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# Determination of the novel SNPs in UQCC gene among three different cattle breeds in Pakistan

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## Abstract

For farm animals, the quality of meat is of great economic importance. It is influenced by the environment and by the multi-genes. In recent decades, developments for molecular genetics have assisted in the discovery of genes or markers associated with traits that affect the quality of meat. The aim of this study was to investigate the novel SNPs in UQCC gene that may affect the meat quality. Initially, UQCC gene were amplified from cattle DNA samples and visualized on gel electrophoresis. Later, these PCR products were sent for DNA sequencing followed by SNP analysis. Study showed that five mutations were spotted in Exon 1 region of UQCC gene in all three cattle breeds. Based on genetic code, three different types of mutation were observed, namely, silent, missense and delete mutations. Silent mutation was common between Dhanni and Red Sindhi cattle breeds. Missense mutation was observed only in Red Sindhi cattle breed whereas delete mutation was very common among all three cattle breeds. Missense mutation observed in Red Sindhi cattle may be consider as a good sign and can produce good impact on meat traits as it codes for essential amino acids. These results suggest that Red Sindhi cattle breed can be implied for breed admixture and marker assisted selection in cattle breeding.

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## Introduction

Single nucleotide polymorphisms (SNPs) remain among the frequent genetic mutation in an organism's genome and it is recognized that the technique used to classify SNPs plays a huge role in influencing the outcomes of population genetics. It was anticipated that the SNPs identified as part of the Bovine HapMap Project (BHP) would have some ascertainment bias. It is due to the SNPs were primarily characterized to compare the taurine's reference sequence. Analyzing the effect of ascertainment bias in these SNPs is significant, since they are beginning to be included in genetic research of biologically and commercially relevant traits (Neto & Barendse, 2010).

Ubiquinol-cytochrome c reductase complex chaperone (UQCC) gene plays an important role in the maintenance of bone and cartilage. These genes found close to the bone forming cells which regulate the bone morphogenetic proteins involves in transcription of several morphogenesis gene products and bone growth. In a genome-wide search, UQCC was chosen as a potential gene for its involvement in chondrocyte multiplication and bone development.

In beef production, weight measurement traits have significant importance since these are the main factors which regulate the quantity and quality of meat in cattle. Induced mutations in cattle has considerable role for getting commercially valuable traits (Imabayashi *et al*., 2003). However, studied in mouse demonstrated that inaccurate mutation could lead to strange skeleton and ligaments formation (Harada *et al*., 2007). Moreover, UQCC was found in the abnormal development of joint (Erlacher *et al*., 1998; Francis-West *et al*., 1996).

Human genome has been comprehensively studied to find out useful traits in the *Homo sapiens* (Lettre *et al*., 2008; Sanna *et al*., 2008). Mouse and human UQCC gene studies showed that this gene is essential for the development of bone, growth of chondrocytes and morphogenesis. Therefore, it could be useful gene for bovine body's measurement characteristics. Moreover, role of UQCC gene in regulating the important characteristics of human body such as bone development, expression of tissues and morphogenesis are extensively studied in humans.

In contrast to human UQCC gene, data on bovine UQCC polymorphism is limited (Voight *et al*., 2006). To our best knowledge, few studies have been carried out in China to determine the UQCC gene's role in controlling the traits of pigs and cattle's meat. In comparison to China, knowledge gap on UQCC gene is wider in Pakistan as very negligible research has been carried out to improve the meat quality and quantity. Therefore, this study was carried out to study novel mutations in the local cattle breads known as Dhanni, Sahiwali and Red Sindhi cattle breed using SNP method (Zhang *et al.,* 2018). Moreover, this study was aimed to determine and compared the percentage of mutations among the indigenous cattle breeds centered on genetic code for improving the characteristics of local cattle breeds.

## Materials and methods

*Collection of blood samples*

Thirty blood samples were collected from indigenous cattles, namely, Dhanni, Sahiwali and Red Sindhi cattle breed. The animals used in this study were at the age ranging from 1.5 to 2 years and maintained according to Canadian Council for Animal care. Using a disposable syringe, blood sample (5ml) was collected from jugular vein of all the three cattle breeds and transferred into a EDTA (0.5M) tubes. Later, collected samples were stored at 4 $^{o}$C for subsequent extraction of DNA.

*DNA extraction*

After collecting the blood samples, DNA was extracted from these samples to amplify the target gene. Genomic DNA extraction kit (Thermo Scientific) was used to extract DNA from whole blood samples as per manufacturer's guidelines.

*Quantification of Extracted DNA*

To ensure that extracted DNA concentration was present in sufficient amount for amplification, DNA concentration was measured of each extracted sample using nanodrop machine at Genome research Centre,

HEJ, University of Karachi. DNA purity was also measured at 260/280 nm ratio.

*Primer Designing*

UQCC gene sequence was retrieved from NCBI website and was subjected to primer designing through online primer 3 software. Later primer sequences (Table 1) were blast in NCBI website for checking specificity.

*PCR amplification*

To amplify the UQCC gene, reaction mixture was prepared in an Eppendorf tube. The reaction mixture was prepared using the optimized concentration of all components. Subsequently, a 20µl reaction mixture was pipetted in each PCR reaction tube that contained 7µl of 2X Red PCR Master Mix, 2µl of each Forward and Reverse Primer (10pmol/µl), 5µl (100ng) genomic DNA and 4µl of ddH2O. PCR amplification was performed using thermo cycler machine (Bio Rad S1000, USA) and thermal cycling conditions were: 1) pre denaturation for 5 min at 94°C, 2) 34 cycles of denaturing at 94°C for 35s, annealing at 58°C for 45s, extension at 72°C for 1 min, and 3) final extension at 72°C for 5 min. PCR reaction was performed under standard protocol as described by Zhang *et al.*, 2007.

**Table 1.** Sequences of forward and reverse primers for amplifying UQCC gene.

| Gene | Forward primer (5′ → 3′) | Reverse primer (5′ → 3′) | bp | Tm |
|---|---|---|---|---|
| UQCC gene | GACGCTCCTGACCGAGACTA | GTTGTCTTTTCGCCTGTTGG | 177 | 58 |

*Gel electrophoresis*

For visualization, the amplified PCR products were run on agarose gel (1.5%) containing Ethidium bromide, a DNA intercalating agent. PCR product (4µl) and DNA loading dye (1µl) were mixed and pipetted in each well of agarose gel and in one well 1kb DNA ladder was loaded. Afterwards, agarose gel was completely submerged in 1X TBE buffer and an electric current was applied at 70 V for 45 min. Subsequently, the PCR products that migrated toward anodic field were visualized using the UV transilluminator (Bio rad, USA).

*Sequencing, purification, and Data analysis*

For sequencing, amplified PCR products were sent to The Advance Bio Science International company. The sequence obtained was analyzed online through ensemble.org genome browser by blasting on sequence alignment tool.

## Results

*Quantification of Extracted DNA*

Nanodrop measurement of extracted DNA samples revealed that the quantity of DNA was in the range of 30-103 ng/µl which is sufficient for PCR amplification. Nano drop also determined the purity of DNA was also analyzed analysis To evaluate DNA purity, measure absorbance from 230nm to 320nm to detect other possible contaminants. The most common purity calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm. Good-quality DNA will have an A260/A280 ratio of 1.7–2.0

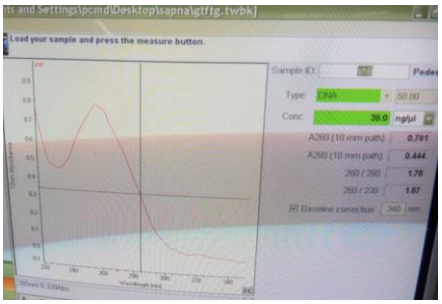


**Fig. 1.** DNA quantification using nanodrop method.

*PCR amplification and Gel electrophoresis*

In all three breeds a UQCC gene was PCR amplified and size was confirmed (177bp) through marker 100 kb as shown in Fig. 2.

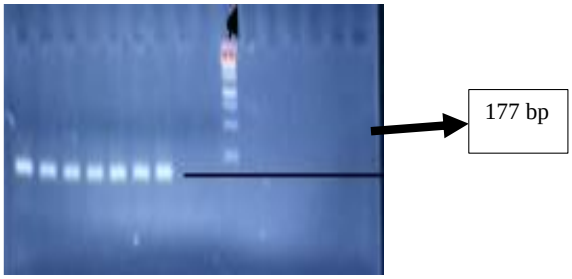


**Fig. 2.** PCR product of UQCC gene.

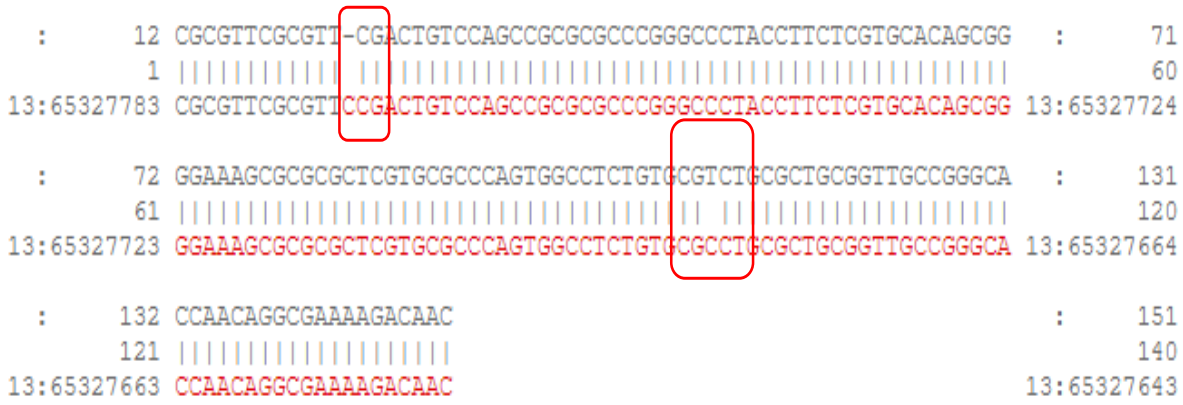


**Fig. 3.** SNPs identification through Sequence alignment of the bovine UQCC gene.

*Sequencing and Identification of Single Nucleotide Polymorphism (SNPs)*

After PCR amplification sequencing of amplified product was performed. The sequence of UQCC gene obtained is shown in Fig. 3. The sequence was analyzed online through ensemble.org by sequence alignment tool. Alignment graph shows the following mutations based on genetic code.

A total of 05 mutations were identified in Exon 1 region of UQCC gene in all three cattle breeds. Reportedly, 02 SNPs were identified in Dhanni cattle breed, 02 SNPs in Red Sindhi Cattle breed and only 01 SNP was found in Sahiwal cattle breed. Based on genetic code five mutations obtained were classified into following three types of mutations namely, Silent mutation in Dhanni cattle breed, delete mutation in all three breeds and missense mutation in Red Sindhi breed.

Based on Genetic code, five mutations were classified into Silent mutation (01) in Dhanni cattle breed where CGT mutated to CGC, one (01) delete mutation observed in all three breeds where CCG modified to -CG and one missense in Red Sindhi cattle Breed, which causes change of codon GAG that was coding for Non-essential amino acid (Glutamic acid) to GGG that codes for conditionally essential amino acid (Glycine ).

The details of the mutations and their frequencies are mentioned in Table 1.

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

| Sample name | Number of change nitrogenous base | Original codon | Changed codon | Original amino acid | Changed amino acid | Type of Mutation | Total% of Mutation |
|---|---|---|---|---|---|---|---|
| Dhanni D1 | 99 | CGT | CGC | Arginine (Essential) | Arginine (Conditionally Essential) | Silent Mutation | 0.56% |
| Dhanni D2 | 13 | CCG | -CG | | Proline | Deletion Mutation | |
| Red Sindhi R1 | 36 | GAG | GGG | Glutamic acid (Non-Essential) | Glycine (Conditionally Essential) | Missense mutation | 1.12% |
| Red Sindhi R2 | 13 | CCG | -CG | | Proline | Deletion Mutation | |
| Sahiwal S1 | 13 | CCG | -CG | proline | | Deletion Mutation | 1.12% |

**Discussion**

DNA markers have a major potential role in animal breeding programs. The use of DNA markers has a revolutionary effect on gene mapping and generally on the genetics of all animals and plants Single nucleotide polymorphisms, often referred to as SNPs (most commonly called "snips"), are the most common type of genetic variation among animals. Each SNP represents the difference in one strand of DNA called the nucleotide. Most often these variations are found in DNA between the genes. They can act as biological markers, helping scientists to find genes that are related to the expression of traits or disease in animals. When an SNP occurs within the gene or in the regulatory area near the gene, they may have a more direct role in the function of the gene which may affect the health, disease and other characteristics such as meat and milk production (Dodgson, Cheng, & Okimoto, 1997).

In this study the novel SNPs in UQCC gene were investigated that may affect meat characteristics such as meat quality and quantity. The results of this study provide the complete genetic analysis of the Exon 1 region of bovine UQCC and demonstrates the comparison of SNPs types and their percentage among all three indigenous cattle breeds of Sindh, Pakistan namely, Dhanni cattle breed, Red Sindhi cattle breed and Sahiwali cattle breed. In this study five mutations were found in Exon 1 region of UQCC gene in all three cattle breeds namely Dhanni cattle breed (two), Red Sindhi Cattle breed (two) and Sahiwal cattle breed one with the help of PCR-Gel Electrophoresis and DNA Sequencing techniques.

Silent mutation was very rare and this was only observed in Dhanni cattle breed which will not affect the meat quality and quantity traits because of intramolecular change in the genetic codon would not affect the expression of gene as amino acid coded by this gene remains same. Delete mutation was common in all three breeds, namely, Dhanni cattle breed, Red Sindhi breed and Sahiwal cattle breed. This type of mutation will negatively impact the meat characteristics because no amino acids will be coded by the modified codon this in turn will shorten the protein structure therefore meat quantity will be reduced. Missense mutation was also rare and one mutation was noted in Red Sindhi breed, which is very good sign and can create a very good impact on meat quantity as well as meat quality of this cattle breed because modified codon caused change of non-essential amino acid (Glutamic acid) into essential amino acid (Glycine) hence it will not only increase the meat quality but also meat quantity (Liu *et al.*, 2010; Lettre *et al.,* 2008). These results are in consistent with the study of Liu *et al.* (2010) and contrast with study of Lettre *et al.* (2008). Furthermore, Red Sindhi cattle breed and Dhanni cattle breed showed 1.12% of SNPs whereas Sahiwali only 0.56%. If the percentage of mutations in any population is less than one and it is simply called as a mutation. However, if the percentage of mutations in any population is greater than one then it is known as polymorphisms thus it suggests that Dhanni cattle breed and Red Sindhi cattle breed can be very much useful for breed admixture to enhance milk and meat characteristics.

## Conclusion

In this study three different types of mutation observed among three different cattle breeds. Among these three mutations, delete mutation was common in all three breeds and these mutations are not found useful as these might reduce meat characteristics. However, missense mutation in Red Sindhi breed could be useful for enhancing the meat and milk production. Therefore, this breed of cattle can be selected for breeding admixture and marker assisted selection in cattle breeding.

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