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Comparative Study of Genetic Polymorphism of Uncoupling Protein-1 Gene in Three Indigenous Goat Breeds of Khairpur District, Sindh, Pakistan

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Abstract

The UCP-1 gene is linked with obesity-related characteristics, non-shivering thermogenesis, and the quality and amount of milk and meat produced. The goal of this research was to discover genetic variations in the UCP-1 gene in three indigenous goat breeds from Sindh, Pakistan, with the hope of improving their breeding prospects. According to the results of this study, there were eight single-nucleotide variants found in the UCP-1 gene across all three goat breeds. Three mutations in the Sindh Desi goat breed were discovered using PCR-Gel Electrophoresis and DNA sequencing methods, while three mutations in the Barbari goat breed were identified, and two mutations in the Tapri breed were uncovered using DNA sequencing techniques. Depending on their genetic coding, these mutations may be classified as one of three kinds of point mutations. For example, in the Sindh Desi goat breed, two missense mutations and one silent mutation have been identified; in the Barbari goat breed, there has been one missense mutation and two silent mutations identified; and in the Tapri goat breed, there has been one missense mutation and one silent mutation identified. Our results may be used as a reference and guidance for choosing high-quality milk and meat for industrial purposes and when crossbreeding and genetic improvement activities are carried out on the animals.

Keywords: UCP1, DNA sequencing, PCR, Goat, SNPs

Introduction

Mitochondrial energy is used to establish an electrochemical proton gradient across the inner membrane, which is subsequently used by an enzyme called ATP synthase to produce ATP. UCP-1 is a brown fat mitochondrial inner membrane protein that transforms stored energy into heat in this electrochemical proton gradient. This aids in the conversion of ADP to ATP. UCP-1 and brown fat activity play a substantial role in systemic energy expenditure in rats and mice (Dalgaard and Pedersen 2001). UCP-1 is a mitochondrial membrane transport protein that is required for the production of non-cold heat and temperature regulation. UCP-1 is a protein found in brown adipocytes that causes uncoupling of respiration in response to fatty acids. The role of UCP-1 in respiratory uncoupling and adaptive thermogenesis has been investigated using pharmacological, physiological, biochemical, and genetic approaches. Exposing rats to cold is the simplest technique to induce UCP-1 production (Rousset et al., 2004).

UCP-1, originally known as thermogenin, was discovered around thirty years ago. BAT mitochondria were shown to have abnormal ionic conductivity in early investigations conducted in the 1960s and 1970s, leading researchers to speculate that mitochondrial membrane proteins were to fault. The UCP1 gene was identified in 1978. (Heaton et al., 1978). UCP-1 has been shown to be the protein that causes uncoupling. UCP-1 was initially purified and then cloned in 1988 for the first time (Kozak et al., 1988). UCP1, UCP2, UCP3, UCP4, and UCP5 are members of the uncoupling protein family, and humans have copies of all five of these genes. Various organs contain these proteins, which serve a wide range of purposes (Boss et al. 2000; Erlanson-Albertsson 2002). First discovered was UCP1, a BAT-specific UCP (Nicholls et al., 2001). The UCP-1 gene has a mass of 32 kilodaltons (kilodaltons). UCP-1 has the same six exons and five introns in all animals. In cattle, the UCP-1 gene may be located only on chromosome 17. Kappes and Sonstegard (Sonstegard and Kappes, 1999). Aside from that, the S NP in this gene has never been related to carcass features until this work was done. With an isoelectric point of 9.7, UCP-1 protein contains 306 amino acids, 28 of which are essential and 19 of which are acidic. The nine positive charges listed below are thought to be necessary for integration into the phospholipid environment (cardiolipin) (Modriansky et al., 1997).

Weight measurement parameters are essential in beef production because they influence the amount and quality of meat produced by cattle. Induced mutations contribute significantly to the development of economically valuable traits in goats. (2003) (Imabayashi et al.). UCP1 is expressed in African American families' mammalian islet cells and is involved in the acute insulin response to glucose (Sale et al., 2007). Four SNPs are found in the UCP1 coding area, with c.214GA (val72Met) and c.273CT on axon 2. Additionally, the 5th axon of ovine UCP1 has c.624CT and c.757GA (Ala253Thr) (Yuan et al., 2012). Additionally, it was shown that the two significant mutations responsible for the amino acid change had a minimal impact on protein production—the Nellore cattle UCP1 SNPs associated with carcass characteristics (Ferraz et al., 2009). UCP-1 is compared to other mitochondrial carrier protein families. UCP-1 contains three firmly coupled cardiolipin molecules that contribute significantly to the protein's stability and serve as monomers binding to a single purine nucleotide. The phylogenies indicate that UCP-1 originated from dicarboxylate carriers and gained a few changes to enable it to function in thermogenesis, a late development (Crichton et al., 2017). The research gap on the UCP-1 gene in Pakistan is much more significant since relatively little study has been conducted to improve the quality and quantity of milk and meat. Consequently, the purpose of this research was to identify polymorphisms in the UCP-1 gene, determine the proportion of mutations, and determine the genetic effect of mutations utilizing genetic code from indigenous goat breeds in Khairpur Sindh, Pakistan. Our results may be used as a reference and guidance for choosing high-quality milk and meat for industrial purposes and when crossbreeding and genetic improvement initiatives are undertaken.

Methodology

sampling

For the study, thirty blood samples were taken from the jugular veins of three different indigenous goat breeds, including the Tapri goat breed, the Sindh Desi goat breed, and the Barbari goat breed. From 1.5 to 2 years old, they were all cared for per the Canadian Council on Animal Care (CCAC) standards. Blood samples (5ml) were obtained and stored in EDTA (0.5M)-containing tubes using

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syringes with a capacity of 5 to 10cc. Blood samples were kept at 0oC for future DNA extractions, and they were then processed.

DNA Extraction and Quantification

DNA was extracted from blood leucocytes using Thermo Scientific's Gen JET genomic DNA purification micro kit# K0781. This kit aims to eliminate toxic phenol-chloroform and finish the alcohol precipitation process utilizing a silica membrane to extract high-quality genomic DNA from the whole spin column. After cell lysis releases the highly pure DNA, the whole process takes around twenty to thirty minutes. Nanodrop machines at the Genome Research Centre (HEJ, University of Karachi) evaluated the extracted sample DNA concentration to ensure enough amplification. DNA purity was also assessed using a 260/280 nm ratio.

Primer Designing and PCR Amplification

The Ensemble.org genome browser was used to obtain the UCP-1 gene sequence and primers were designed online through website and were finally synthesized from Marogen Korea (https://dna.macrogen.com/). The specificity of the subsequent primer sequences is given under Table 1.

Table. 1 Sequences of forward and reverse primers for amplifying UCP-1 gene.

Primers	Forward 5` --- 3`	Reverse 5`--- 3`	bp	Tm
UCP-1 gene	CTGGAGAGATGCCCAGATTG	GATCCAGGTCTCTGGGGATT	210	56
	CGCGCTTGTTTAAGGGACTT	GATCCAGGTCTCTGGGGATT	210	56

UCP-1 gene was PCR amplified in a reaction mixture made in an Eppendorf tube. All the components were at their optimum concentrations when the reaction mixture was prepared. An additional 20l of a 20l reaction mixture, including 7l of 2X Red PCR Master Mix, 2l of each Forward and Reverse Primer (10 pmol/l), and 100ng of genomic DNA was pipetted into each PCR reaction tube after that. Thermal cycling conditions were as follows: 1) pre-denaturation for 5 minutes at 94°C, 2) 34 cycles of denaturing at 94°C for 35s, annealing for 45s, and extension for 1 minute at 72°C; and 3) final extension for 5 minutes at 72°C. The PCR amplification was carried out on a thermocycler machine (Bio-Rad S1000, USA) following the standard procedure established by Zhang et al., 2007.

Gel Electrophoresis

The amplified PCR products were run on a 1.5 percent agarose gel with EtBr, a DNA intercalating agent for visualization. A 1kb DNA ladder was pipetted into one well of an agarose gel, along with PCR product (VL) and DNA loading dye (1l). The agarose gel was then wholly submerged in 1X TBE buffer for 45 minutes before subjected to a 70V electric current.

The UV transilluminator was then used to visualize the PCR products that moved toward the anodic field (Bio-rad, USA).

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Data analysis, sequencing, and purification

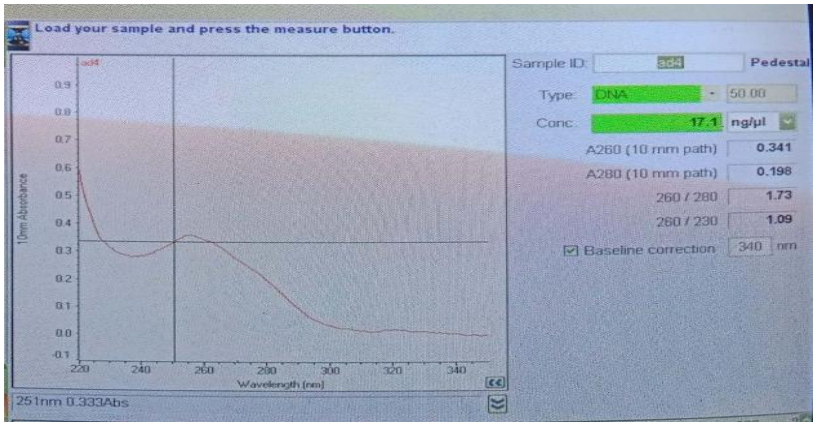
Amplified PCR products were submitted to Macrogen Korea (https://dna.macrogen.com/) for sequencing. By blasting on the sequence alignment tool, the sequence obtained was examined online using the ensemble.org genome browser.

Results

Quantified DNA

The amount of DNA in extracted DNA samples was measured using a nanodrop spectrophotometer, and the Gel Documentation system was found to be in the range of 30-103 ng/l, which is adequate for PCR amplification. The results of DNA quantification are given
under Graph 1.

Graph 1: Quantification of DNA using the Nanodrop Technique.

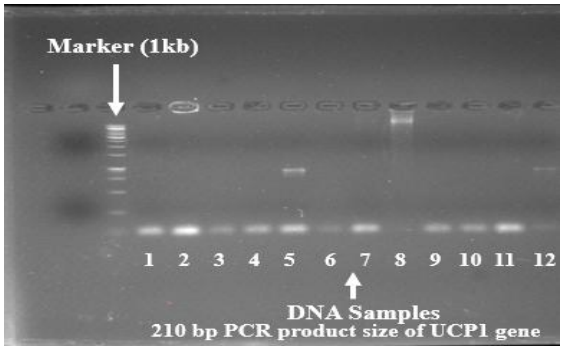


Identification of Single Nucleotide Polymorphism (SNPS)

A UCP-1 gene was PCR amplified in all three breeds, and size was verified (210bp) and SNPs were identified.

Following PCR amplification, the amplified product was sequenced. The resulting sequence for the UCP-1 gene is shown in Fig. 1. The sequence alignment tool ensemble.org was used to evaluate the sequence online. The alignment graph depicts the following mutations following the genetic code.

Fig 1 PCR amplified Product of UCP1 gene



UCP-1 gene was PCR amplified and size was confirmed (210bp)

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There were eight different single nucleotide variations found in the UCP-1 gene across the three goat breeds studied. These UCP-1 gene variants can be found in all three Sindh native goat breeds. The Sindh Desi goat has three new SNPs, the Barbari goat has three new mutations, and the Tapri goat has two new variations. A point mutation is a small alteration in the genetic code that has no effect on the outcome. The 94th base pair in the Sindhi goat's DNA is where cytosine is converted to thymine. It was discovered that the non-essential amino acid alanine was changed to the essential amino acid valine by a nonsense mutation that affected the start codon GCG, which codes for alanine. At nucleotide 101, guanine takes the place of cytosine to produce the second mutation. However, the amino acid glycine is now encoded by codon GGG, rather than its original form of GGC.

Adenine was converted to Cytosine by a single base pair mutation in the Sindh Desi goat breed. The original leucine amino acid-coding codon, TTA, was missense altered to phenylalanine amino acid-coding codon, TTC. In the Barbari goat breed, two different alleles were found on the 114th and 138th base pairs. This altered the amino acid bases from CCC to GTG to GGG to encode the amino acids proline, valine, and glycine. The base pairs that encoded these amino acids remained the same. The 92nd and 99th nucleotides of the Tapri goat genome had two single nucleotide polymorphisms (SNPs). Before, GCG encoded the amino acid Alanine, but now it encodes the required amino acid Valine, which is encoded by the base pair GCG instead of Alanine. It was decided to use the nucleotide pair GGG instead of GGC to encode the amino acid Glycine. Table 2 lists the mutations and the frequency with which they occur.

Table 2. Detailed information about the types of identified Mutations based on Genetic code.

Name of Samples	Position of changed nucleotide	Original codon	Original amino acid	Changed codon	Mutated amino acid	Types of point mutation
AD1 Sindh Desi	94	**GCG**	**Alanine** (Non-Essential)	**GTG**	**Valine** (Essential)	Missense Mutation
	101	**GGC**	**Glycine** (Non-Essential)	**GGG**	**Glycine** (Non-Essential)	Silent Mutation
AD2 Sindh Desi	341	**TTA**	**Leucine** (Essential)	**TTC**	**Phenyl alanine** (Essential)	Missense Mutation
AB2 Barbari	114	**CCC**	**Proline** (Non-Essential)	**CCG**	**Proline** (Non-Essential)	Silent Mutation
	131	**GCG**	**Alanine** (Non-Essential)	**GTG**	**Valine** (Essential)	Missense Mutation
	138	**GGC**	**Glycine** (Non-Essential)	**GGG**	**Glycine** (Non-Essential)	Silent Mutation
AT1 Tapri	92	**GCG**	**Alanine** (Non-Essential)	**GTG**	**Valine** (Essential)	Missense Mutation
	99	**GGC**	**Glycine** (Non-Essential)	**GGG**	**Glycine** (Non-Essential)	Silent Mutation

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Discussion

DNA markers have the potential to play a significant role in animal husbandry planning. The use of DNA markers has had a revolutionary impact on genetic mapping, as well as on the genetics of all animals and plants in general. Single nucleotide polymorphisms (sometimes known as "snips") are the most prevalent type of genetic variation found in mammals. Each SNP reflects a nucleotide variation in a DNA strand. The majority of the time, these differences are detected in the DNA between genes. They can be employed as biomarkers to aid researchers in the discovery of genes linked to animal features or disease expression. When SNPs exist in regulatory areas within or near genes, they may have a more direct effect on gene function, affecting health, sickness, and other traits including meat and milk production (Dodgson, Cheng, & Okimoto, 1997).

The UCP-1 gene of the three goat breeds has a total of 08 single nucleotide changes, according to the findings. This genetic variant of the UCP-1 gene was discovered in three indigenous goat breeds from Pakistan's Sindh Province. Three SNPs were discovered in the Sindhi goat breed, three mutations in the Barbary goat breed, and two mutations in the Tapuri goat breed, according to PCR basic analysis. These changes are classed as point mutations in the genetic code. The 94th base pair of cytosine in the Sindhi goat breed was transformed to thymine, resulting in this alteration. The non-essential amino acid alanine was replaced by the required amino acid valine, and the original codon GCG was modified to GTG. The mutation is incorrect. At nucleotide 101, there is still another mutation. When cytosine is replaced by guanine, the original GGC codon, which codes for glycine, mutates to GGG, which codes for the same amino acid.

Another single mutation occurred in the Sindhi goat breed, where adenine was changed to cytosine at base pair 341. The TTA codon, which codes for the amino acid leucine, is changed to TTC, which codes for the amino acid phenylalanine. This modification is incorrect. Two variants were discovered in Barbari goat base pairs 114, 131, and 138. The amino acids alanine and glycine are altered in the proline, valine, and glycine encoded by CCG, GTG, and GGG, whereas the base pairs CCC, GCG, and GGC encode proline. There are two SNPs in the Tapri goat breed, one at nucleotides 92 and 99 and the other at nucleotides 92 and 99. The GCG base pair, which originally coded for alanine, has been transformed into valine, an important amino acid. The GGC nucleotide pair that originally encoded the amino acid glycine has been changed to GGG. Table 2 lists the mutations and their frequencies in detail. The current study's findings are in line with those of Ujjan et al. (2011).

Conclusion

In this study, eight gene/SNP alterations in the UCP-1 gene were discovered in three goat breeds. We also discovered three mutations in the Sindh Desi goat breed, three mutations in the Barbari goat breed, and two mutations in the Tapri goat breed using DNA sequencing technologies and PCR gel electrophoresis. These mutations are characterized as point mutations in the genetic code. 02 nonsense mutations and 01 silent mutations are regarded advantageous genetic mutations in meat properties in Sindhi goat breeds, 01 nonsense mutations and 02 silent mutations in Barbary goat breeds, and 01 nonsense mutations and 01 silent mutations in Tapri goat breeds. Furthermore, our findings suggest that three different goat breeds, including the Sindhi goat, Barbary goat, and Tapuri goat, may be beneficial for meat properties.

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References

1. **Boss, O., Hagen, T. and Lowell, B.B.,** 2000. Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes*, *49*(2), pp.143-156.
2. **Crichton, P.G., Lee, Y. and Kunji, E.R.,** 2017. The molecular features of uncoupling protein 1 support a conventional mitochondrial carrier-like mechanism. *Biochimie*, *134*, pp.35-50.
3. **Dalgaard, L.T. and Pedersen, O.,** 2001. Uncoupling proteins: functional characteristics and role in the pathogenesis of obesity and Type II diabetes. *Diabetologia*, *44*(8), pp.946-965.
4. **Dodgson JB, Cheng HH, Okimoto RO.** 1997. DNA marker technology: a revolution in animal genetics. Poultry Science **1; 76(8),** 1108-1114.
5. **Erlanson-Albertsson, C.,** 2002. Uncoupling proteins a new family of proteins with unknown function. *Nutritional neuroscience*, *5*(1), pp.1-11.
6. **Ferraz, J.B.S., Pinto, L.F., Meirelles, F.V., Eler, J.P., De Rezende, F.M., Oliveira, E.C.M., Almeida, H.B., Woodward, B. and Nkrumah, D.,** 2009. Association of single nucleotide polymorphisms with carcass traits in Nellore cattle. *Genet. Mol. Res*, *8*(4), pp.1360-1366.
7. **Heaton GM, Wagenvoord RJ, Kemp A, Jr., and Nicholls DG.** Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. *European journal of biochemistry / FEBS* 82: 515-521, 1978.
8. **Imabayashi H, Mori T, Gojo S, Kiyono T, Sugiyama T, Irie R, Isogai T, Hata JI, Toyama Y, Umezawa A.** 2003. Redifferentiation of dedifferentiated chondrocytes and chondrogenesis of human bone marrow stromal cells via chondrosphere formation with expression profiling by large-scale cDNA analysis. Experimental cell research. **288(1),** 35-50.
9. **Kozak, L.P., Britton, J.H., Kozak, U.C. and Wells, J.M.,** 1988. The mitochondrial uncoupling protein gene. Correlation of exon structure to transmembrane domains. *Journal of Biological Chemistry*, *263*(25), pp.12274-12277.
10. **Modriansk**ý**, M., Murdza-Inglis, D.L., Patel, H.V., Freeman, K.B. and Garlid, K.D.,** 1997. Identification by site-directed mutagenesis of three arginines in uncoupling protein that are essential for nucleotide binding and inhibition. Journal of Biological Chemistry, 272(40), pp.24759-24762.
11. **Modriansky, M.**, et al., Identification by site-directed mutagenesis of three arginines in uncoupling protein that are essential fo r nucleotide binding and inhibition. J Biol Chem, 1997. 272(40): p. 24759-62.
12. **Nicholls, D.G.,** 2001. A history of UCPI. *Biochemical Society Transactions*, *29*(6), pp.751-755.
13. **Rousset, S., Alves-Guerra, M.C., Mozo, J., Miroux, B., Cassard-Doulcier, A.M., Bouillaud, F. and Ricquier, D.,** 2004. The biology of mitochondrial uncoupling proteins. *Diabetes*, *53*(suppl 1), pp.S130-S135.
14. **Sale, M. M., Hsu, F. C., Palmer, N. D., Gordon, C. J., Keene, K. L., Borgerink, H. M., ... & Norris, J. M.** (2007). The uncoupling protein 1 gene, UCP1, is expressed in mammalian islet cells and associated with acute insulin response to glucose in African American families from the IRAS Family Study. *BMC Endocrine Disorders*, *7*(1), 1-10.

15. **Ujan, J. A., Zan, L. S., Wang, H. B., Ujan, S. A., Adoligbe, C., Wang, H. C., & Biao, S. F.** (2011). Lack of an association between a single nucleotide polymorphism in the bovine myogenic determination 1 (MyoD1) gene and meat quality traits in indigenous Chinese cattle breeds. Genetics and Molecular Research, 10(3), 2213-2222.