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Identification of Genetic Polymorphism in Resistin (RETN) Gene and its Influence on Reproduction and Production Traits of Indian Dairy Cattle

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ABSTRACT

The present study was executed to elucidate the genetic polymorphism in resistin (*RETN*) gene and its association with reproduction and production traits in Sahiwal cattle. A fragment of exon-2 region of *RETN* gene from genomic DNA of Sahiwal cattle was amplified by PCR that resulted in amplicon of 338 bp. The obtained amplicon was subjected to Single Stranded Confirmation Polymorphism (SSCP) technique for identification of genetic polymorphism which revealed two genotypes, homozygous AA and heterozygous AB genotypes. In the studied population of Sahiwal cattle AA was most frequent genotype (67.24%) than AB genotype (32.76%). The frequency of A and B alleles were 0.836 and 0.164, respectively. The association study of RETN/SSCP assay revealed significant influence of genotypes on birth weight (BW), dry period (DP) and lactation period (LP). The RETN/SSCP polymorphism revealed higher BW in AA than AB genotype. The study showed significantly longer DP in AB than AA genotype in second lactation while significantly longer LP was observed in AA compared to AB genotypes in first lactation of Sahiwal cows. In conclusion, PCR-SSCP assay was found to be capable of detecting genetic polymorphism in exon-2 region of RETN gene and its association with reproduction and production traits suggests that A allele of this gene might serves as candidate genetic marker for selection of Sahiwal cattle with better reproductive and production traits. However, further studies are warranted to discover this genetic polymorphism in another breed and population of cattle.

Keywords: RETN gene, PCR-SSCP, Reproduction, Production, Sahiwal cattle

Resistin (RETN) is an important adipokine secreted from adipocytes of dairy cows (Ebner *et al.,* 2013). It plays crucial role in regulation of energy metabolism by affecting insulin sensitivity in peripheral tissues thereby influences immunity, reproduction and cardio-vascular function (Waki *et al.,* 2003). The natural expression patterns of RETN in follicular and luteal cells of the bovine ovary also reported to affect the physiological status of the ovary (Maillard *et al.,* 2011). The significant role of RETN in energy metabolism and ovarian function suggests that this adipokine could affect production and reproduction in dairy animals.

Moreover numerous studies have been carried out to unearth the associations between quantitative trait loci (QTL) and genetic markers recognized from the genome and targeted to determine genetic variants associated to production traits of beef cattle (Casas *et al.,* 2000; Li *et al.,* 2004; Gao *et al.,* 2007). In addition several single nucleotide polymorphisms (SNPs) have been documented in the promoter, intron and 3'UTR (untranslated region) regions in RETN gene so far and genetic association of these SNPs were reported to affect resistin levels, BMI, obesity, type 2 diabetes mellitus and blood pressure (Mattevi *et al.,* 2004; Osawa *et al.,* 2007; Suriyaprom *et al.,* 2009; Onuma *et al.,* 2010; El-Shal *et al.,* 2013). Moreover SNPs in intron and exon regions of RETN gene were also identified and found associated with carcass and meat quality traits (Park *et al.,* 2007; Gao *et al.,* 2011) of

beef cattle. Taking into consideration the association of RETN gene with meat quality traits as well as the function of the encoded proteins in energy metabolism, this study was aimed to identify genetic polymorphism in RETN gene and its association with reproduction and production traits of Sahiwal cattle.

MATERIALS AND METHODS

Animals and DNA extraction

Sahiwal cattle maintained at Instructional Livestock Farm Complex (ILFC), College of Veterinary Science and Animal Husbandry, Mathura, were used for the study. Approximately 5 ml of blood was collected from jugular vein in vacuitainer tubes containing EDTA as anticoagulant and genomic DNA was isolated as per the standard phenol-chloroform isolation protocol (Sambrook and Russell, 2001). Quality and quantity of DNA was determined using spectrophotometer by measuring optical density at wavelength of 260 and 280 nm.

PCR amplification

To amplify the genomic sequence of exon-2 region of bovine RETN gene (AY618903), following primer set was used.

Table 1: Primer set used for amplification of ADIPOQ gene

Gene	Primer sequence	Region	Reference
RETN	F:5'– CCAACCCAACGC CAATCT-3'	Exon-2 (AY618903)	Gao *et al*., 2011
	R:5'–AACGGAGTTCTGTA CCTACC-3'		

The PCR reaction was performed in thermocycler (Bio-Rad, USA) in a 25µl reaction mixture containing 5 pM of each primer, 250 µM of each dNTP, 1 Unit of Taq DNA polymerase, 2.5 µM of reaction buffer and 50-100 ng of DNA as template. The PCR program for RETN gene was 95°C for 5 min, followed by 35 cycles of 94°C for 30s, 57.9°C for 35s and 72°C for 35 sec. The final step prolonged for 10 min at 72°C.

PCR-SSCP of RETN gene

For determination of genetic polymorphism, the obtained PCR products were subjected to SSCP technique (Sambrook and Rusell, 2001) with some modification as used by Gao *et al.,* (2011). In this technique, double-stranded DNA amplicons were denatured to single-stranded (ss) DNA by mixing 2.5 µl PCR product and 7.5 µl of a formamide loading dye and heated at 99°C for 10 minutes in the thermal cycler machine. After boiling, tubes were immediately immersed in ice-chilled box and kept in -20°C deep freeze for 10 minutes. Subsequently samples were loaded on 12% gel of polyacrylamide gel (PAGE) under non-denaturing conditions in 0.5 × TBE buffer (pH 8.3) and electrophoresis was carried out first at 80V for 30 min then at 65V for 18 hrs at 4°C in refrigerator. After completion of the electrophoresis the gel was separated from the plates and subjected to silver staining to visualize SSCP band patterns.

Statistical analysis

The allele and genotype frequencies of RETN/SSCP polymorphism was examined for deviation from Hardy-Weinberg equilibrium using χ^2 test and statistical significance was determined by student's *t*-test using SPSS software for Windows (version 16.0). The data were presented as the mean ± SE and a p value <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Gene and genotypic frequency of RETN gene locus

The amplification of exon-2 fragment of RETN gene revealed ampilcons of 338 bp which were confirmed on 1.0% agarose gel (Fig. 1). The RETN/SSCP assay revealed two types of genotypes, the samples showing two bands in the gel were considered as homozygous (AA) while others showing three bands in the gel were considered as heterozygous (AB) (Fig. 2). The results revealed that studied population of Sahiwal cattle was polymorphic in nature with two types of alleles A and B. The most frequent genotype observed in the population was AA (67.24%) and other less frequent was AB (32.76%). The frequencies of A and B alleles were 0.836 and 0.164, respectively. However, the selected population of Sahiwal cattle was not found in Hardy- Weinberg equilibrium.

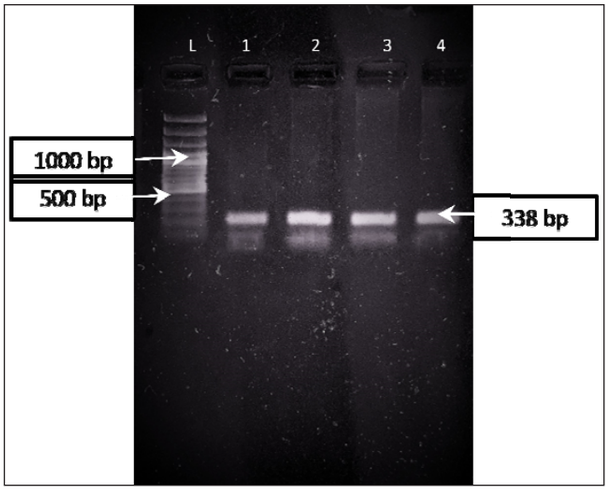
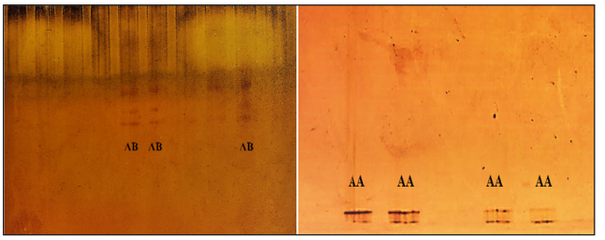


Fig. 1: Agarose gel electrophoresis (1%) of RETN PCR product showing 338 bp products in all lanes (1-4), L= Ladder (100 bp)



(a) **(b)**

Fig. 2: RETN-SSCP assay on 12% PAGE showing **(a)** heterozygous (AB) and **(b)** homozygous (AA) genotypes

Similar to the present findings, Gao *et al.,* (2011) also reported two genotypes in Luxi and Simmental cattle breeds of China, substantiates the findings of present study. However they reported three genotype viz. AA, AB and BB in Nanyang and Xia'nan cattle breeds of China. In present investigation the genotypic frequency of AA and AB genotypes were 67.24 and 32.76%, respectively. In agreement with the present report Gao *et al.,* (2011) also reported higher genotypic frequency of AA genotype i.e. 78.13, 70.83 and 94.34% in Luxi, Jiaxian and Lxi X Simmental breeds of cattle, respectively. The variation in frequency of genotypes might be due to population size and geographical distribution of the animals.

Association of RETN/SSCP genotypes with reproduction and production traits

The results of association of RETN/SSCP genotypes with reproduction and production traits for four lactations of Sahiwal cattle are presented in Table 2 and 3, respectively. The RETN/SSCP assay revealed noteworthy effect of genotypes on birth weight (BW), dry period (DP) and lactation period (LP) of Sahiwal cattle. The assay revealed significantly higher BW in AA genotype compared to AB genotype. The results also showed significantly shorter DP in AA than AB genotype in second lactation. Similarly the LP of cows showed significant association with genotypes in second lactation which revealed longer LP for AA genotype compared to AB genotype. Moreover the RETN/

Table 2: Association of RETN/SSCP genotypes with reproduction traits

Lactation	Genotype	n	BW (kg)	AFS (days)	AFC (days)	GP (days)	DP (days)	CI (days)
I (N=36)	AA	28	19.19 ± 0.60	1167.10 ± 105.12	1641.30 ± 78.16	277.23 ± 3.26	188.96 ± 19.80	611.54 ± 32.29
	AB	18	21.11* ± 1.18	1070.90 ± 82.18	1590.80 ± 91.51	280.44 ± 2.47	194.00 ± 30.18	522.61 ± 26.53
II (N=34)	AA	22				283.91 ± 2.38	119.23 ± 13.40	520.14 ± 23.27
	AB	12				281.50 ± 2.84	150.58* ± 33.21	505.58 ± 36.48
III (N=21)	AA	13				283.69 ± 2.79	152.46 ± 17.45	494.23 ± 19.36
	AB	8				283.75 ± 2.43	140.62 ± 36.46	492.38 ± 40.51
IV (N=19)	AA	11				282.55 ± 2.82	156.82 ± 30.72	447.91 ± 26.92
	AB	8				280.00 ± 2.17	135.88 ± 25.30	473.62 ± 30.23

Means bearing asterisk (*) in a column for one lactation differ significantly ($P < 0.05$); BW: Birth weight; AFS: Age at first service; AFC: Age at first calving; GP: Gestation period; DP: Dry period; CI: Calving interval.

Table 3: Association of RETN/SSCP genotypes with milk production traits

Lactation	Genotype	n	LP (days)	TMY (liters)	AMY (liters)	MYPP (liters)	MY300 (liters)	PY (liters/ day)	DRPY (days)
I (N=36)	AA	28	422.39 ± 23.75	2114.10 ± 179.31	4.84 ± 0.17	79.93 ± 4.55	1452.80 ± 51.42	7.18 ± 0.28	40.75 ± 2.93
	AB	18	328.72* ± 15.19	1672.90 ± 127.84	5.01 ± 0.28	77.39 ± 7.21	1506.40 ± 84.28	8.00 ± 0.51	43.78 ± 4.34
II (N=34)	AA	22	403.18 ± 21.79	2305.50 ± 132.69	5.80 ± 0.23	119.77 ± 5.67	1738.20 ± 67.29	9.36 ± 0.45	40.50 ± 2.54
	AB	12	353.75 ± 18.30	2143.20 ± 174.47	6.09 ± 0.48	103.12 ± 10.33	1832.20 ± 145.64	8.54 ± 0.64	36.92 ± 4.08
III (N=21)	AA	13	346.23 ± 22.33	1902.80 ± 170.44	5.47 ± 0.31	96.85 ± 11.21	1640.30 ± 93.78	8.77 ± 0.48	38.38 ± 3.62
	AB	8	377.25 ± 33.32	2390.10 ± 215.24	6.35 ± 0.36	126.19 ± 16.13	1910.90 ± 108.95	10.63 ± 0.72	38.25 ± 3.33
IV (N=19)	AA	11	288.09 ± 15.66	1595.00 ± 194.13	5.40 ± 0.54	85.09 ± 9.39	1624.50 ± 162.67	8.59 ± 0.95	49.73 ± 9.52
	AB	8	341.12 ± 14.84	2113.80 ± 121.26	6.29 ± 0.48	109.88 ± 12.95	1886.00 ± 143.17	9.94 ± 0.83	42.38 ± 6.58

Means bearing asterisk (*) in a column for one lactation differ significantly ($P < 0.05$); LP: Lactation period; TMY: Total milk yield; AMY: Average milk yield; MYPP: Milk yield in periparturient period (21 days); MY300: Milk yield in 300 days; PY: Peak yield; DRPY: Days to reach peak yield.

SSCP assay did not show significant association with other reproduction (AFS, AFC, GP, CI) and production (TMY, MYPP, AMY, MY300, PY and DRPY) traits of Sahiwal cattle.

This was the first kind of study that elucidated the influence of genetic polymorphism in RETN gene on reproduction and production traits of dairy cattle. The only study conducted so far for determination of genetic polymorphism at the exon-2 region of the RETN gene documented their significant association with carcass traits in cattle breeds of China (Gao *et al.,* 2011). In addition Park *et al.* (2007) also reported significant association of genetic polymorphism in RETN gene on carcass traits of Korean cattle however they identified genetic polymorphism at different region of the RETN gene. Moreover no other reports are available documenting the genetic polymorphism at exon-2 region of RETN gene and their influence on production and reproduction traits of dairy animals.

Thus it can be concluded from the study that PCR-SSCP is capable of identifying genetic polymorphism in exon-2 fragment of RETN gene and association of this polymorphism with production and reproduction traits of Sahiwal cattle indicated that this gene might be used as genetic marker for selection of dairy cattle with better production potential. However additional investigations are required to authenticate this genetic polymorphism in another breed and population of dairy cattle.

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