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# Identification of Polymorphism in Intron 2 of Adiponectin (*ADIPOQ*) Gene in Indian Sahiwal Cattle

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**ABSTRACT**

Adiponectin (*ADIPOQ*) gene regulates the glucose metabolism, insulin sensitivity, fatty acid oxidation, immunity and reproduction. In the present investigation, PCR-RFLP method was conducted to identify the genetic polymorphism in intron 2 region of adiponectin (*ADIPOQ*) gene in Indian Sahiwal cattle. PCR of intron 2 of *ADIPOQ* gene fragment produced amplicon of 961 bp which was subsequently digested with *Rsa*I restriction enzyme. The *Rsa*I/PCR-RFLP assay revealed monomorphic pattern only, TT genotype (wild type homozygote) in Sahiwal cattle population which was confirmed by sequencing. The obtained sequences of *ADIPOQ* after aligning revealed absence of *Rsa*I recognition site GTAC and consequently, the association study with economic traits could not be performed.

**Keywords:** Adiponectin gene, Genetic polymorphism, PCR-RFLP, Sahiwal cattle

Adiponectin (*ADIPOQ*) is one of the most abundant circulatory adipokines which is reported to play crucial role in regulation of glucose metabolism, insulin sensitivity, fatty acid oxidation, immunity and reproduction (Waki *et al.,* 2003; Brochu-Gaudreau *et al.,* 2010). Moreover, *ADIPOQ* gene is reported to be located within bovine chromosome (BTA) 1 region harboring QTL which documented to affect carcass traits in crossbred cattle (Cai *et al.,* 2004; Kim *et al.,* 2010). Furthermore, several polymorphic sites in *ADIPOQ* gene and their significant association with meat quality traits have been documented in various breeds of beef cattle (Davis *et al.,* 1998; Casas *et al.,* 2000; Li *et al.,* 2004; Morsci *et al.,* 2006; Zhang *et al.,* 2009). Considering lack of information regarding genetic polymorphism in *ADIPOQ* gene in Indian cattle breeds, the present study was undertaken to explore the polymorphism in intron 2 region of *ADIPOQ* gene in Indian Sahiwal cattle.

## MATERIALS AND METHODS

### Animals and DNA extraction

Approximately 5 ml of blood was collected in EDTA vials from 69 adult Sahiwal cows maintained at Livestock Farm Complex (LFC), College of Veterinary Science and Animal Husbandry, Mathura. The genomic DNA from blood was isolated as per the standard phenol-chloroform isolation protocol (Sambrook and Russell, 2001). The concentration of DNA was determined using Nanodrop (Sigma, USA) by measuring optical density at wavelength 260 and 280 nm. The quality of DNA was evaluated on 0.7% agarose gel electrophoresis in 1X TAE buffer stained with ethidium bromide (1 µg/µl) (Fermentas, USA).

### PCR amplification

To determine the genetic polymorphism, the primer pair (F:5'TTGTGGCTGACAGTGTGTAT3' and R:5'TAACACAGGCTCCCATAAAT3') reported by Shin and Chung (2013) was used to amplify the genomic sequence of intron-2 region of bovine *ADIPOQ* gene (Gene Bank accession number DQ156120). About 100-150 ng of template DNA was amplified in a total volume of 25 µl PCR mix in thermocycler (Bio-Rad, USA). The PCR mix contained: 2.5 µl of 10X PCR dream buffer, 2.5 µl of 2 mM dNTPs, 0.5 µl from each primer (10 pmol/µl) and 1 U *Taq* DNA polymerase. The PCR cycling condition for this fragment of *ADIPOQ*-gene involved initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30s, 58.5°C for 30s and 72°C for 1 min with a final extension for 5 min at 72°C. The final PCR product is confirmed by running on 1% agarose gel electrophoresis.

### PCR-RFLP assay

For detecting the genetic polymorphism in *ADIPOQ* gene, the PCR products were digested using Fast Digest *Rsa*I enzymes (Thermo Scientific, USA). Each digestion reaction contained 10 µl PCR products, 1.5 µl Buffer (10X), 0.5 µl (0.5 U) restriction enzyme and 3.0 µl nuclease free water. The restriction digestion was carried out at 37°C for 5 minutes in water bath and subsequently, the fragments were separated by 2% agarose gels electrophoresis.

Shin and Chung (2013) by *Rsa*I/PCR-RFLP analysis in intron-2 region of bovine *ADIPOQ* gene reported that the wild-type homozygote (T/T) generated 2 fragments (516 and 445 bp) and was referred as AA genotype while SNP at 2606T>C generated three fragments (100, 416 and 445 bp) which was designated as BB genotypes (polymorphic genotype C/C).

## RESULTS AND DISCUSSION

The amplification of fragment of intron-2 region of *ADIPOQ* gene revealed amplicon of 961 bp observed on 1% agarose gel electrophoresis (Fig. 1). The *Rsa*I/PCR-RFLP assay revealed only one type of banding pattern in all the animals. This result indicated absence of polymorphic *Rsa*I site at one location (2606T>C) out of two locations, produced a single cut in amplicon generating two bands of 516 and 445 bp (Fig. 2), the wild type homozygote (T/T). The result revealed that Sahiwal cattle used in this study were monomorphic in nature and DNA sequencing also confirmed the absence of the restriction site for *Rsa*I at one location (2606T>C) out of two locations in PCR products. The obtained sequence of *ADIPOQ*/*Rsa*I after aligning revealed the wild type homozygote (T/T) with no substitution (T→C) at 2606 position.

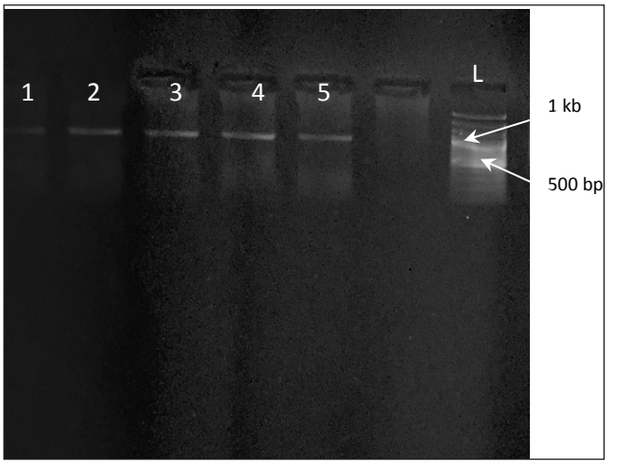


**Fig. 1:** Agarose gel electrophoresis (1%) of ADIPOQ PCR product showing amplicons of 961bp in all lanes (1-5), L= Ladder (100bp)

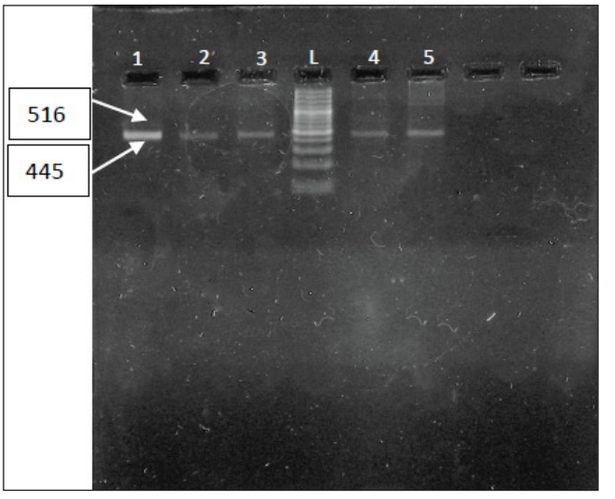


**Fig 2:** *ADIPOQ*/*Rsa*I PCR-RFLP assay pattern in 2.0% agarose gel; Lane 5: Undigested PCR product; Lane 1-5: Monomorphic pattern after PCR-RFLP assay (TT genotype); Lane L: DNA Ladder (100 bp)

*ADIPOQ*/*Rsa*I assay in the present study revealed monomorphic banding pattern the wild type homozygote (T/T) in studied population of Sahiwal cattle. However, Shin and Chung (2013) reported SNP at same location (2606T>C) in intron-2 region of bovine *ADIPOQ* gene in Hanwoo (Korean) cattle population and revealed three genotypes *viz*., CC, CT and TT. The monomorphic pattern of polymorphism in this study indicates that low mutation rates during evolution may be responsible for conserving this region of ADIPOQ gene in Sahiwal cattle.

In addition several authors reported genetic polymorphism in different regions of ADIPOQ gene. Morsci *et al.* (2006) documented genetic polymorphism at promoter region of bovine *ADIPOQ* gene using *Tsa*I/PCR-RFLP in Angus cattle population and reported three genotypes viz, major, minor and heterozygous genotypes. Furthermore Shin and Chung (2013) also reported SNP at promoter region of bovine *ADIPOQ* gene by *Tsa*I/PCR-RFLP in Hanwoo (Korean) cattle population and revealed three genotypes *viz*., CC, CT and TT. A novel 5 bp deletion polymorphism in the promoter region of the bovine ADIPOQ gene was also reported by Zhang *et al*. (2009). Additionally Choi *et al*. (2015) determined the SNPs and insertion (67bp) polymorphism in the promoter region of the bovine adiponectin (ADIPOQ) gene and their genetic effects on carcass traits of Hanwoo cattle and confirmed a 67 bp insertion (I, allele) that began at nucleotide positions 81966364 to 81966429 and nucleotide substitutions at positions 81966235 (C>T) and 81966377 (T>C) in the promoter region of 5'UTR. Similarly Fonseca *et al.* (2015) investigated the genetic polymorphism of ADIPOQ gene by *Bsr*I PCR-RFLP assay and reported three genotypes viz. TT, CT and CT in Nellore cattle.

## CONCLUSION

This is the first report on (2606T>C) polymorphism in intron 2 region of *ADIPOQ* gene in Indian cattle breeds, in which T>C polymorphism in intron 2 region of *ADIPOQ* gene revealed 100% TT genotypes. The results revealed that T allele of *ADIPOQ* gene appears to be fixed in screened cattle population. Consequently, association between genotype and milk production traits could not be established due to monomorphic pattern of animals. However, further studies are warranted to examine SNP status in other diversified cattle population and at different regions of this gene and their relationship with economic traits required to be verified.

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