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Research Article

Identification of Associations Between Ucp2 and Ucp3 Gene Polymorphisms and Meat Quantity Traits in Three Indigenous Cattle Breeds of Pakistan

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Abstract

In identifying mutations occurring in distinct cow breeds, genetic elements must be taken into consideration. More recently, these hereditary features have gained attention throughout the world. As in many underdeveloped nations, to bridge the deficit in molecular genetics, multiple solutions are required. The inner membrane anion carrier superfamily contains the uncoupling proteins (UCPs), vital to energy regulation. Research on heredity has shown that variations in the Ucp2 and Ucp3 genes are connected to obesity and metabolic syndrome. This research aimed to investigate if any mutation in the Ucp2 and Ucp3 genes are related to many characteristics in Pakistan's three indigenous cattle breeds. The results of this study revealed 07 variations in the Exon 4 region of the Ucp2 gene and 03 variants in the Exon 3 region of the Ucp3 gene using Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) followed by Sequencing among 215 indigenous cattle breeds. The association study revealed that the g.C35G mutation in the Ucp3 gene is strongly related to meat quantity characteristics such as carcass weight and drip percentage (P0.05), but not with body height or hip-width (P > 0.05). Sequence analysis showed five distinct diplotypes: AA, BC, AC, CC, and CD. Cattle with the novel heterozygous diplotype BC perform better in carcass trait and drip percentage than animals with other genotypes. The study's findings suggest that the Ucp3 gene may be utilized for marker-assisted selection (MAS) and breed mixing in Pakistan cattle breeds to aid in the country's economic growth.

Keywords: DNA; PCR; Gel Electrophoresis; Variations; Ucp2 Gene; Ucp3 Gene

Introduction

Energy is the fundamental requirement of cells for many life processes, and the primary energy source is within the cell mitochondria. During the Redox reaction, ATP is synthesized inside mitochondria, a method called phosphorylation, Mitochondria, Adenosine triphosphate (ATP) synthesis is perceived. Although the reactions were not detected as combined processes, the transport families inside the mitochondria were not present. The family of this carrier is known as the UCP uncoupling protein [1]. In the inner membranes of mitochondria, the UCPs "uncoupling proteins" of the carrier protein family are widely encountered [2].

UCPs are categorized as "UCP1, UCP2, UCP3, UCP4 and UCP5 and UCP plant" into six separate groupings. Intrinsic mitochondrial proteins with a molecular weight of roughly 31kDa-34kDa are uncoupling proteins. The mass of BMCP1 and uncoupling protein 4 (UCP4), the larger proteins, is 3638 kDa. Unconnected proteins are regarded to be the essential proteins around which they are isoelectric. Its structure is tripartite; in each repetition, two hydrophobic sections are accurate to a-helices. Six crossings of the lipid bilayer are performed in the polypeptide chain, and the amino carboxylic ends reach beyond the mitochondria's internal membrane [3]. In each repetition, the two helices are connected to the protein matrix by a hydrophilic hoop. The functional unit is a dimer and is composed of two comparable subunits. It has been shown that two monomers are coupled in tandem with functionally capable units for other carrier proteins [4].

Uncoupling proteins 2, located mainly in the central nervous system, intestines, lungs, spleen, kidneys, uterine and immune cells, were detected in mammals [5]. It has become widespread in life processes, including mainly ROS products ("reactive species of oxygen"), feeding mechanisms, insulin regulation, immune

systems, and various illnesses, e.g., atherosclerosis, cancer, diabetes mellitus, and injury to neurons [6]. UCP3 mainly was found in skeletal muscles and the heart and "BAT" very little [7]. The central body mass is the muscles of the skeleton, and they contribute to thermogenesis and metabolism [8]. The protein uncoupling-3 is the one responsible for the thermal genesis in the muscles of the skeleton. The uncouple proteins – 3 are strongly controlled during fasting and starvation in skeletal muscles when energy consumption for metabolism is substantially demanded [9].

The estimated cattle population in Pakistan is 29.66 million. Almost 49 percent are present Punjab, Pakistan. At the same time, the remaining 23% of the population in Sindh is 20% in NWFP. In Balochistan, just 8% are located (GOP, 2006). These percentages have varied since 1996 in various surveys. Recently, Punjab's share grew by 2.8 percent, and Sindh declined by 3.3 percent. In other Pakistani provinces, the general percentage of the livestock population is not equal [10]. The humped-type (zebu) and Bos endemic Pakistani animals (Cattle). There are about 15 legitimate livestock in the country and 43% of the cattle population [10].

While many researchers have been involved in regulating energy metabolism genes UCP2 and UCP3, less genetically modified information on UCP2 and UCP3 and a percentage of unique mutations in three indigenous breeds of Sindh Pakistan cattle is known. Thus, the study attempted to detect UCP2 and UCP3 gene polymorphisms and analyze their new mutation in 3 native Sindh Pakistan cattle. The results of this research may indicate a broader assumption for more research impact of UCP2 and UCP3 genes and for MAS based livestock.

Material and Methods

Blood collection

In this investigation, blood was obtained from 215 animals of the Sahiwal cattle breed, ranging from one to five years. Five mL of blood samples were collected from each in EDTA tubes. The animals were cared for following Canada's established protocol [11].

DNA extraction and quantification

Mini DNA extraction kit (K0781) of Thermo Scientific was used to obtain the DNA from the whole blood. It is particularly made for the speedy and efficient separation of purified genomic DNA from whole blood. A silica-based membrane in a spin column is included in the package to avoid costly and dangerous phenol-chloroform isolation and prolong the alcohol precipitation procedure. After losing the cells, the technique takes around 20 minutes to complete and executes the highly pure DNA [12].

Primer Design and Synthesis

Primer Premier 3 software version 0.4.0 was used to design PCR primers for both Ucp2 and Ucp3 genes. Primers were finally synthesized from the Macrogen company of Korea. Table 1 shows two primer pairs built using the Ensemble database: UCP2 having Acc. ENSBTAG00000003692 and UCP3 with Acc. ENSBTAG00000005259 [13].

Primers	Primer sequence	Tm (°C)	Location	Amplicon size
Ucp2 Primer	F: 5-TGCAGATCCAAGGAGAAAGG-3	57	5' UTR region	620 (bp)
	R: 5-GCTTGACGGAGTCGTAGAGG-3			
Ucp3 Primer	F-GCCTCTACGACTCCGTCAAG-3	58	Exon 3, Intron 2, Exon 4	950(bp)
	R-CTCCTCATGCTTCAGCTTCC-3			

Table 1: Shows the Primer details for Ucp2 and Ucp3 genes.

PCR amplification and single-stranded conformation polymorphism (SSCP)

The PCR reaction was performed in a 20 µL PCR tube with 50 ng/uL of DNA sample, 10 pmol of each primer, 0.2 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (Biomolecules, China). When carrying out a PCR reaction, the following conditions should be followed: initial denaturation phase was performed for 5 min. at 95 ° C, followed by 35 cycles including denaturation at 94 °C for 30 seconds, hybridization at 58 ° C for 45 seconds, and extension at 72 °C for 60 seconds and final extension at 72°C for 5 min. Genotyping of SSCP was performed by combining 5 uL of PCR product with 5

uL of denaturing solution, heating at 98 °C for 10 minutes and immediately cooling on ice [14].

Gel electrophoresis

Agarose gel (1.5%) and Polyacrylamide gel (10%) was used for electrophoresis of amplified PCR product. The gel was then put into an electrophoresis chamber, which contains 1X TBE buffer. A constant electric current of 70 V was applied for 45 min. Finally, the PCR product was observed under the UV Gel Doc system (Bio-rad, USA), [15].

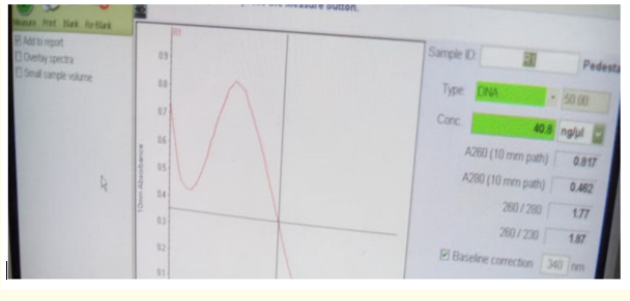
Purification, sequencing, and bioinformatic analysis

Amplified PCR products were sent to the Macrogen company of Korea for purification and Sequencing. The sequence results were analyzed online through the ensemble.org genome database by blasting on the sequence alignment tool. At the same time, the percentage of the mutation was calculated by using Microsoft excel sheet. The data for meat quantity association traits were analyzed through SPSS software [16].

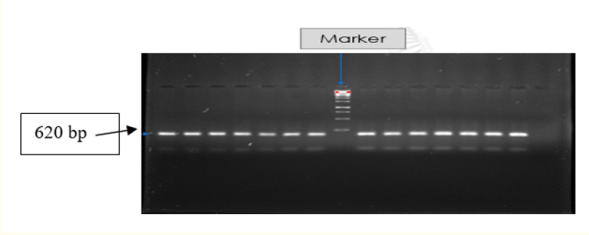
Results

Genetic analysis of UCP2 and UCP3 and identification of mutations

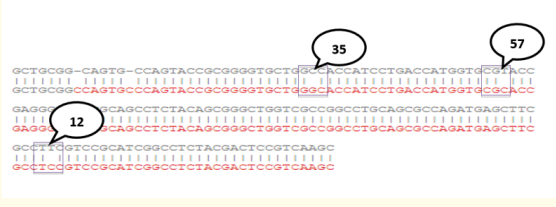
Through analyzing DNA sequence alignments on ensemble.org, mutations were discovered. The results of the quantification of DNA are depicted in graph 1. PCR amplified product gel photo is also shown under graph 2. Results of the type of identified mutation, its percentage, type of amino acid deduced are given in table 2 and 3. Moreover, the position of SNPs and location are described.



Graph 1: Shows the DNA quantification for UCP 2 gene.



Graph 2: Shows the Amplified PCR product of the UCP2 gene.

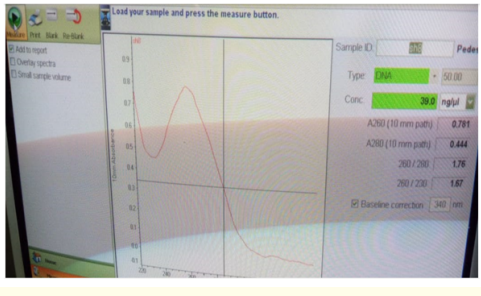


Graph 3: Shows the position of the mutation in the UCP2 gene.

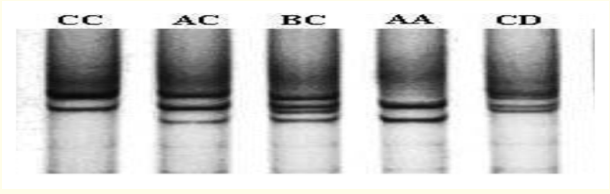
SNPs description in UCP2

Seven distinct variations were discovered with respect to our query sequence when queried for the cow gene UCP2 known as ENSBTAG00000003692 and shown under graph 3

- Transversion mutation (Guanine to Cytosine) was noted at nt. 35bp.
- Transition mutation (Cytosine to Thymine) was noted at nt.57bp.
- Transition mutation (Cytosine to Thymine) was noted at nt.125bp.



Graph 4: Shows the DNA quantification for UCP3 gene.



Graph 5: Shows the Amplified PCR product of the UCP3 gene on 10% PAGE.

Graph 6: Shows the position of the mutation in the UCP3 gene.

SNPs description in UCP3

Three distinct variations were discovered concerning our query sequence when queried for the cow gene of UCP3 cow gene ENS-BTAG00000005259 as shown in the sequence alignment.

- Transition mutation (Adenine to Guanine) was observed at nt.18bp
- Transversion mutation (Thymine to Guanine) was noted at nt. 35bp.
- Transition mutation (Adenine to Guanine) was observed at nt.119bp.

Name of Gene	Name of Breed	Found SNP	Percentage method	Total percentage
UCP2	Dhanni	2	2 × 100/192=	1.04%
	Cholistani	2	2 × 100/192=	1.04%
	Sahiwal	3	3 × 100/192=	1.562%
UCP3	Sahiwal	3	3 × 100/192=	1.910%
	Cholistani	0	0	0
	Dhanni	0	0	0

Table 2: Percentage calculation of mutations among three cattle breeds.

According to the definition of Polymorphism in genetics, if the rate of change of mutation is greater than 1% (Percentage) in any population, it is concluded as Polymorphism. If the rate of change is less than 1% in any population, it is concluded as just mutation.

Sample ID	Position of mutation (bp)	Original codon	Modified codon	Original amino acid	Modified amino acid	Identified mutation
UCP2.2D1	27	GTG	GTA	Valine(E)	Valine (E)	Silent Mutation
	41	CTG	CGG	Lucine (E)	Aginine(E)	Missense Mutation
UCP2.2C1	60	CCC	CCT	Proline (N)	Proline (N)	Silent Mutation
	80	GCT	GTT	Alanine(N)	Valine(E)	Missense Mutation
UCP2.2S1	35	GGC	GCC	Glycine(N)	Alanine(N)	Missense Mutation
	57	CGC	CGT	Arginine(E)	Arginine(E)	Silent Mutation
	125	TCC	TTC	Serine(N)	Phenylalanine(E)	Missense Mutation
UCP3.3S1	18	GGA	GGG	Glycine(N)	Glycine(N)	Silent Mutation
	35	CTG	CGG	Leucine(E)	Arginine(E)	Missense Mutation
	119	CAT	CGT	Histidine(E)	Arginine(E)	Missense Mutation

Table 3: Genetic code based identified mutations and deduced amino acids.

Association analysis

The association analysis of CGG mutation at exon 3 of the UCP3 gene shows all 05 diplotypes with 04 meat quantity traits in the Sahiwal cattle breed. Cattles with Genotype BC had higher body height than AA. Animals having BC genotypes were significantly linked with carcass weight (P=0.04). Significant association of carcass weight and Drip percentage was observed for CGC polymorphism in the UCP3 gene and non-significant association between body height and body width. The meat quantity controlling traits such as carcass weight were measured according to the evaluation of [17].

Diplotypes (Mean ± SE)	Meat Quantity Traits			
	BH (cm)	HW (cm)	CW (Kg)	DP (%)
AA (n = 40)	139.3 ± 1.5	46.8 ± 1.04	257.03 ± 9.9^{a}	52.3 ± 1.1^{a}
AC (n = 47)	138.7 ± 1.2	46.07 ± 0.8	266.6 ± 7.8^{ab}	53.5 ± 0.9^{ab}
BC (n = 56)	141.1 ± 1.0	46.8 ± 0.6	281.2 ± 6.5^{b}	55.5 ± 0.5^{b}
CC (n = 46)	139.8 ± 1.2	46.8 ± 0.8	255.1 ± 8.2^{a}	53.1 ± 0.9^{ab}
CD (n = 26)	139.0 ± 1.5	48.6 ± 1.04	252.04 ± 9.9^{a}	54.5 ± 1.1^{ab}
P-value	0.5	0.45	0.04	0.11

Table 4: Effect of UCP1 gene polymorphism (P < 0.05) on meat quantity traits.
Note: Superscripts (a, b) used in the above table shows significant difference (P < 0.05), BH: Body Height, HW: Hipwidth, CW: Carcass Weight, DP: Dressing Percentage and SE: Standard Error of Means

Discussion

Genes that control metabolism and energy distribution are the source of the genetic variation in farm animals, which may be advantageous when improving productivity. Various research has proved that UCP2 and UCP3 are engaged, by way of physiological and pathological processes, in the control of metabolized energy [18], Body mass index [19], Oxidative stress [20], obesity [21]. Gene mutation was also linked to human obesity, insulin resistance, and meat quality in past research [22-24] pig meat yield [25-27] According to the results of these above studies, Ucp2 and Ucp3 genes may be considered the crucial gene that affects cattle meat yield characteristics.

In the current study through PCR-SSCP, DNA sequencing and Gel electrophoresis were performed to identify seven mutations in the UCP2 gene and three in the UCP3 gene. Three silent mutations and two missense mutations are found on the fourth exon of the UCP2 gene. two missense mutations are found on exon 3 of the UCP3 gene, whereas one silent mutation is found and no changes have been made to the protein sequence. Among Dhanni cattle, 27bp of UCP2 GTG has changed to GTA; both these variants code for Valine, and the change was quiet mutation because of that. The coding of another amino acid, Leucine, has changed from CTG to CGG, and this alteration was not manifested. The missense mutation has occurred. In Cholistani, in the 60bp domain of UCP2, there is an undetectable CCC mutation (proline) and an observable GCT mutation (alanine) resulting in an effectively silent amino acid change. In the 80bp domain, there is an observable GCT mutation (valine) and an undetectable GCT mutation (tryptophan) that causes an observable amino acid change. However, results of only silent mutations and missense mutations are shown in both graphs of mutations. Whereas previous studies also support the role of silent mutations for gene function and expression as reported by [28].

Furthermore, our results revealed a significant association of mutation in UCP3 gene for Drip percentage trait and carcass weight trait whereas, no significant relationship was found for body height and body width traits in bovine. The genotype BC appears to be favorable compared with other genotypes for growth and carcass trait performance. While comparing with the already published results of this study are inconsistent with work of [29,30].

In summary, correlations between genetic polymorphisms and carcass weight, Drip % body height, and body width characteristics in cattle are critical for understanding the genetics of complex economically significant variables. While the findings of this research indicate that these genes affect economically significant characteristics, the processes by which these genetic alterations occur remain unknown since most of them do not result in amino acid changes. They may affect gene transcription, splicing, mRNA stability, or even translation. More information on how noncoding

sequences influence gene activity becomes known, it may become clear how these SNPs contribute to variance in these characteristics. Additionally, they may be associated with other causal mutations that have not been identified yet.

Conclusion

The research identified ten mutations in total, seven from UCP2 and three mutations from UCP3. A significant association of UCP3 gene mutation for carcass weight and Drip percentage traits and a non-significant association with body height and body width traits in bovine was noted. The genotype BC appears to be favorable than those with other genotypes for growth and carcass trait performance. The data can be used by the Veterinary and Animal Sciences Department to enhance cattle breeds for the betterment of the bovine industry.

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