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ASSOCIATION OF ATP1B2 GENE POLYMORPHISM WITH MILK YIELD, MILK COMPOSITION AND HEAT RESISTANCE TRAITS OF CATTLE BRED IN IRAQ

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Abstract

The present study aimed to identify genetic variations and SNPs in ATP1B2; among Local Iraqi cattle, Holstein and their crosses. The primer used in this study amplified 705-bp fragments from the ATP1B2 gene. The results showed the presence of 8, 1 and 3 polymorphic sites leading to the construction of 3, 2 and 2 different haplotypes for local, Holstein and crosses respectively. Haplotype and nucleotide diversity were 0.377, 0.571 and 0.285 and 0.0028, 0.0009 and 0.0014 respectively. The Haplotype network showed that haplotype 1(H1) found in all breeds, branch represents H2 shown by local and Holstein cows. while also Local breed included in H3 and the crosses in H4. AMOVA showed that variation within breed (between individuals) was higher (94.39%) than between breeds (5.61%). Neutrality test Tajima's D revealed that local cows recorded the highest negative values (-1.636). Whereas, the Holstein cows recorded positive values (1.444). Those of Fu's Fs were 1.667, 0.966 and 1.514 for local, cross and Holstein cattle respectively. The results showed that Haplotype 3 recorded the highest percentages of protein when compared to other haplotypes.

Key words: ATP1B2 gene, Iraqi cattle, milk yield, Genetic Diversity.

Introduction

Heat stress in animals is one of the main causes of production loss, especially in dairy cows under hot climatic conditions in the tropics and subtropics (Verma *et al.*, 2017). There are more than fifty proteins other than HSPs, which are also expressed in response to heat shock, ATP1B2 is also among those proteins which are expressed when an animal exhibit heat stress (Jayakumar, 2014). ATP1B2 (ATPase, Na+/K+ transporting, beta 2 polypeptides), the Na^+ /K^+, ATPase (NKA), is a transmembrane carrier protein widespread in eukaryotic cell membranes and is responsible for the transport of hydronium ion, energy metabolism and adjustment of body temperature of the organisms and also, it regulates the balance of Na and K ions across the cell (Xu and Zhang, 2004) and therefore low NKA activity is associated with impairing the hydronium transport leading to disturbances in the normal energy metabolism of cells (Vague *et al.*, 2004).

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The ATP1B2 gene is located at chromosome 19 of cattle and buffaloes, it consists of 7 exons and 6 introns and The length of the gene is about 4310bp of genomic DNA (Wang *et al.*, 2011). The ATP1B2 protein is composed of 20 amino acids (Wang *et al.*, 2009). The objectives of the present study were to detect the single nucleotide polymorphism (SNP) of *ATP1B2* in cattle of cattle bred in Iraq by DNA sequencing and to investigate the association between a polymorphism in *ATP1B2* gene and milk yield, milk composition in the cow.

Materials and Methods

This investigation was carried out in the laboratory of Genetic Engineering in the Department of Animal Production, College of Agriculture, University of Basrah, Iraq.

Animals and genomic DNA isolation

This study included the use of 35 cows (10 local 15 Holstein and 10 Crosses). Blood samples (10ml/cow) collected from the jugular vein. Genomic DNA was

extracted from whole blood using the gSYNC™ DNA Extraction Kit manufactured by the Taiwanese Geneaid Company. A fragment (705bp, intron 2) of the ATP1B2 gene in cattle was amplified by using the primer F: 5'-AGGTGGTTGAGGAGTGGAAGGAGTT-3' and R: 5'-CACTCCCTCCGCTTGTGACATCT-3' (Wang, 2011). The PCR amplifications were conducted in a 50 µl volume containing 6 µl genomic DNA, 25 µl of Master Mix, 4 µl both primer and 15 µl free water. The amplification conditions were as follows: initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 30 sec., annealing at 56°C for 40 Sec and extension at 72°C for 30 Sec, followed by the final extension at 72°C for 5 min. The PCR product was detected by 2% agarose gel electrophoresis, stained with Ethidium bromide and visualized by ultraviolet light. For sequencing, the PCR product was sent to Yang ling Tianrun Aoka Biotechnology Company, China.

Bioinformatics analysis

The sequencing results of the ATP1B2 gene were compared with accession number NC_007317.3 at GenBank by BioEdit 7.0 software (Hall, 1999). Haplotype diversity (Hd) and nucleotide diversity (π) were analyzed using DnaSP v5. 10 software (Librado and Rozas, 2009); Watterson's theta estimator for the studied species separately using haplotypes sequences was obtained. Nucleotide diversity (π) is based on the average number of nucleotide differences between the sequence and theta are based on the total number of segregating sites in the sequence (Kantanen *et al.*, 2009). The haplotypes network was drawn using Network 5.0.0.0 software (Bandelt *et al.*, 1999). Molecular variation (AMOVA) and neutrality tests were analyzed using Arlequin 3.5.1.2 software (Excoffier and Lischer, 2010).

Statistical analysis

The Completely Randomized Design (C.R.D) was used to analyze the production data studied within the SPSS, (2016) Statistical program V.24. Means were compared by the Least Significant Difference Test within the program.

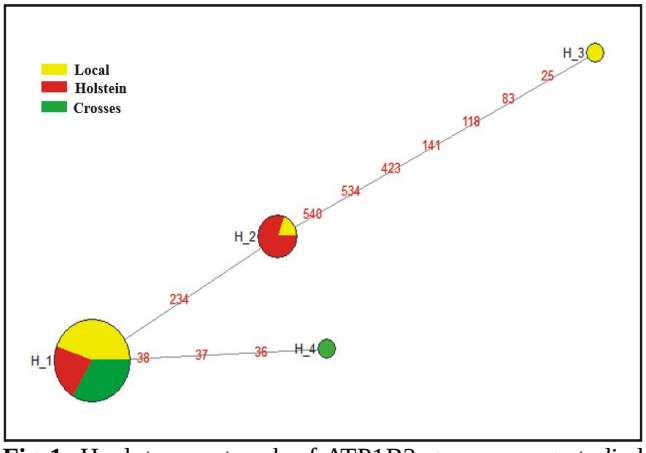

Fig. 1: Haplotype network of ATP1B2 gene among studied cattle.

Table 1: Genetic Diversity of *ATP1B2* Gene among Different Cattle Breeds.

Breeds	N	H	NH	Hd	π
Local	10	3	8	0.377	0.0028
Crosses	7	2	3	0.285	0.0014
Holstein	8	2	1	0.571	0.0009

N: Number of Sequences; H: Haplotype; NH: Number of polymorphic; Hd: Haplotype Diversity; π: Nucleotide Diversity

Results

Genetic Diversity

The number of ATP1B2 sequences was 25 (Table, 1). Teen, seven and eight of sequences belonged to local cattle, cross and Holstein respectively. Haplotypes number (H) were four distributed as 3 for local, 2 for crosses and Holstein. Local, crosses and Holstein cattle showed a polymorphism (NH) of 8, 3 and 1 respectively. Holstein revealed the highest value of haplotype diversity (Hd) (0.571), followed by the local breed (0.377) and the cross cattle (0.285). On the contrary, local cattle recorded the highest nucleotide diversity (π) followed by cross and Holstein cattle (0.0028, 0.0014 and 0.0009 respectively).

Haplotype Network

A total number of haplotypes of the ATP1B2 gene showed by different breeds were four (Fig. 1). The central circle represents Haplotype 1 (H1) found in all breeds. Two branches appeared from H1, the first branch represents H4 which differed from H1 by three bases (36, 37 and 38) and included the crosses cattle only. The other branch represented H2 shown by local and Holstein cows and differed from H1 by 234 bases. Whereas, the haplotype H3 represented the local cattle and differed from H2 by 25, 83, 118, 141, 423, 534 and 540 bases.

Analysis of Molecular Variation (AMOVA)

AMOVA of ATP1B2 gene among studied breeds of cattle resulted in between breed variation of 5.61% and within breed variation of 94.39% (Table 2).

Table 2: Analysis of Molecular Variance (AMOVA) of ATP1B2 Gene for Studied Breeds.

Source of Variation	Degree of Freedom	Sum Squares	Variance Components	Variation %
Between Breeds	2	1.189	0.03367	5.61
Within Breeds	22	12.471	0.56688	94.39
Total	24	14.160	0.60088	

Table 3: Neutrality test of ATP1B2 gene for studied cattle breeds.

Breeds	Tajima's (D) Test	Fu's Fs Test
Local	-1.636	1.667
Cross	-1.358	0.966
Holstein	1.444	1.514

Neutrality test

The results of the neutrality test (Tajima's D) of the ATP1B2 gene (Table 3) showed negative values for local breeds (-1.636) and cross (-1.358), but it was positive (1.444) for Holstein cows. Those of Fu's Fs were 1.667, 0.966 and 1.514 for local, cross and Holstein cattle respectively.

ATP1B2 Polymorphisms and Milk Chemical Contents

Different haplotypes associated significantly (P<0.05) with (fat, protein, lactose and SNF) % (Table, 5). The association study showed that H3 has a significant effect on milk Protein percentage of 3.92% (p<0.05). Other Haplotypes showed no effect on milk Chemical Contents.

Discussion

Only a few studies were carried out of the ATP1B2 gene polymorphism in farm animals. Most of the studied regions have been introns 1,2,4 and 5 in the ATP1B2 gene (Deb *et al.*, 2015). The identification of different gene variants (haplotypes) and to uncover the associations between these haplotypes and different production traits is of great importance for improving that trait in animals (Faraj *et al.*, 2019a). However, the association of ATP1B2 gene variants *i.e.* haplotypes with milk yield traits has not been studied in Iraqi cattle. Some studies regarding the ATP1B2 gene had also been conducted. detected two novel SNPs, G2258A and C2833T, in the intron 2 and intron 4, respectively in Chinese Holstein cows. The mutation G2258A significantly affected milk fat content and 305-day milk yield, but not milk protein content, While the mutation C2833T significantly affected milk protein content and 305-day milk yield, but not milk fat content (Wang *et al.*, 2010). Jayakumar, (2014) detected three SNPs in the ATP1B2 gene of Murrah. The two SNPs were transition type A1457G, A2607G and one was Transversion type T2775G. As for Sahiwal and Karan Fries cows, it has been reported two SNPs in intron 2 of ATP1B2 gene G2243A and T2366C (Verma, 2015). Tajima's D test compares the difference between many segregating sites and the average number of pairwise (Tajima, 1989). Under neutrality Tajima's D value is assumed to be zero; under positive selection, there is an excess of rare polymorphisms and Tajima's D value is negative. Negative D values can also be due to population expansion. If there is balancing selection, intermediate frequency genetic variants are kept and Tajima's D value is positive. The partitioning of the genetic variation from an AMOVA revealed that 5.61% of the variation was between breeds. A similar pattern of variance partitioning was observed in similar studies (Faraj *et al.*, 2019b; Öner *et al.*, 2019), in which 90% or more of the variation is contained within breeds.

Table 5: Association of ATP1B2 Haplotypes and milk chemical components.

Haplotypes	Protein (%)	Lactose (%)	Fat (%)	SNF (%)
H1	3.59±0.15	3.95±0.24	3.64±0.22	7.31±0.30
H2	3.08±0.04	4.34±0.33	2.87±0.19	6.91±0.70
H3	3.92±0.02*	5.00±0.10	3.52±0.20	6.62±0.48
H4	3.32±0.26	3.81±0.37	2.70±0.25	7.91±0.20
*Significant at (P>0.05)				

Conclusions

In the present study, intron 2 of the bovine ATP1B2 gene polymorphisms were screened by DNA sequencing technique. We have reported here for the first time single nucleotide polymorphisms of the ATP1B2 gene for intron 2 in cattle bred in Iraq. We concluded that the identified SNPs lend themselves readily for further research regarding physiological impacts such as productive, reproductive and heat tolerance traits in cattle**.**

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