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IGFALS Gene Polymorphisms in Simmental Breed Cattle

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ABSTRACT

Defining new genetic markers to understand the population structure and genetic basis of cattle breeds, increase production and improve yield quality is of great importance in the field of modern breeding technology. In this study, it was aimed to identify new molecular marker polymorphisms by using DNA sequence analysis method on the Insulin-Like Growth Factor-Binding Protein, Acıd-Labile Subunit (IGFALS) gene, which affects different yield characteristics in cattle. 20 samples from PCR products with various base sizes were randomly chosen for sequence analysis. The study revealed that the IGFALS gene's first exon region, which is 492 bp long, contains the g.1368491 A/G polymorphism. A 463 bp length region of the second exon was where the g.1369854 -/G insertion was found.

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Introduction

Inheritance of economically important traits is done by identifying genes that underlie the genetic variability of selected traits. Genomic data obtained by different methods are used in genetic evaluation of animals and genetic breeding programs. To identify and classify populations that can be used as genetic resources in establishing the genetic structure affecting yield in farm animals, many methods including Marker Assisted Selection (MAS), QTL (Quantative trait locus), GWAS (Genome-wide association study), RFLP (Restriction fragment length polymorphism), SSCP (Single-strand conformation polymorphism), and gene sequencing are used. (Sönmez, 2017; Özdemir, 2021). The IGFALS (Insulin-Like Growth Factor-Binding Protein, Acıd-Labile Subunit) gene, which is a member of the IGF (Insulin-like growth factor) gene family and has a metabolism regulation function, has a high potential to be used as a marker in the field of animal breeds.

IGF is stimulated in the ternary complex, weighing 150 kDa and consisting of two receptors (IGF-I receptor (IGFIR) and IGF-II receptor (IGFIIR) and two ligands (IGF-I and IGF-II), and each molecule of IGF-I synthesized by the liver, IGF binding protein (IGFBP 3-5), and labile acid subunit (ALS) molecules (Baxter, 1994; Domene et al., 2011). The labile acid subunit (ALS) mRNA of the GH hormone is secreted in the liver parenchymal cells and the proximal kidney tubule, (Rechler 1993; Boisclair et al., 2000a; Boisclair et al., 2000b,). It is known that IGF-I secreted in the endocrine glands constantly decreases as a result of null mutation of the labile acid subunit (ALS) of the GH hormone, and accordingly, the body weight decreases, pubertal delay, insulin insensitivity, and reduced bone mineral density, making it difficult for IGF-I to be released and reach the relevant tissues (Ueki et al., 2009; Lai et al., 2014; Jasper et al.2016). The IGFALS gene, which affects growth, udder development, yield traits, bone growth and milk production, in cattle and controls postnatal growth (Ueki et al., 2009; Lai et al., 2014; Zhi et al. 2019), consists of two exon regions with a length of 2.949 base pairs on chromosome 25 and encodes 611 amino acids weighing 65.97 Daltons. It has been reported that the reductions and mutations in *IGFALS* gene expression in humans affect growth and lead to changes in the mineral density rates of bones, osteoporosis, reduced IGF1 gene expression, late maturity, uneven muscle development, and diseases such as short stature. Likewise, it has also been reported that different expression levels of mRNA and proteins caused by mutations in the *IGFALS* gene in human placental tissue have a functional effect on growth traits (Domene et al., 2011; Iniguez et al., 2011; Hess et al., 2013; Storr et al., 2015; Högler et al., 2014; Castilla et al., 2017; Ramirez et al., 2020).

In studies investigating the effects of gene function and mutations in the gene on farm animals, it is known that there are significant relationships between polymorphic regions identified in the *IGFALS* gene and some weight, growth data

and biometric traits and gene expression effect of stimulated prenatal muscle growth and development in sheep (Alizadeh et al., 2020, Gauvin et al.2020). Different polymorphic structures observed in the gene affect the height at withers and the width of the chest circumference, the regulation of corticosteroid hormone secretion, retinoid binding, unsaturated fatty acids, and steroid metabolism. The IGFALS gene expression deficiency in cattle breeding is primarily associated with gluconeogenic gene expression, food intake and reduced body weight (Flannery et al., 2013; Liu et al., 2014; Akbar et al., 2015; Pareek et al., 2019; Fang and Pausch et al., 2019). IGFALS gene expression effect of severe energy balance on cattle (McCarthy et al. 2010; Wathes et al. 2011) The IGFALS gene may be a candidate gene linked to yield qualities in cattle breeding when all these traits are taken into consideration.

In this study, the genotypic structures of the *IGFALS* gene in the "Simmental" breed of cattle raised in Turkey will be examined, and the single nucleotide polymorphisms (SNPs) of the relevant gene will be determined using the gene sequence analysis method.

Material and Methods

Material

The blood of 65 unrelated heads of "Simmental" cattle, which were raised in a combined way in a private enterprise in Erzurum province, was used as the material. The selected breed of cattle were raised on the same farm, under the same conditions, and subjected to the same feeding.

Ethical Protocol: Ethical Protocol was approved by the experimental animal ethics committee of Atatürk University with its session dated 27.10.2022 and decision numbered 257.

Genomic DNA was obtained using the commercial DNA isolation kit. Before starting PCR applications to amplify the DNA obtained, the qualitative determination of DNA was performed using 1% agarose gel electrophoresis, and the quantitative determinations were performed by taking measurements by a MaestroNano device.

Primers

The primers (ARS-UCD1.2 (GCF_002263795.1)) were designed using the Primer 3.0 program to include both exons according to the relevant reference sequence. The primers designed for the IGFALS gene was presented in Table 1.

PCR

To complete the total volume to 30 µl for PCR amplification, 1 µl of each primer, 0.5 units of Taq DNA polymerase, 2 µl dNTPmix, approximately 200 ng template DNA, 1 µl of 25 mM MgCl2, 3 µl of 10x PCR Buffer and ddH2O were added. After PCR amplification conditions were set as 50 seconds at 96 °C, 50 seconds at 62 °C, and 50 seconds at 72 °C for the 492 bp long 1st exon region of the IGFALS gene and as 5 minutes at 96 °C, 45 seconds at 61 °C, and 45 seconds at 72 °C for the 463 bp long 2nd exon region so that final elongation temperatures would be 10 minutes and 1 cycle at 72 °C under the same conditions. The initial denaturation temperatures were determined as 1 cycle and denaturation cycle 33 at 96°C for 5 minutes with the same, the amplicon conditions for both gene regions. The gene frequencies of each Simmental breed were calculated by counting POPGENE software v1.32 (Yeh et al., 1999).

DNA Sequencing

One-way sequence readings of a total of 40 different PCR samples, including 20 different from each region, with the sequences giving strong bands randomly under UV light from among the PCR products of 65 heads of Simmental cattle that we amplified with a PCR device separately for each exon region, were performed by sending them to the relevant commercial company. Sequencing results showed FinchTV2000 programs. According to reference sequences, the sequences showing polymorphism were determined using MEGA 7.0 (Kumar et al., 2016) and BioEdit 7.2.6 (Hall, 2011) software programs.

Table 1. IGFALS Gene Primers

	(5'->3')	Base pair(bp)
Primers: Exon 1		
F1	GGC ATC CTT TGT CCA GTG AT	492 bp
R1	TTA AGA GAA GGC CCT GCT GA	
R3	GTA GAG TTT CTG GAG CTT GG	
Primers: Exon 2		
F4	GCT CAA CCT CTC CAG CAA	463 bp
R4	GGT CCT CAG CGA GTT GTT TC	

Results and Discussion

Two percent agarose gels were prepared according to the band sizes of the PCR products, and the PCR products were run to determine whether the amplification occurred after the PCR process. The PCR products of the relevant genes were observed under UV light, and imaging was performed (Figure 1).

The Simmental cattle 492 bp DNA sequence analysis that we carried out led to the discovery of the g.1368491 A/G polymorphism on the first exon region of the IGFALS gene. With this base change observed in the 1st exon region, the amino acid codons differed, and the *CAG* Glutamine amino acid codon was transformed into the *CGG* codon and led to the encoding of the Arginine amino acid (Gln-Arg). As a result of the 463 bp long DNA sequence analysis, including the 2nd exon region, g.1369854 -/G insertion was detected (Figure 2)

As a result of eliminating the samples with sequence reading errors among the 20 samples on which sequence analysis was performed in the Simmental breed of cattle, the alleles and genotypes of the remaining 13 samples were counted by direct counting on the FinchTV2000 program (Figure 3), and the results are presented in Table 2.

Table 2. *IGFALS* 1st and 2nd exon polymorphic regions allele and genotype frequencies

	Genotype	N
g.1368491 A>G	AA	5
	AG	7
	GG	3
Allele Frequences	p: 0.57	q:0.43
g.1369854 -/G+ insertion	6	
g.1369854 -/G-	7	

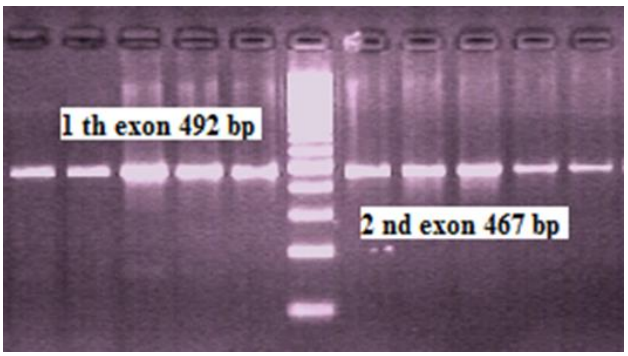


Figure 1. IGFALS gene (standard Genesta 100 bp DNA marker) 1st exon 492 bp and 2nd exon region 463 bp

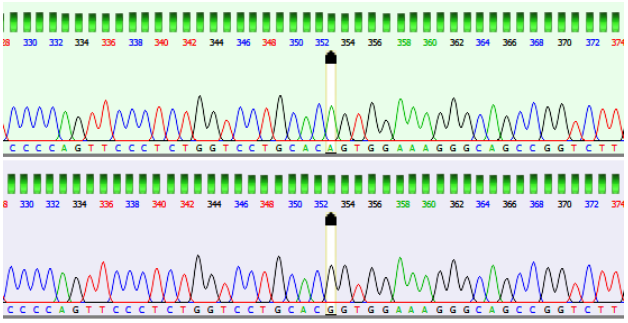
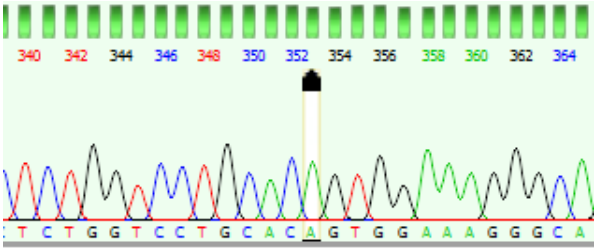
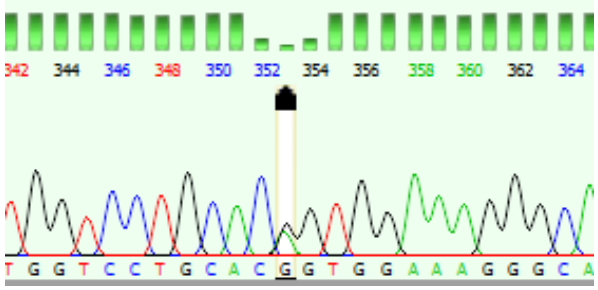


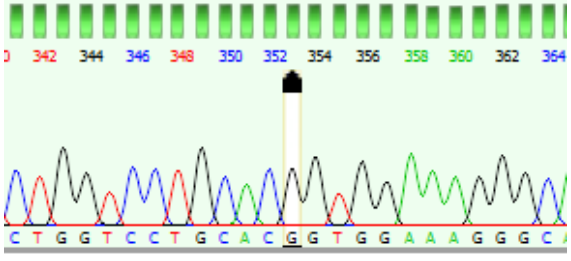
Figure 2. IGFALS gene 1st exon region g.1368491 A/G and 2nd exon region g.1369854 -/G polymorphic sequence



AA



AG



GG

Figure 3. IGFALS gene 1st exon g.1368491 A/G polymorphic region genotypes AA, AG and GG

In cattle, the IGFALS gene is carried on chromosome 25 and is composed of two exon regions. Unlike the polymorphic regions reported in the DNA sequence analyses in this study and the DNA sequence analyses previously identified on this gene, an SNP (g.1368491 A>G) in the first exon region and an insertion base (g.1369854 -/G) in the second exon region were detected. Liu et al. (2014) detected 4 SNPs (g1219: TNC, g1893: TNC, g2612: GNA, and g2696: ANG) in the 2nd exon region of the *IGFALS* gene in the Qinchuan beef cattle breed. In the META analysis study in which the genotypes and phenotypes of 4578 Brown bulls were analyzed, it was reported that rs133315098 SNP on the 1,455,150 bases of the *IGFALS* gene located at the locus of chromosome 25 had strong pleiotropic effects on height, live weight, milk yield, fat yield, protein yield, and the recovering ability of the lactating cow after calving. Since we did not have yield records for the breed we examined, the correlation analysis with yield and the phenotypic effect of SNPs could not be determined. In the studies conducted to determine SNPs and new markers in *Bos Taurus* breeds, Liu et al. (2014) detected 4 SNPs (g1219: TNC, g1893: TNC, g2612: GNA, and g2696: ANG) and 12 haplotypes in the 2nd exon region of the *IGFALS* gene in the Qinchuan beef cattle breed. They determined that SNP g2696: ANG SNP led to converting the asparagine amino acid into the aspartic acid in the leucine-rich region of the carboxyl-terminal domain *IGFALS*. Only g.1369854 -/G insertion was detected in the 2nd exon region in our study.

As a result of the 492 bp DNA sequence analysis in Simmental cattle, g.1368491 A/G polymorphism was detected on the 1st exon region of the *IGFALS* gene. With this base change observed in the 1st exon region, the amino acid codons differed, and the *CAG* Glutamine amino acid codon was transformed into the *CGG* codon and led to the encoding of the Arginine amino acid (Gln-Arg). As a result of the 463 bp long DNA sequence analysis, including the 2nd exon region, g.1369854 -/G insertion was detected.

The base change observed at g.1368491 A/G position on the 1st exon region of the *IGFALS* gene probably led to a silent mutation in codon sequences. The conversion of Glutamine amino acid, which is a polar amino acid and can quickly convert to other polar amino acids due to its amino group, into the asparagine amino acid, which belongs to other polar amino acid group (Gln-Arg), by differentiation of the CAG codon and conversion to the CGG codon may have led to a change in the function of the *IGFALS* gene.

Conclusions

The sequence analysis we carried out on the NCBI (ARS-UCD1.2 (GCF_002263795.1)) revealed that the IGFALS gene's first exon region, which is 492 base pairs long, contains the g.1368491 A/G polymorphism, and the 2nd exon region, which is 463 base pairs long, contains the g.1369854 -/G insertion. It is advised that research to be carried out in animal breeding analyze the qualities of being a marker in breeding programs and examine the effects of the identified polymorphic areas on various breeds and with various performance traits.

This study is an excerpt from the master thesis titled Determination of IGFALS Gene Polymorphisms in

Simmental Breed of Cattle By The Dna Sequencing Method, which was conducted in 2021.

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