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The Identification of Novel Single Nucleotide Polymorphisms in Calpain 1 *(CAPN1)* Gene of Japanese Quail *(Coturnix coturnix japonica)*

Japon Bıldırcınlarında Calpain 1 *(CAPN1)* Geni Tek Nükleotid Polimorfizmlerinin Tanımlanması

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Keywords:

CAPN1 Gene, DNA sequencing, *Coturnix japonica, quail*.

ABSTRACT

Objective: Calpains, in particular μ-calpain, are responsible for the post mortem proteolysis processes in muscle tissue and have main influences on meat quality. The *CAPN1* gene that codes for large subunit of μ-calpain is revealed as a candidate gene related with meat quality and tenderization traits for livestock. For this reason, in this study it is aimed to investigate the genetic variation of *CAPN1* gene in Japanese quails.

Material and Methods: In this study, the genetic variation of *CAPN1* gene was analyzed via DNA sequencing of 35 (13 males and 12 females) Japanese quails which were reared in Tekirdağ Namık Kemal University, Animal Research Unit.

Results: Some genetic variants which are found in the 4th and 5th exons are as g.103969C>T in the 4th exon region and eight novel SNPs as g.104116A>T, g.104118T>G, g.104148G>C, g.104169G>C, g.104172A>G, g.104179C>G, g.104181G>A, g.104184T>C in the 5th exon of *CAPN1* gene. The novel DNA polymorphisms of *CAPN1* gene in Japanese quails are reported for the first time in this study and these sequences were deposited to NCBI GenBank Database, with the accession numbers MK496828-MK496837, respectively. g.103969C>T transversion which is localized in the 4th exon region and g.104148G>C, g.104169G>C transversions and g.104172A>G, g.104181G>A, g.104184T>C transitions which are localized in the 5th exon region have not caused an amino acid change. Instead, g.104116A>T, g.104118T>G tranversions caused the change from Threonine to Serine amino acid. Similarly, C→G transversion which was observed on the 104179th position caused the amino acid change from Proline to Alanine.

Conclusion: These observed SNPs may have an effect on meat yield and tenderness in quails, so further researches are needed to demonstrate this hypothesis and these SNPs may be candidate SNPs for quails breeding.

Anahtar Kelimeler:

CAPN1 Geni, DNA dizi analizi, *Coturnix japonica*, bıldırcın.

ÖZ

Amaç: Kalpainler, özellikle μ-kalpain, kas dokusunda ölümden sonraki proteoliz işlemlerinden sorumludur ve et kalitesi üzerinde ana etkiye sahiptir. Büyük μ-kalpain alt birimi tarafından kodlanan *CAPN1* geni, çiftlik hayvanlarında et kalitesi ve lezzet özellikleri ile ilgili aday gen olarak belirlenmiştir. Bu nedenle Japon bıldırcınlarında *CAPN1* geni genetik varyasyonunun tanımlanması amaçlanmıştır.

Materyal ve Metot: Bu çalışmada *CAPN1* geni genetik varyasyonu Tekirdağ Namık Kemal Üniversitesi Hayvan Araştırmaları Birimi'nde yetiştirilen 35 adet (13 erkek ve 12 dişi) Japon bıldırcında DNA dizi analizi yöntemi ile belirlenmiştir.

Bulgular: *CAPN1* genindeki genetik varyasyonlar 4. ve 5. ekzonlarda olmak üzere; g.103969C> T varyasyonu 4. ekzon bölgesinde, ve g.104116A>T, g.104118T>G, g.104148G>C, g.104169G>C, g.104172A>G, g.104179C>G, g.104181G>A, g.104184T>C olmak üzere sekiz yeni SNP 5. ekzonda görülmüştür. Japon bıldırcınlarındaki *CAPN1* genindeki yeni DNA varyasyonları ilk kez bu çalışmada bildirilmiştir ve bu diziler sırasıyla MK496828-MK496837 erişim numarasıyla NCBI GenBank veri tabanına kaydedilmiştir. 4. ekzon bölgesinde görülen 103969C> T transversiyonu ve 5. ekzon bölgesindeki g.104148G> C, g.104169G> C transversiyonları ve g.104172A> G, g.104181G> A, g.104184T> C transisyonları amino asit değişikliğine neden olmamıştır. Ancak g.104116A>T, g.104118T>G transversiyonları, Threonin'den Serin amino asidi değişimine neden olmuştur. Benzer şekilde, 104179. pozisyonda C→G transversiyonu, Prolin'den Alanin amino asidi değişimine neden olmuştur.

Sonuç: Bu çalışmada tespit edilen SNP'lerin bıldırcınlardaki et verimi ve lezzeti üzerinde bir etkisi olabileceği düşünülmektedir. Bu nedenle bu hipotezi doğrulamak için daha fazla araştırmaya ihtiyaç duyulmaktadır. Tespit edilen SNP'lerin bıldırcın ıslahında aday gen ve SNP'ler olarak kullanılabilirlikleri değerlendirilmelidir.

INTRODUCTION

Calpains, in particular μ-calpain, are responsible for the post mortem proteolysis processes in muscle tissue (Koohmaraie, 1992) and they have main influence on meat quality parameters in animals (Geesink and Koohmaraie, 1999a,b; Barendse, 2002; Sentandreu et al., 2002; Koohmaraie and Geesink, 2006; White et al., 2008; Rasouli et al., 2013). Proteolysis of myofibrillar proteins that are responsible for two antagonistic processes, myofibrillar protein synthesis and degradation are the primary reason for ultrastructural alterations in skeletal muscle related with meat tenderness (Goll et al., 2003). μ-calpain and m-calpain are two isozymes of calpain that are activated by high concentrations of Ca^{2+} (Ishiura et al., 1978).

The expression of *CAPN1, CAPN2* and *CAPN3* genes were identified in poultry (Okumura et al., 2005). *CAPN1* gene is the most active calpain gene in poultry. The *CAPN1* gene that codes for large subunit of μ-calpain was introduced as a candidate gene related with meat quality and tenderization traits (Ropka-Molik et al., 2014). The *CAPN1* gene consist of 20 exons and it has about 22.367 bp. The *CAPN1* gene is located between 97751th- 120117th bp on *Coturnix japonica* whole genome shotgun sequence (Genbank Accession number NW_015439879.1). *CAPN1* protein has 641 amino acid residues.

In many domestic animals including poultry, studies are handled to identify the genetic origin of modifications in the structure of calpain protein variation. The relation of the *CAPN1* gene with the tenderness process of meat post-mortem has been demonstrated in several species. In the bovine, Page et al. (2002; 2004) revealed 38 polymorphisms localized in coding and non-coding regions of *CAPN1* gene and, affirmed their relations with significant beef quality traits. Similarly, many polymorphisms in exons, introns, and 3′ untranslated (3′UTR) region of CAPN1 gene were identified associated with carcass meat content in porcine (Yang et al., 2007; 2008; Li et al., 2009). Negro et al (2016) revealed that a novel SNP (g.68G>A) for *CAPN1* gene in goose. Also, g.68G>A was statistically related with carcass, meat quality traits and meat tenderness female goose of the Toulouse breed ($p = 0.043$). Zhang et al. (2008) detected three SNPs which were found in meat-type chicken populations. They found that some of the haplotypes were related with carcass weight, breast muscle weight and leg muscle weight ($p < 0.05$).

Maeda et al. (1990) investigated low degradation rate of myofibrillar protein revealed in the line selected for high body weight in the Japanese quail (*Coturnix coturnix japonica*). Afterwards, Maeda et al. (1991) revealed that the Japanese quail line selected for small body weight was characterized by a high calpain activity in skeletal muscle. Alike, Johari et al. (1993) showed that layer chickens have the higher m-calpain activity and muscle protein turnover rate than in broiler chickens. For this reason, it is obvious that calpain activity associated with turnover of muscle protein. In quail, only one research was found on calpain gene (Palmer et al., 1999). The Japanese quail is a source of poultry protein and investigation for improving its meat quality is therefore important.

The aim of this study is to determine polymorphism of the *CAPN1* gene in Japanese quails via DNA sequencing method.

MATERIAL and METHOD

Animal Material

In this study, a total of 35 blood samples were used which were collected from Japanese quails that were reared in Tekirdağ Namık Kemal University Animal Research Unit.

Method

Samples were collected to 5 mL of vacuum tubes, including EDTA as anticoagulant and stored at −20 °C till DNA extraction. Genomic DNAs were extracted using a commercial DNA isolation kit (Invitrogen, Life Technologies) according to manufacturer's instructions.

Primer sequences of *CAPN1* gene was designed based on the quails sequence retrieved from GenBank (Accession number NW_015439879.1) using Primer-BLAST algorithm (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primer sequences of *CAPN1* gene are F: 5′- TGG AGT GCT TTG CTG GAG AAA GCC TA-3 and R: 5′- TGG TAG AGG TCT GCG GGG GGC TTG CG-3′ (Rasouli et al., 2013). The 25 μL PCR volume contained: 50 ng genomic DNA, 0.5 μM of each primers, 1× PCR Buffer, 200 μM dNTP, 2 mM $MgCl_2$ and 1 U of Taq DNA polymerase (Invitrogen, Life Technologies). The cycling protocol was 5 min at 95 ^{0}C, 35 cycles of 95 ^{0}C for 30 sec, 57 ^{0}C annealing for 1 min, 72 ^{0}C for 2 min, with a final extension of 72 ^{0}C for 10 min. Afterwards, the PCR products of *CAPN1* gene were checked with 2 % agarose gel electrophoresis, and the gels were stained with SafeView™ Classic (Applied Biological Material

Inc. Canada) and photographed in Vilber Lourmat gel imaging system.

229 bp *CAPN1* gene was sequenced on an Applied Biosystems 3500XL Genetic Analyzer System (Applied Biosystems, USA) in order to identify the *CAPN1* gene sequence. The sequences of *CAPN1* fragments were aligned by using the MEGA6 software (Molecular Evolutionary Genetics Analysis, version 6.0, Tamura et al., 2013).

RESULTS

The genetic variation of *CAPN1* gene was investigated by DNA sequencing and the comparison with the DNA sequences taken from GenBank (Accession number NW_015439879.1). *CAPN1* gene sequence that is investigated in this study located between 103964th-104192nd bp at NCBI GenBank database (Accession number NW_015439879.1). In this study, the studied gene region has spanned between 4th-5th exon and 4th intron in quails and it includes 49 amino acids. The *CAPN1* gene sequences investigated in this study were deposited to NCBI GenBank database, with the accession number MK496828-37.

229 bp of *CAPN1* gene was amplified (Figure 1) and nine novel genetic polymorphisms were determined at position 103969th, 104116th, 104118th, 104148th, 104169th, 104172th, 104179th, 104181th, 104184th of *CAPN1* gene in quails (NW_015439879.1). The SNPs which were found as g.104116A>T, g.104118T>G, g.104148G>C, g.104169G>C, g.104172A>G, g.104179C>G, g.104181G>A, g.104184T>C were localized in the 5th exon region. The SNP which was found as g.103969C>T was localized in the 4th exon region. The Genbank accession numbers and variation sites of the *CAPN1* gene region of *Coturnix japonica* are given in Table 1.

The *CAPN1* gene region which is identified in this study has spanned between partial 4th, 5th exon and 4th intron and it includes 49 amino acids between 229 base pairs. The novel genetic variations as g.103969C>T, g.104116A>T, g.104118T>G, g.104148G>C, g.104169G>C, g.104172A>G, g.104179C>G, g.104181G>A, g.104184T>C in the 4th and 5th exon were detected in *CAPN1* gene, and it was contrasted with the GenBank record (Accession number NW_015439879.1). The *CAPN1* gene sequence, which was identified firstly in current study, was deposited to NCBI GenBank database, with the accession number MK496828-37.

g.103969C>T transversion which is localized in the 4th exon region is a synonymous mutation which has not caused any amino acid change. Similarly, g.104148G>C, g.104169G>C transvesions and g.104172A>G, g.104181G>A, g.104184T>C transitions which is localized in the 5th exon region have not caused the altered codon to produce an amino acid change. But, g.104116A>T, g.104118T>G tranversions caused an amino acid change from Threonine to Serine. Also, C→G transversion was observed on the 104179th position caused an amino acid change from Proline to Alanine.

Table 1. The Genbank accession numbers and variation sites of the *CAPN1* gene region of *Coturnix japonica*.
Çizelge 1. *Bıldırcın (Coturnix japonica) CAPN1 geninin varyasyon bölgeleri ve Genbank aksesyon numaraları*

DNA nucleotide positions		103969**	104116**	104118**	104148**	104169**	104172**	104179**	104181**	104184**
Haplotypes/ Reference	**GenBank Accession Numbers**									
Reference Sequence	NW_015439879.1[1]	C	A	T	G	G	A	C	G	T
New Sequences	MK496828*	T	T	G	C	C	G	G	A	C
	MK496829*	-	T	G	C	C	G	G	A	C
	MK496830*	T	-	G	C	C	G	G	A	C
	MK496831*	T	T	-	C	C	G	G	A	C
	MK496832*	T	T	G	-	C	G	G	A	C
	MK496833*	T	T	G	C	-	G	G	A	C
	MK496834*	T	T	G	C	C	-	G	A	C
	MK496835*	T	T	G	C	C	G	-	A	C
	MK496836*	T	T	G	C	C	G	G	-	C
	MK496837*	T	T	G	C	C	G	G	A	-

[1]NW_015439879.1 of *Coturnix japonica* whole genome shotgun sequence.* MK496828-37- 229 bp of *CAPN1* gene of Japanese quails found in this study. - indicates identical nucleotides at that site. ** Variable sites that are newly found in this study.

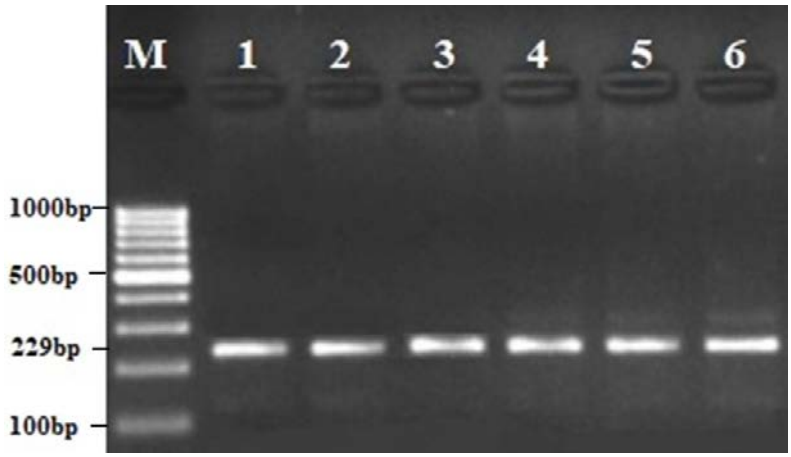

Figure 1. PCR products of the *CAPN1* gene. M; Marker

Şekil 1. *CAPN1 geni PCR ürünleri. M; Marker*

DISCUSSION and CONCLUSION

In this study we have identified the calpain gene in quails which were found in association with meat tenderization and traits in farm animals. Calpain gene was studied and DNA sequencing method was used in order to find out the genetic variation in this gene region of quails. In this study, a genetic variant as g.103969C>T in the 4th exon region and eight novel SNPs are identified as g.104116A>T, g.104118T>G, g.104148G>C, g.104169G>C, g.104172A>G, g.104179C>G, g.104181G>A, g.104184T>C in the 5th exon region of *CAPN1* gene in Japanese quail. Rasouli et al. (2013) found that the genotypes of the *CAPN1* gene in the 217-bp region were significantly related with yellowness and shear force. Soria et al. (2009) and Smith et al. (2009) reported similar findings and declared that when the myoglobin content of breast muscle was low, the yellowness of the meat could increase. The shear force of breast meat; because of high calpain proteolytic activity, TT genotype was significantly lower than the other genotypes in birds. Alike, Zhang et al. (2008) reported that the tenderness of breast meat in TT genotype was higher than the other genotypes and the myofibrillar degradation with allele T is higher than with allele C.

Very few studies have been carried out on CAPN gene variations and their relations on meat quality traits in quail. In this study, new polymorphisms are found which will implement beneficial information for improving meat quality and tenderization in Japanese quail based on marker-assisted selection.

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