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ORIGINAL ARTICLE

# Association between single nucleotide polymorphism in ovine Calpain gene and growth performance in three Egyptian sheep breeds

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**Abstract** The aim of the present study was to assess the association of single nucleotide polymorphisms (SNPs) of Calpain (*CAPN*) gene with birth weight (BW), final weight (FW) and average daily gain (ADG) in three Egyptian sheep breeds: Barki, Rahmani and Ossimi. Blood samples were collected from 108 animals representing the three breeds. DNA was isolated using salting out procedure and then the quality and quantity of DNA extracted were measured. A 190 bp of *CAPN* was amplified by PCR using specific primers. The allele and genotype frequencies for all the identified SNPs were calculated. The PCR products corresponding to each genotype were sequenced to identify SNPs associated with the traits in question. Two SNPs (C→T) were detected in the nucleotides 44 and 154. For each SNP, the two mentioned alleles were named C and T, respectively. The sequenced *CAPN* segments were subjected to nucleotide blast at NCBI, which revealed 99% identity with that reported for sheep in Genbank. The TT was the least common genotype, whereas frequencies of CT and CC genotypes were fluctuated in the three sheep breeds under study. Animal carrier TT genotype had higher BW, FW and ADG than those with CT genotype, while the lowest values were associated with CC genotype. For the three traits under study, Rahmani had the highest estimates followed by Ossimi and Barki. Males exhibited heavier BW and FW as well as higher ADG compared with females. The results generated provide preliminary indication of the functional diversity present in Barki, Rahmani and Ossimi sheep and the possibility of using this polymorphism in Egyptian sheep genetic improvement.



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## 1. Introduction

Recent developments in molecular biology and statistics have provided the possibility of using genomic variation to accelerate the rate of genetic improvement of livestock. Application of marker-assisted selection (MAS) can be more effective in traits that are expressed late in the life of the animal or controlled by a few genes [1].

Calpains (*CAPN*) encode intracellular calcium-activated cysteine proteases that have been involved in several physiological and pathological processes [2]. Calpains are regulated by a variety of factors, including a 30-kDa small subunit [3], calcium and phospholipids [4], and calpastatin, a widely distributed calpain-specific inhibitor [5] and [6]. In living muscle, calpains are responsible for remodeling proteins that maintain the structure of skeletal muscle (myofibrillar linkage proteins, MLP) such as titin, nebulin and desmin [7]. The calpain activity is basically required for myoblast fusion as well as cellular proliferation and growth [8]. Polymorphism in *CAPN* gene has been identified in different breeds of sheep [9–11]. Page et al. [12] proposed *CAPN* as a potential candidate gene for meat tenderness in cattle, causing degradation in myofibrillar proteins postmortem. Calpain substrates include a variety of enzymes such as cytoskeletal proteins [13], kinases and phosphatases [14], and epidermal growth factor receptors [15]. The extent of physiological cleavage of these and other proteolytic proteins depends mainly on the presence and the activity of specific cell inhibitors.

The most common sheep breeds in Egypt are Barki, Ossimi and Rahmani. Barki is originated from the Libyan province Barka, and is spread along the coastal Mediterranean zone form west of Alexandria to the eastern provinces in Libya. Ossimi name is derived from a village near to Cairo, called Ossim, Giza governorate. It is the major breed in the Nile Valley and Delta zones, where the breed is generally more productive in middle Egypt rather than in Upper Egypt. Rahmani breed originates mainly from Northern Syria and Southern Turkey. The breed is most popular in the North and Middle of the Nile Delta. All breeds are classified as fat tailed animals producing coarse wool, well adapted to harsh environmental conditions and are raised basically for lamb production, followed by wool and milk. Generally animals belonging to the three sheep breeds are usually kept by small holders where herd size is of 1–3 heads, fed on low quality diets, bred through natural service, herd books and breed registration are generally not available [16].

The objective of the present study is to determine the polymorphism of calpain gene in three Egyptian sheep breeds and to evaluate the association of these SNPs with birth and final weights as well as average daily gain.

## 2. Material and methods

### 2.1. Animals

The present study was performed on 108 animals (males and females), representing the three Egyptian sheep breeds (Barki, Rahmani and Ossimi), animals were reared in the Agricultural Experiment Station, belonging to Faculty of Agriculture, Cairo University. Animals were classified into heavy and light final body weight (56 animals per breed per phenotype). A 10-ml blood sample was collected through vein puncture from each animal in a tube containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant, and sent to the laboratory under cooled conditions. Traits measured were birth weight (BW), final weight at slaughter (FW) and average daily gain (ADG).

### 2.2. DNA extraction

Genomic DNA was extracted from the whole blood using salting out procedure described by Miller et al. [17]. Ultraviolet Spectrophotometer and 0.8 per cent agarose gel electrophoresis were used to check the quantity and quality of DNA.

### 2.3. Polymerase chain reaction (PCR)

The DNA fragment of the studied gene was amplified through PCR technique. This amplified fragment covered a part of exon 2, intron 2, exon 3 and a part of intron 3. The primer sequence of *CAPN* was the previously designed by Nassiry et al. [18].

A 20 μl PCR cocktail consisted of 20 pmol forward (AACATTCTCAACAAAGTGGTG) and reverse primers (ACATCCATACAGCCACCAT), 0.2 mM dNTPs and 1.25U of *Taq* DNA polymerase. The cocktail was aliquoted into PCR tubes with 100 ng of ovine DNA. The reaction was cycled with the following conditions; initial denaturation for 5 min at 94 °C followed by 35 cycles of denaturation at 94 °C, annealing at 60 °C and extension at 72 °C, each step for 1 min and the final extension for 5 min at 72 °C. The amplification was verified by electrophoresis on 2% agarose gel (w/v) in 1X TBE buffer. The size of PCR product was measured using Gene Ruler of 100-bp ladder as a molecular weight marker. The gel was stained with ethidium bromide and visualized under UV transilluminator.

### 2.4. Sequence analysis

The PCR products corresponding to distinct patterns were purified and got sequenced by Macrogen Incorporation (Seoul, South Korea). Sequence analyses and alignment to reveal nucleotide substitutions were carried out using nucleotide blast at NCBI (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi) for sequence homology and comparison searches in public databases [19].

### 2.5. Statistical analyses

Animals were assumed to be unrelated. Deviations from the Hardy–Weinberg equilibrium were tested by the $\chi 2$-test. The genotypic and allelic frequencies were calculated using XLSTAT [20]. The program was used to test the significance of the fixed effects on the studied traits (BW, FW and ADG). Marker genotypes were considered to be independent in all analyses. Association analysis of the single genotypes at different loci in *CAPN* gene was performed individually for the three studied traits in Barki, Rahmani and Ossimi breeds.

Statistical analyses were performed for BW, FW and ADG. The model used to analyze the data was as follows: