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Single Nucleotide Polymorphisms in Exon 11 of *LHCGR* Gene in Iraqi Jenoubi Cattle

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Abstract: The bovine luteinizing hormone receptor (*LHCGR*) gene has been widely used as a candidate gene for molecular selection to improve cattle's reproductively traits. In present study, DNA sequencing methods were applied to detect the single nucleotide polymorphisms (SNPs) in the LHCGR gene of Iraqi local cattle (Jenoubi). Blood samples from all of ٨٦ Jenoubi cows were obtained for DNA isolation. The nucleotide and amino acid sequences of exon 11 of the LHCGR gene were registered for Jenoubi cattle in the National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ), and the European Nucleotide Archive (ENA) under the accession numbers of LC516717 and BBU53735, respectively. The results showed the presence of one polymorphic site leading to the construction of two different haplotypes. Haplotype diversity was 0.679, while nucleotide diversity was 0.00271. One SNP was detected in the LHCGR gene at exon 11 (LC516717: c.1401 T > C) that has changed the three-dimensional protein structure result of the changed the amino acid from Valine (GTG) to Alanine (GCG). LHCGR gene in Jenoubi cattle showed polymorphism and potential for molecular selection in the breeding program.

Keywords*: LHCGR* gene, Iraqi local cattle, Single nucleotide polymorphism, Protein 3D

The Jenoubi cattle are included in Bos indicus breed that capable to adapt and live the hot areas well in the south of Iraq (Paulson and Thompson 2015, Alshawi et al 2019). Increased attention has been paid to the genetic improvement of cattle, in particular local breeds, in order to improve production and preserve their genetic structures (Felius et al 2014). The investigation and understanding of genomic regions related to production traits and breed differentiation has been made possible thanks to advances in molecular genetics and software technologies (Öner et al 2018). The gene polymorphisms at specific genome sites have become one of the important signs in the detection of animal characteristics (Faraj et al 2020a). Among the genes that have multiple polymorphisms are LHCGR (Arifin et al 2019). LHCGR (also named LHR), luteinizing hormone/ choriogonadotropin receptor. The LHCGR gene is located on chromosome 11 of cows, it consists of 10 exons and 11 introns and encodes the transmembrane receptor (Arslan et al 2017). The Luteinizing Hormone Receptor (LHR) is a subfamily member of glycoprotein hormone receptors in the G-protein coupled receptor (GPCR) superfamily / seven transmembrane domain receptors (Amitosh 2018). The mature receptor size is 80-90 KDa, out of which the carbohydrate chains contribute some 15 KDa (Dufau 1998). LHCGR is transmembrane receptors necessary for hormonal functioning during reproduction, mainly found in ovaries, testes and uterus (Omer et al 2016). The luteinizing hormone receptor (LHR) plays a critical role in the functioning of the luteinizing hormone because it binds to a specific receptor located in the plasma membrane of the target cells through its ability to increase steroidogenesis. LH hormone is critical for the development of the follicles, ovulation, corpus luteum and embryonic preimplantation (Yu et al 2012). The aim of this study was to determine single nucleotide polymorphisms and analyze protein structure for the LHCGR gene using DNA sequencing methods, and to use bioinformatics tools in Iraqi cattle.

MATERIAL AND METHODS

The study was conducted at University of Basrah, Iraq. The study covered the use of 86 Jenoubi cattle. The blood samples (10ml / cow) from the jugular vein were collected and transported immediately to the laboratory in an ice-containing cool box and stored at −20° C until further analysis. Genomic DNA was extracted from whole blood using DNA Extraction Kit (Genaid, Taiwan). Nanodrop device was used to determine the concentration and purity of DNA. The DNA concentration ranged between 24.8-69.3 ng/ul. The purity OD (optical density) (260/280) was 1.67`-1.95. A fragment (303bp) of *LHCGR* gene in cattle by using the primer F: 5′-CAAACTGACAGTCCCCCGCTTT-3′ and R: 5′-CCTCCGAGCATGACTGGAATGGC-3′ (Marson et al 2008).

The extracted DNA samples were taken as 3µl, then added 1µl primer F, 1µl primer R, 12.5µl PCR mix and 7.5 µl free water. The amplification conditions were: initial denaturation at 94°C for 5 min followed by 37 cycles of denaturation at 94°C for 45 seconds, annealing at 61°C for 45 seconds, and extension at 72°C for 45 seconds, and then the final extension at 72°C for 10 min. The PCR results were extracted using apparatus at 1% agarose gel with the visualized by contact with ultraviolet light. The PCR product was sequenced by Yang ling Tianrun aoka Biotechnology Company. The sequencing results of the *LHCGR* gene were compared with accession number XM_027555221.1 at the NCBI by BioEdit 7.0 software (Hall 1999). Haplotype diversity (HD) and nucleotide diversity (π) were estimated using DnaSP V5. 10 software (Librado and Rozas 2009). The Geneious prime (version 2020.0.4) program was used to detect genotypes. To design the three-dimensional structure of the luteinizing Hormone Receptor, the Phyre2 V. 2.0 and EzMol V.1.22 software on the website were used (Kelley et al 2015, Reynolds et al 2018).

RESULTS AND DISCUSSION

The accession numbers (LC516717 and BBU53735), the nucleotide and amino acid sequences in exon 11 of the LHCGR gene were recorded for Jenoubi cattle in the National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ) and the European Nucleotide Archive (ENA).

Genetic diversity: The results of the genetic diversity of *LHCGR* gene showed that their total number of sequences (N) was 86 and the number of haplotypes (H) were 2 haplotypes resulting in 1 genetic polymorphisms (NH). The values of haplotype and nucleotide diversity were 0.679 and 0.00271, respectively. The analysis of nucleotides and protein in exon 11 recorded one SNP; thiamin (T) to cytosine (C) in position 1400 (Table 1 and Fig. 1, 2). Thus, the amino acids changed to 460Val>Ala.

Three-dimensional protein structure: A three-dimensional protein structure for the LHCGR protein was drawn to locate the change in amino acid. The LHCGR protein carries several important functional regions. On the intracellular domain, one single-nucleotide polymorphism (SNP) was found. The change from Valine to Alanine has led to some changes in the structure of three-dimensional proteins (Fig. 3).

Transmembrane helices: The transmembrane (TM) helices in both XP_027411012.1 and BBU53735 presented in Figure 3. The LHCGR gene has two TM helices (S1-S2). The different positions between XP_027411012.1 and

Table 1. Type of amino acid change in the LHCGR gene in Jenoubi cattle

Location of mutation	Nucleotide change	Amino acid change	Type of mutation
460	GTG>GCG	Val > Ala	nonsynonymous

Val: Valine, Ala: Alanine

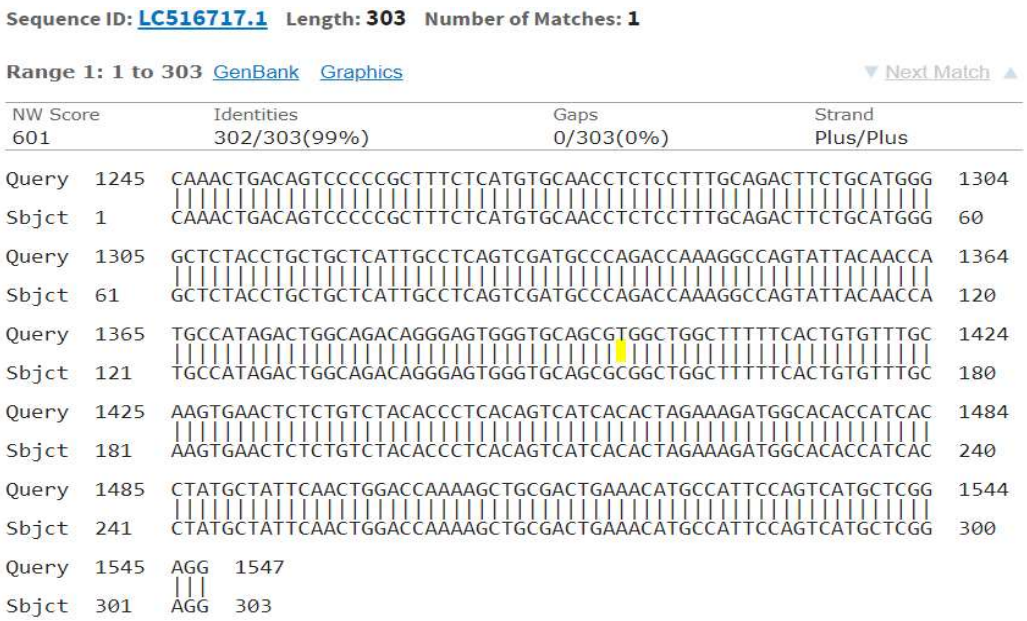

Fig. 1. Sequencing of *LHCGR* gene in Jenoubi cattle in gene bank (LC516717.1) vs. reference sequencing (XM_027555221.1)

BBU53735 were on S1 (9-28 and 9-29) and S2 (49-76 and 49-74) both in the extracellular and cytoplasmic.

Single-nucleotide polymorphisms (SNPs) markers are the most effective molecular markers for assessing genetic diversity, population differentiation, breeding relationships, and determining animal population parentage (Yang et al 2013). Identifying genetic variation and uncovering the associations between these polymorphisms and the different characteristics of production is of great importance for improving that characteristic in animals (Faraj et al 2020b). In this study, identified a Single-Nucleotide Polymorphisms (SNP) and the Tridimensional Protein Structure Prediction in

Sequence ID: BBU53735.1 Length: 101 Number of Matches: 1

Range 1: 1 to 101 GenPept Graphics ▼ Next Match ▲

NW Score	Identities	Positives	Gaps
526	100/101(99%)	100/101(99%)	0/101(0%)

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Query  410  KLTVPRFLMCNLSFADFCMGLYLLLIASVDAQTKGQYYNHAIDWQTGSGCSVAGFFTVFA  469
            KLTVPRFLMCNLSFADFCMGLYLLLIASVDAQTKGQYYNHAIDWQTGSGCS AGFFTVFA
Sbjct  1    KLTVPRFLMCNLSFADFCMGLYLLLIASVDAQTKGQYYNHAIDWQTGSGCSAAGFFTVFA  60

Query  470  SELSVYTLTVITLERWHTITYAIQLDQKLRLKHAIPVMLGG  510
            SELSVYTLTVITLERWHTITYAIQLDQKLRLKHAIPVMLGG
Sbjct  61   SELSVYTLTVITLERWHTITYAIQLDQKLRLKHAIPVMLGG  101
```

Fig. 2. Sequencing of *LHCGR* gene in Jenoubi cattle in gene bank (BBU53735) vs. reference sequencing (XP_027411012.1)

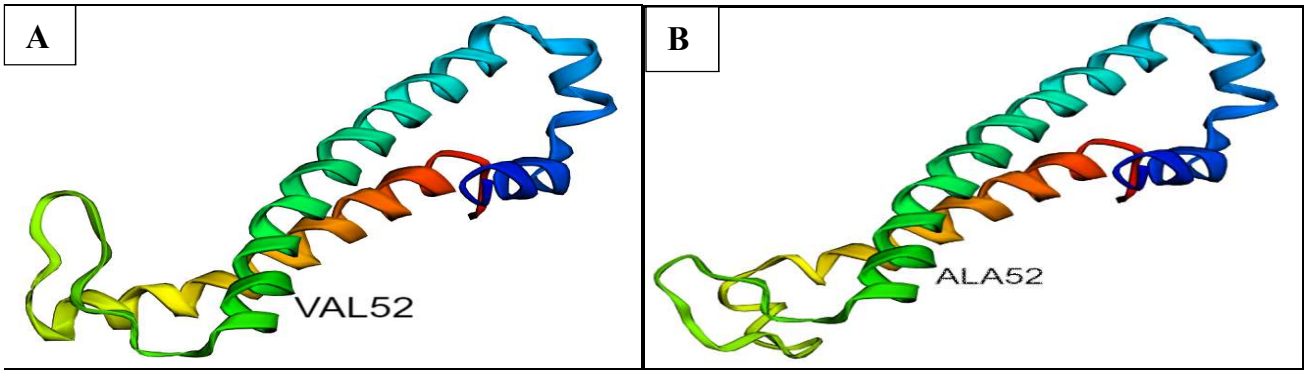

Fig. 3. Changes in the protein structure of LHCGR of Jenoubi cattle. The amino acid residue at 52 was valine acid (Val) for Type A molecule (left figure), and alanine (Ala) for Type B molecule (right figure)

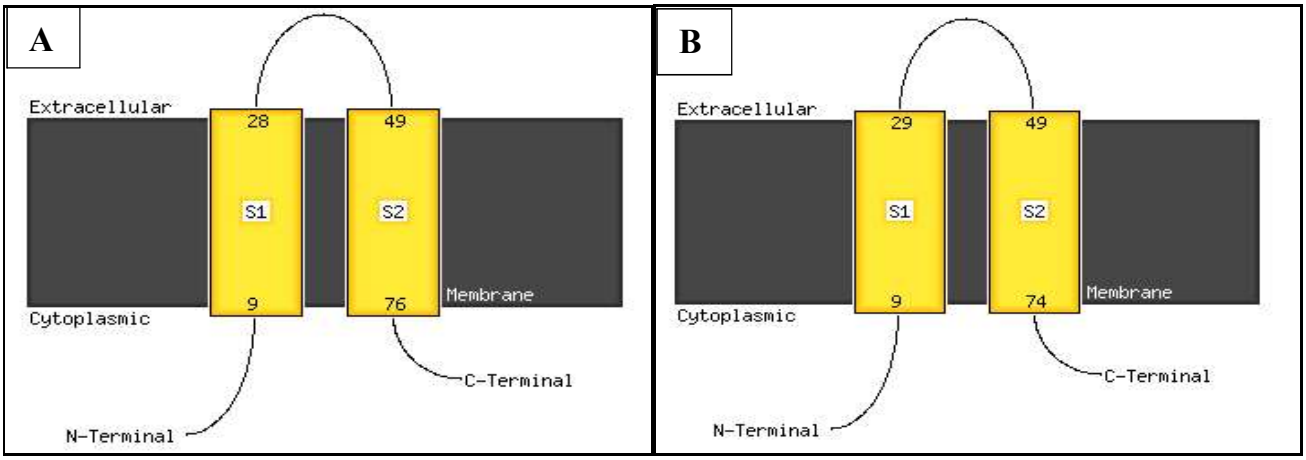

Fig. 4. Transmembrane helices of the LHCGR protein of XP_027411012.1 (A) and BBU53735 (B)

the LHCGR gene of Iraqi local cattle. These nucleotide variations were localized in exon 11. There are no previous studies in cattle in which the functional effect of SNP was evaluated at the level of the predicted three-dimensional protein structure. The LHR gene were previously studied for its association with puberty timing in Angus cattle (Lirón et al 2012) for superovulation traits (Yu et al 2012) and ovarian follicular cysts (Abdi et al 2017). The 1401 T>C substitution results in a change in amino acids (valine to alanine), which may influence the structure and biological function of the LHR protein (et al 2015, Amitosh, 2018), but further verification is needed. In the present study on the three-dimensional structure of LHR, it is likely that the alteration of an amino acid residue from Val to Ala may change the structure of the LHR molecule, which may influence the interaction between LH and the receptor. The alteration of the amino acids occurred in exon 11, important for binding with LH, implying that the amino acid change may alter the binding ability of the molecule with its receptors.

CONCLUSION

The LHCGR gene in Jenoubi Cattle showed a high genetic diversity. The one mutation at site 1401 was nonsynonymous and amino acid change was (V>A). This change has raised some differences in the three-dimensional protein structure. Therefore, the results of this study can be used as a reference to the selection program improving genetic quality in the reproductive traits of Iraqi cattle.

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