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Candidate SNP in Genomic sequence of Cacna2d1 gene and their association with production performance traits in Hardhenu cattle (*Bos indicus* × *Bos taurus*)

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Abstract
The present study was conducted to identify polymorphisms in Cacna2d1 gene and their association with production performance traits in Hardhenu cattle. The SNPs *viz*., A38795904G in intron 10 of Cacna2d1 gene were targeted and genotyped by PCR RFLP for the presence of polymorphism. Statistical analysis was performed to identify association of identified SNP with first lactation production performance traits in Hardhenu cattle. PCR amplified product of 386 bp harboring A38795904G mutation in particular intron 10 of Cacna2d1 gene resolved into three possible genotypes that were defined by three distinct banding patterns i.e. GG (229, 157 bp), AG (386, 229, 157 bp) and AA (386 bp) in present study. The genotypic frequency of GG genotype (GG= 62.5) with allelic frequencies as G = 0.79 was found to be predominant. While, corresponding allelic frequency of A (A=0.21) was reported to be rare in population at A38795904G locus. Chi-squared tests showed that A38795904G SNP meet with the Hardy–Weinberg equilibrium in our studied population. Least square analysis revealed significant association (p<0.01) between different genotypes of A38795904G SNP of Cacna2d1 gene with LMY-305 and FLL with the highest value of association of AG genotype with LMY-305 (2986.46±156.10 kg). While, it was highest for AA genotype with FLL (432.00±31.09 days). The association of this SNP with production performance traits can be utilized as an aid to selection after validation of results on large number of animals.

Keywords: Cacna2d1 gene, Hardhenu, production performance traits, SNP

1. Introduction
Cattle performance traits are a quantitative trait controlled by many genes, each one of them with small additive effect. Advancement in molecular genetics provides valuable information which could contribute to the knowledge of genes underlying quantitative traits (Czarnik *et al*., 2007; Matejicek *et al.,* 2007) [3, 8]. Marked assisted selection is a better alternative for early selection leading to higher genetic gain of targeted traits. Recent advances in molecular biotechnology provided great opportunities to incorporate molecular information into the traditional genetic evaluation models and to improve selection accuracies in livestock populations. These advancements have enabled the detection of some of the genes that contribute to genetic variation in economically important quantitative traits. The calcium channel, voltage-dependent, alpha-2/delta subunit 1 (Cacna2d1) gene encodes for a member of the alpha2/delta subunit family, a protein in the voltage-dependent calcium channel complex. The cattle Cacna2d1 gene contains 39 exons and 38 introns and has been mapped to BTA 4q18 (Buitkamp *et al.,* 2003) [2]. Cacna2d1 gene are a group of voltage gated ion channels found in excitable cells i.e. muscle, glial cells, neurons, etc. with a permeability to the ion Ca^{2+} (Hille and Bertil, 2001; Purves, 2001) [7, 9]. It plays a great role in muscle physiology i.e. in contraction. Furthermore, Bagheri *et al*. (2013) [1] reported SNPs of Cacna2d1 gene and their association with milk yield in Iranian Holstein cattle, but no research work has been performed so far in *Bos taurus* ×*indicus* cattle. Although, little work has been done in past regarding associationship of the above gene with production performance traits in cattle. Keeping all these points in view, the objective of the present study was to study association of Cacna2d1 gene with production performance traits in Hardhenu (*B. taurus* × *B. indicus*) cattle.

2. Materials and Methods
The experimental animals for the present study were taken from the herd of Hardhenu cattle maintained at Cattle Breeding farm (CBF), Department of Animal Genetics and Breeding, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

Blood samples were collected from a total of 40 randomly selected animals completed third parity to screen for the presence of polymorphisms in the genomic region of CACNA2D1 gene and association of different genotypes with the production traits. About 10 ml of blood collected from each animal aseptically in a sterile vial containing 0.5% EDTA (10 µl/ml of blood) from Jugular vein puncture. The blood stored in the freezer at 5 ^{o}C before the DNA isolation. DNA isolated from blood using protocol of Sambrook and Russell (2001) with few modifications. Quality of DNA was checked by electrophoresis by loading 2-5 µl DNA on 0.8% agarose in horizontal mini electrophoresis unit using 1X TBE as running buffer. After electrophoresis gel was stained with ethidium bromide solution (0.5 µg/ ml). The quality of DNA was estimated by comparing the bands with reference DNA in electrophoresis gel and the quantity determined by UV spectrophotometry. Optical density (O.D.) determined at 260 nm and 280 nm wavelengths in spectrophotometer against distilled water as blank sample. Primers designed online using Primer 3.0 software with the relevant reference sequences for the segmentation of entire gene comprising all exonic, intronic and promoter region and the annealing temperature calculated using the Wallace formula. The PCR parameters *viz*. concentration of genomic DNA and $MgCl_2$ and annealing temperature was optimized to obtain a specific amplified product in sufficient quantity. The reaction volume was kept constant at 25 µl. Thermal cycler programmed to carry out the PCR amplification.

The amplified PCR product checked by running in 1.5% agarose gels in 1X TBE along with suitable DNA marker. The amplified product was visualized, size estimated, and documented under gel documentation system. The PCR amplified products were subjected to restriction digestion with Taq-1 enzyme. The restriction digested fragments separated on 2.5% agarose gels and resolved by ethidium bromide. Photographs were taken using gel documentation system to screen for restriction fragment length polymorphism in the population to be studied. Allelic and genotype frequencies were calculated by direct counting method (Falconer and Mackay, 1998) [4]. Suitable model used for association analysis.

3. Results and Discussion

3.1 SNP identification and allele frequencies

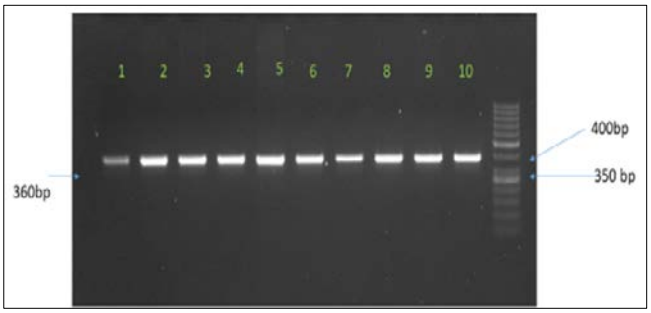
The SNPs *viz*., A38795904G in intron 10 of Cacna2d1 gene were targeted and genotyped by PCR RFLP (Reference sequence: ENSBTA00000020569). The 386 bp amplicon harboring A38795904G mutation in intron 10 of Cacna2d1 gene was digested with the *Taq* I restriction enzyme, the three possible genotypes were defined by three distinct banding patterns i.e. GG (229, 157 bp), AG (386, 229, 157 bp), and AA (386 bp) were observed in present study (Plate 1 and 2). This finding appeared in conformity with the observations of Yuan *et al.* (2011) in Chinese resource population [(Holstein-73, Sanhe- 78, Simmental-89 (N=240)] and Bagheri *et al.*, (2013) [1] in Holstein cows. This variation between breeds might be due to selection pressure and breed differences

The genotypic and allelic frequencies were obtained as GG = 62.5, AA = 5, AG = 32.5 with allelic frequencies of G = 0.79 and A = 0.21 at A38795904G locus. Chi-squared tests showed that A38795904G SNP meet with the Hardy–Weinberg equilibrium (p<0.01) in crossbreed cattle population (Table 1).

3.2 Association of Genotypes of Cacna2d1 gene with production performance traits

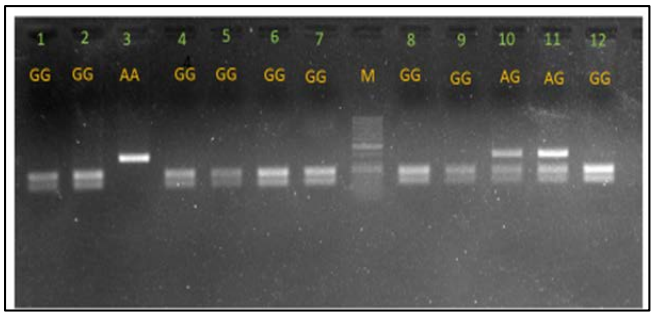
The association between different genotypes of A38795904G SNP of Cacna2d1 gene and production performance traits were estimated. The association between different genotypes of A38795904G SNP of Cacna2d1 gene and production performance traits revealed significant association (p<0.01) with LMY-305 and FLL with the highest value of association of AG genotype with LMY-305 (2986.46±156.10 kg). While, it was highest for AA genotype with FLL (432.00±31.09 days). Moreover, LMY, PY and AFC did not find any significant association with genotype (Table 2).

Different research workers reported association of allele G of Cacna2d1 gene with the somatic cell count and susceptibility to mastitis. Bagheri *et al.* (2014) [1] reported allele G of Cacna2d1 gene to be predominant and allele A which is rare was found to be highly associated with milk yield. On the other hand, Yuan *et al.* (2011) reported the corresponding genotypic frequency as (AA=0.42, AG=0.16, GG=0.42) in Chinese Holstein population. While, slightly lower values for different genotypes (AA=0.44, AG=0.22, GG=0.34), (AA=0.45, AG=0.17, GG=0.38) than present study were reported in Sanhe and Simmental cattle. Whereas, corresponding lower allelic frequency of G (G=0.49, 0.46, 0.47) were reported in all three Chinese source population than the present study. In present study, AG genotype has highest value of association with LMY-305. So, we have to made compromise either for high milk yield and susceptibility to mastitis in selection programmes, however, validation of results on large number of animals is warranted.



Lane 1-9: PCR Product (386 bp)
Lane M: 50bp DNA ladder

Plate 1: PCR amplified product in intron 10 of CACNA2D1 Gene in Hardhenu cattle



Lane 3: AG Genotype 386 bp,
Lane 11,12: AG Genotype 386, 229, 157 bp
Lane 1-2,3-8,9: GG Genotype 229, 157 bp
M: 50 bp Marker

Plate 2: PCR-RELP of intron 10 of CACNA2D1 gene using *Taq* 1 restriction enzyme in Hardhenu cattle

Table : Estimates of gene and genotypic frequency of Cacna2d1 gene in cattle

Genotype Code	Genotypes	No. of Genotype	Allele frequency	Chi-square value
1	GG	25 (62.5)	0.7875	0.0335^{NS}
2	AA	02 (5.0)	.2125	
3	AG	13 (32.5)		

Figures in parenthesis showing genotypic frequency

Table 2: Estimates of association of genetic variants of Cacna2d1 gene with various production traits

Genotype and codes		Production performance Traits				
Genotype Code	**Genotypes**	**AFC**	**FLMY**	**FPY**	**FLL**	**LMY-305**
1	GG	1236.88±40.78	2799.56±194.76	16.12±0.70	303.20^{a}±9.93	2675.32^{a}±185.71
2	AA	1242.50±15.55	3451.50±266.29	15.85±0.95	432.00^{b}±31.09	2644.50^{a}±165.99
3	AG	1198.54±43.61	3187.62±175.14	16.55±0.69	326.00^{a}±13.88	2986.46^{b}±156.10

4. Conclusion

Several studies have been performed to explore the candidate SNPs associated with production and disease resistance traits in cattle. As these are quantitative traits controlled by number of genes, therefore, proper validation and implementation is the perquisite for any successful breeding programs. In the present study, different genetic variants of A38795904G in intron 10 of Cacna2d1 gene were targeted and genotyped by PCR RFLP. It was revealed that in Hardhenu cattle with respect to the particular region, G allele is predominant with allelic frequencies of G = 0.79 at A38795904G locus. In present study, AG genotype has highest value of association with LMY-305 and AA genotype has highest association with first lactation milk yield but having high lactation length. So we can use this as a marker to select animals having high LMY-305 and susceptibility to mastitis. Since the detection of this association is based on a relatively small sample size, further work are necessary to study these SNPs in larger populations and other breeds to better clarify the role of these SNPs on production performance traits in cattle.

5. References

1. Bagheri M, Ashtiani RM, Shahrbabak MM, Javaremi AN, Pakdel A, Bosstel UV S *et al*. Selective genotyping and logistic regression analyses to identify favorable SNP-genotypes for clinical mastitis and production traits in Holstein dairy cattle. Livestock science 2013;151:140-15.
2. Buitkamp J, Ewald D, Masabanda J, Bishop MD, Fries R. FISH, RH mapping of the bovine alpha(2)/delta calcium channel subunit gene (CACNA2D1) Anim. Genet 2003;34:302-318.
3. Czarnik U, Zabolewicz T, Strychalski J, Grzybowski G, Bogusz M,Walawski K *et al*. Deletion/insertion polymorphism of the prion protein gene (PRNP) in Polish Holstein-Friesian cattle. Journal of Applied Genetics 2007;48:69-71.
4. Falconer DS, Mackay TFC. Introduction to Quantitative Genetics Longman, Essex, U.K., 1996, 4th ed. edition.
5. Gabashvili IS, Sokolowski BH, Morton CC, Giersch AB. Ion channel gene expression in the inner ear. Journal. Assoc. Res. Otolaryngol 2007;8:305-328.
6. Gene id ENSBTA00000020569 mRNA and lncRNA profiles in extracellular vesicles secreted from LIM1683 CRC cells @SciReports
7. Hille and Bertil. Ion channels of excitable membranes. Sunderland, Mass; 2001 Sinauer.ISBN 0-87893-321-2.
8. Matejicek A, Matejickova J, Nemcova E, Jandurova OM, Stipkova M, Bouska J *et al*. Joint effects of CSN3 and LGB genotypes and their relation to breeding values of milk production parameters in Czech Fleckvieh. Czech J Anim. Sci 2007;52:83-87.
9. Purves D, Augustine GJ, Fitzpatrick D. The Role of Calcium in Transmitter Secretion. Neuroscience. 2nd edition. Sunderland (MA), 2001; Sinauer Associates.
10. Sambrook J, Russel DW. Commonly used techniques in molecular cloning Appendix 8, in Molecular Cloning, 3rd edition. Cold Spring Harbor Laboratory Press, 2001; Cold Spring Harbor, NY, USA.
11. Vinci VA, Byng G. Strain improvement by non-recombinant methods. Manual of Industrial Microbiology and Biotechnology, (Demain, A. & Davies, J. eds.) 2001; pp. 103-113. ASM Press, Washington, D.C.
12. Yuan Z, Li J, Liu L, Zhang L, Chen C, Chen X *et al*. Single nucleotide polymorphism of CACNA2D1 gene and its association with milk somatic cell score in cattle. Molecular Biology Reports 2011a;38:5179-5183.