance.

Pearson's correlation amongst various phenotypic traits: The Pearson**'s** correlations showed significant association of breed with F%, SNF, P%, L%, Parity and FOM; F% was significantly correlated with L%; SNF was found highly significant with P% and L%. Correlation analysis showed significant association between P% and breed whereas, highly significant association with L% and SNF; L% showed significant association with breed and SCC; The mastitis indicator traits i.e. SCC, SCS and FOM were significantly associated with breed, L%, AMY and FOM. AMY was significantly correlated with F% while highly significant towards breed, SCS and FOM (Table 3). Correlation results are in agreement with the study of Ptak *et al.* (2012), El-Moghaz*yet al.* (2015) and Sourabh *et al.* (2017).

Table 1: Allele and genotype frequencies and Hardy–Weinberg equilibrium test of identified SNPs

SNP	Base position	SNP	Genotype frequency			Allele frequency		χ^2 Test P value
SNP1	2kb 104010804	A/G rs110894017	AA	AG	GG	A	G	0.15
			0.343 (n=69)	0.443 (n=89)	0.214 (n=43)	0.56 (n=227)	0.43 (n=175)	
SNP2	2kb 104010868	C/T rs109927115	CC	CT	TT	C	T	0.03
			0.617 (n=84)	0.398 (n=80)	0.184 (n=37)	0.61 (n=248)	0.38 (n=154)	

Table 2: Effect of SNPs on mastitis indicator and production traits

Marker	Genotype	AMY	SCC	SCS	FOM	F%	L%	P%
SNP1	AA	4164.53 ^{a}	217.39	3.26	0.18^{b}	4.04	4.75	3.14
	AG	3355.05^{ab}	231.74	3.18	0.26^{b}	3.79	4.63	3.15
	GG	2999.12^{b}	191.28	3.26	0.54^{a}	3.58	4.47	2.98
	P Value	0.09	0.27	0.69	0.02	0.38	0.43	0.48
SNP2	CC	2719.84^{b}	189.58	2.93	0.17^{b}	3.91	4.76	3.19
	CT	3625.21^{ab}	256.88	3.42	0.27^{b}	3.64	4.59	3.10
	TT	4490.01^{a}	199.32	3.33	0.61^{a}	3.82	4.45	2.95
	P Value	0.02	0.3389	0.64	0.005	0.54	0.14	0.61
Combine SNPs	AACC	4490.01^{a}	217.39	3.18	0.18^{a}	4.05	4.75	3.15
	AGCT	3682.33^{b}	257.24	3.40	0.28^{a}	3.82	4.60	3.10
	GGCT	2999.12^{c}	199.32	3.33	0.61^{b}	3.63	4.45	2.95
	P value	0.04	0.37	0.70	0.0097	0.59	0.15	0.64

*P value (<0.05), AMY= Annual Milk Yield, SCC=Somatic Cells Count, SCS= Somatic Cells Score, FOM= Frequency of Mastitis, F%= Fats Percentage, L%= Lactose Percentage, P%= Proteins Percentage.

Table 3: Pearson's correlation amongst various phenotypic traits

	BR	F%	SNF	P%	L%	AMY	SCC	SCS	FOM
BR	1	0.52	0.144	0.196	0.148	-0.544	-0.115	-0.133	-0.165
P value		0.01	0.041	0.005	0.036	0.001	0.105	0.061	0.019
F%	0.52	1	-0.096	-0.097	0.288	-0.043	0.021	-0.069	-0.012
P value	0.01		0.176	0.171	0.001	0.544	0.767	0.330	0.865
SNF	0.144	-.096	1	0.882	0.735	0.015	-0.110	-0.114	-0.091
P value	0.041	0.176		0.001	0.001	0.837	0.121	0.107	0.199
P%	0.196	-0.097	0.882	1	0.632	-0.092	-0.102	-0.111	-0.102
P value	0.005	0.171	0.001		0.001	0.193	0.148	0.118	0.151
L%	0.148	0.288	0.735	0.632	1	-.017	-0.179	-0.187	-0.137
P value	0.036	0.001	0.001	0.001		0.812	0.011	0.008	0.053
AMY	-0.544	-0.043	0.015	-0.092	-0.017	1	0.130	0.199	0.284
P value	0.001	0.544	0.837	0.193	0.812		0.067	0.005	0.001
SCC	-0.115	0.021	-0.110	-0.102	-0.179	0.130	1	0.723	0.134
P value	0.105	0.767	0.121	0.148	0.011	0.067		0.001	0.057
SCS	-0.133	-0.069	-0.114	-0.111	-0.187	0.199	0.723	1	0.130
P value	0.061	0.330	0.107	0.118	0.008	0.005	0.001		0.065
FOM	-0.165	-0.012	-0.091	-0.102	-0.137	0.284	0.134	0.130	1
P value	0.019	0.865	0.199	0.151	0.053	0.001	0.057	0.065	

Correlation is significant at the P<0.05.

The results of the present study show that high yielding *Bos taurus* breeds of cattle would face more chances of mastitis compared to the low producing indigenous cattle. These differences are attributed by both genetic and environmental factors. Through genetic interventions the production can be improved without compromising the health of the dairy cattle.

Conclusions: The present study showed that identified SNPs in the 2kb promoter of *CD4* gene individually and in combination had significant effect on mastitis indicator and production traits. The results infer that *CD4* gene should be considered as potential candidate gene and these SNPs are useful molecular markers for mastitis resistance in dairy cattle.

Authors contribution: TU and YW designed the experiment and supervised the project. AR, NA and SZ collected the samples and carried out the lab work. SN, IK, AK and IA performed data analyses and contributed in writing the manuscript. All authors read and approved the paper.

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