



## **Single Nucleotide Polymorphisms in the Promoter of CYP19 Gene in Cattle Bred in Iraq**

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**Abstract:** The present study was undertaken to characterize the genetic diversity of the aromatase cytochrome P450 (*CYP19*) gene in 34 cows (15 local, 14 Holstein, and 5 Crosses) in Iraq. The objectives of the present study are to detect SNPs (mutations) in promoter p1.1 of the *CYP19* gene in cattle bred in Iraq using sequencing techniques. We identified five single-nucleotide polymorphisms (SNP) loci of the *CYP19* gene that were detected, namely G933T, G994C, A1044G, A1062T, and C1468A. The results showed the presence of 3, 4, and 2 polymorphic sites leading to the construction of 4, 5, and 3 different haplotypes for Holstein, local, and crosses respectively. Haplotype diversity were 0.791, 0.752, and 0.700 respectively. While nucleotide diversity was 0.0017, 0.0022, and 0.0013 respectively. Besides, we carried out a phylogenetic analysis of these sequences to address the evolutionary relationship between the animal species. These fragments were assigned in the GenBank database under the accession numbers: LC490756, LC490757, LC491437, LC491438, LC491439, LC491588, and LC491589.

**Keywords:** CYP19 gene, Iraqi cattle, single nucleotide polymorphism, Genetic Diversity, Phylogenetic tree

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### **Introduction**

The tools of molecular genetics, which allow the detection of genes, have major effects on complex traits, such as reproductive performance. These tools can be used as selection criteria for reproductive traits for genetic improvement (Amitosh, 2018). A large number of genes affect the reproductive performance of cattle, one of them is *CYP19*. It is an important gene that is significantly associated with these important traits in cattle (El-Bayomi *et al.*, 2018). *CYP19* gene belongs to the cytochromes P450 genes. P450 genes are part of a multi-gene

superfamily, which contains 27 distinct genetic families, ten of which are dedicated to mammals, one of which is the *CYP19* gene (Jedrzejczak *et al.*, 2011). In mammals, reproduction is mainly regulated by estrogen, which is synthesized in ovaries through the androgen's aromatization (Kowalewska- Luczak, 2010). The *CYP19* gene encodes the cytochrome P450 aromatase enzyme, aromatase P450 catalyzes the last step in the steroidogenesis which converts the androgens (C19) to estrogens (C18) (Damiani & Damiani, 2007), which maps to band q2.6 on

chromosomes 10 and has 11 exons in cattle (Aken *et al.*, 2016). *CYP19* utilizes different promoters in tissue-specific expression in the alternative splicing mechanism (Simpson & Davis, 2001). Different promoter regions correspond to different 5'-UTR transcripts but the coding region is identical for all tissues (Kalbe *et al.*, 2000). The placental expression of the *CYP19* gene is regulated by P1.1 promoter, and A>G mutation has been detected in this region (Keskin *et al.*, 2015). As well as two SNPs in the P1.1 region of the *CYP19* gene was identified, the first mutation was a G>A transition at position 1044 and the second one was an A>G transition at position 1179 (Mohamadnejad-Sangdehi *et al.*, 2015). Several single nucleotide polymorphisms (SNPs) studies in *CYP19* gene have been reported in different livestock species and breeds as; Slovak Simmental cattle (Trakovická *et al.*, 2015), Jersey cattle (Kowalewska-Luczak *et al.*, 2013), Rathi cattle (Amitosh *et al.*, 2017), the crossbred cows in Egypt (Saber *et al.*, 2017), Gyr dairy cows (Vega *et al.*, 2018).

This study aimed at determining single nucleotide polymorphisms in the *CYP19* gene using DNA sequencing methods and using bioinformatics tools to study this fragment in cattle bred in Iraq. As well as determining genetic diversity both within and between breed.

## Materials & Methods

### Animals and genomic DNA isolation

This study included the use of 34 cows (15 local, 14 Holstein, and 5 Crosses). Blood samples (10 ml.cow<sup>-1</sup>) were collected from the jugular vein. Genomic DNA was extracted from whole blood using the gSYNCTM DNA Extraction Kit (Geneaid).

A fragment (657bp) in the P.1.1 promoter region of the *CYP19* gene in cattle was amplified by using our designed primer F: 5'-GGCAAGGGCCTCATATGGTT-3' and R: 5'-TGTCAGGGAATGTGAGGTGC-3'. The PCR amplifications were conducted in a 50 µl volume containing 6 µl genomic DNA, 25 µl of Master Mix, 4 µl both primer, and 15 µl free water. The amplification conditions were as follows: initial denaturation at 94 C for 5 min followed by 35 cycles of denaturation at 94 C for 1 min, annealing at 56°C for 40 Sec, and extension at 72°C for 30 Sec., followed by the final extension at 72°C for 5 min. The PCR product was detected by 2% agarose gel electrophoresis, stained with Ethidium bromide and visualized by ultraviolet light. For sequencing, the PCR product was sent to Yang ling Tianrun Aoka Biotechnology Company, China.

### Data Analysis

The sequencing results of the *CYP19* gene were compared with accession no. Z69241 at the NCBI by BioEdit 7.0 software (Hall, 1999). Haplotype diversity (HD) and nucleotide diversity ( $\pi$ ) were analyzed using DnaSP v5. 10 software (Librado & Rozas, 2009). The haplotypes network was drawn using Network 5.0.0.0 software (Bandelt *et al.*, 1999). The phylogenetic tree was drawn by using the MEGA X software (Kumar *et al.*, 2018).

## Results

The *CYP19* haplotype sequences from cattle bred in Iraq have been assigned in the National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ) and the European Nucleotide Archive (ENA) under the accession

numbers (LC490756, LC490757, LC491437, LC491438, LC491439, LC491588, and LC491589).

**Genetic Diversity**

The total number of sequences (N) and haplotypes (H) were 34 and 7 respectively, resulted in 5 polymorphisms number (NH) distributed to 4 local, 2 crosses and 3

Holstein (Table 1). Holstein revealed the highest value of haplotype diversity (HD) (0.791), followed by the local breed (0.752) and the cross cattle (0.700). On the contrary, local cattle recorded the highest nucleotide diversity ( $\pi$ ) followed by Holstein and cross cattle (0.0022, 0.0017 and 0.0013 respectively).

**Table (1): Genetic diversity of the *CYP19* gene among different cattle breeds.**

Breeds	(N)	(H)	(NH)	(HD)	( $\pi$ )
Local	15	5	4	0.752	0.0022
Crosses	5	3	2	0.700	0.0013
Holstein	14	4	3	0.791	0.0017

N: Number of Sequences; H: Haplotype; NH: Number of polymorphic; HD: Haplotype Diversity;  $\pi$ : Nucleotide Diversity

**Haplotype Network**

A total number of haplotypes of the *CYP19* gene showed by different breeds were seven (Fig. 1). Three haplotypes (H-2, H-3, and H-4) found in all breeds while the remaining four are divided into two for the local breed (H-6 and H-7) and two for Holstein (H-1 and H-5). Each pair of haplotypes (H-2, H-5 or H-3, H-4) differs from each other with a nitrogen base (592bp). The haplotypes (H-2, H-3 or H-4, H-5) differ from each other with a nitrogen base (563bp). The branches represented H-1 of Holstein cattle differed from H-3 by 28 and 89 bases. Whereas, the haplotypes H-6 and H-7 represented the local cattle differed from H-2 by 157 and 139 bases.

The results in fig. (2) observed the analysis of nucleotides in the P.1.1

promoter region of the *CYP19* gene and they recorded five SNPs; guanine to thymine (G933T), guanine to cytosine (G994C), adenine to guanine (A1044G), adenine to thymine (A1062T) and cytosine to adenine (C1468A). All SNPs are recorded first time except (A1044G).

**Phylogenetic tree of *CYP19* gene**

The evolutionary tree of the *CYP19* gene showed that there were three main branches (Fig. 3). The first branch included the cattle bred in Iraq. The second branch included Holstein bred in Germany. While the third branch divided into two branches, the first branch was presented by the Australian cattle and the second Indian cattle.

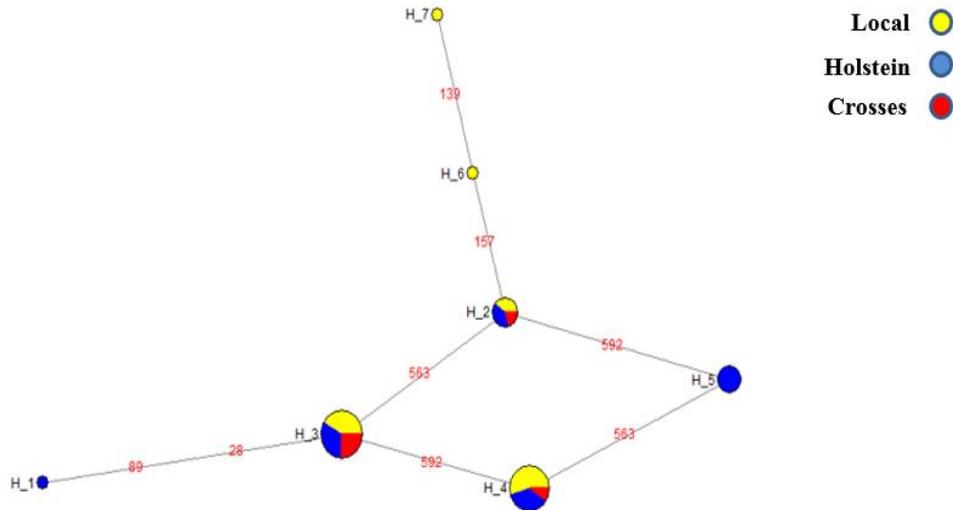
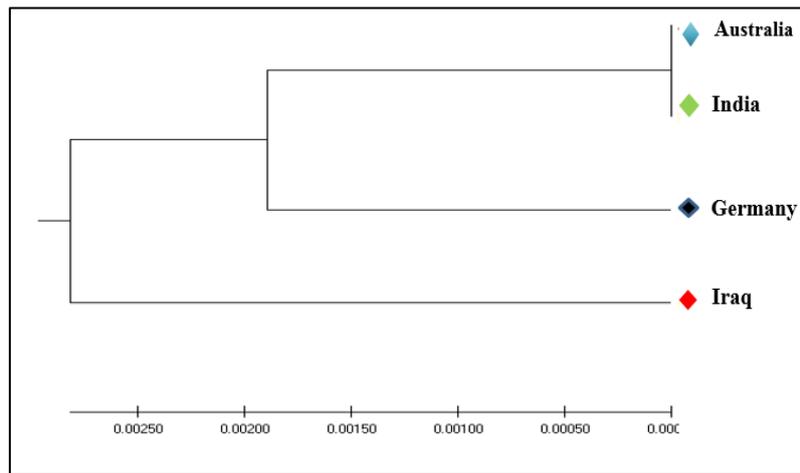


Fig. (1): Haplotype network of *CYP19* gene among studied cattle.

<b>Z69241</b>	905	CTGGCTGGTAATTGTAGATATTAAATTTCTGGAGTGAATACAGTAGTCTGCATGGGCACT	964
<b>Lc491589</b>	2	CTGGCTGGTAATTGTAGATATTAAATTTCTGGAGTGAATACAGTAGTCTGCATGGGCACT	61
<b>Z69241</b>	965	TGCTCTCGATGAGACAGGCTCCCCATGGGAGAAAACCAGAGATCCCATCAGTCTGGATG	1024
<b>Lc491589</b>	62	TGCTCTCGATGAGACAGGCTCCCCATGGGAGAAAACCAGAGATCCCATCAGTCTGGATG	121
<b>Z69241</b>	1025	CCCACTGAGGCTTAGGGCAACTGTGGACAGTGTCAAGTGAAGTGGGTCCCGGACGTGTC	1084
<b>Lc491589</b>	122	CCCACTGAGGCTTAGGGCAGCTGTGGACAGTGTCAAGTGAAGTGGGTCCCGGACGTGTC	181
<b>Z69241</b>	1085	CCTCATTCTCCCTCTAAATATTTCTCACTGGCTCCGCTGGCTGCATAATTTACCACTGTA	1144
<b>Lc491589</b>	182	CCTCATTCTCCCTCTAAATATTTCTCACTGGCTCCGCTGGCTGCATAATTTACCACTGTA	241
<b>Z69241</b>	1145	TCAAGCCTTCCTCATCTGAAAGATGTTGGCGCCCGTGAAAAGTTAAATGACATCAAAGT	1204
<b>Lc491589</b>	242	TCAAGCCTTCCTCATCTGAAAGATGTTGGCGCCCGTGAAAAGTTAAATGACATCAAAGT	301
<b>Z69241</b>	1205	TCAAGGAGATTTCACTTAACAACCTGGAATCACACTCTACTGGTCAACAAAGGTG	1264
<b>Lc491589</b>	302	TCAAGGAGATTTCACTTAACAACCTGGAATCACACTCTACTGGTCAACAAAGGTG	361
<b>Z69241</b>	1265	GTTCTGATCTGGAAAGAGCCTCTGGGACATTGGAAACCTGAAGTAGTAGGTGGATAAAG	1324
<b>Lc491589</b>	362	GTTCTGATCTGGAAAGAGCCTCTGGGACATTGGAAACCTGAAGTAGTAGGTGGATAAAG	421
<b>Z69241</b>	1325	ATCTTAGGAGTTGTAATCCTGCAGGGAGTCCAGAACCCAGCATTGTATTCAAAGTATAT	1384
<b>Lc491589</b>	422	ATCTTAGGAGTTGTAATCCTGCAGGGAGTCCAGAACCCAGCATTGTATTCAAAGTATAT	481
<b>Z69241</b>	1385	TGATTACTGAGTGTCTCCACAGCTTTCATTAGATTCTTAGAATGATGCGTCTGGGGCT	1444
<b>Lc491589</b>	482	TGATTACTGAGTGTCTCCACAGCTTTCATTAGATTCTTAGAATGATGCGTCTGGGGCT	541
<b>Z69241</b>	1445	CTAAAGAGAATGAGAAGAGCCAGCGGAGATGGCCAAACCTGGCCACTTGCAC	1496
<b>Lc491589</b>	542	CTAAAGAGAATGAGAAGAGCCAGCGGAGATGGCCAAACCTGGCCACTTGCAC	593

Fig. (2): Sequencing of the *CYP19* gene in Iraqi Cattle (LC491589) vs. Reference Sequence (Z69241).



**Fig. (3): The Phylogenetic tree of the *CYP19* gene between some cattle bred in Iraq of different countries.**

**Analysis of molecular variance AMOVA**

The results of the AMOVA of the *CYP19* gene between and within cattle breeds showed that genetic variation between breeds was 0.30 % and the variation within

breeds was 99.70 % from the total variance (Table 2). This finding illustrated by the fact that genetic variation within the breed is much greater than genetic variation among breeds.

**Table (2): -Molecular contrast analysis of gene *CYP19* with strains in the world.**

Source of variation	df	Sum Squares	Variance Components	Variation %
Between breed	2	1.650	0.0024	0.30
Within breed	31	24.791	0.7997	99.70
Total	33	26.441	0.8021	

**Discussion**

Molecular markers could be powerful tools in the identification of SNPs and in revealing the current status of genetic diversity within and differentiation between livestock populations (Lenstra *et al.*, 2012). There are a few numbers of studies carried on the *CYP19* gene. The single-nucleotide polymorphisms can be used as a selection criterion for

decreasing fertility problems. The present study was, therefore, aimed at determining both within and between breed genetic diversity of cattle bred in Iraq (local, Holstein, and crosses) using SNPs markers. Single-nucleotide polymorphisms (SNPs) markers are the most efficient molecular markers to evaluate genetic diversity, population differentiation, breed relationships, and

determine parentage in animal populations (Yang *et al.*, 2013). The genetic diversity of cattle has also recently been studied using SNPs markers in Ethiopia (Kukučková *et al.*, 2018) and South Africa (Makina *et al.*, 2016). Single-nucleotide polymorphisms (SNPs) in position 1044 obtained from the analysis of the present study are consistent with the previous studies carried out for promoter p1.1 of the *CYP19* gene in cattle (Keskin *et al.*, 2015; Mohamadnejad-Sangdehi *et al.*, 2015; Zaborski *et al.*, 2014). However, in the present study, other SNPs were found in the position 933, 994, 1062 and 1468. These SNPs are novel to the studied Iraqi cattle. The reason behind that is that Iraq considers as an origin of sheep (Ayied & Zaqeer, 2019), camel (Ayied *et al.*, 2018) and cattle (Faraj *et al.*, 2019; Owaid *et al.*, 2019).

The partitioning of the genetic variation from an AMOVA revealed that 0.30 % of the total genetic variation was between breeds. A similar pattern of variance partitioning was observed in similar studies (Edea *et al.*, 2013; Ngono-Ema *et al.*, 2014; Sanarana *et al.*, 2016; Gororo *et al.*, 2018), in which 90% or more of the variation is contained within breeds. In Ankole cattle breeds, Öner *et al.* (2019) reported within-population diversity to be 92.2 %.

## Conclusion

Cattle bred in Iraq showed a high genetic diversity of the *CYP19* gene. The tree of evolution has shown the presence of cattle bred in Iraq with a separate branch from other cattle breeds. Genetic variation within breed was greater than genetic variation among breeds. *CYP19* gene of cattle bred in Iraq showed five mutations at the sites 933, 994, 1044, 1062 and 1468. Local cattle differ from Holstein cattle by two haplotypes (H1 and H5 for Holstein and H6 and H7 for local).

However, both share three haplotypes (H2, H3, and H4).

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## Conflicts of interest

The authors-declare-that they-have-no-conflict of interests.

## Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

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## تعدد أشكال النيوكليوتيدات الفردية (SNPs) في منظم جين CYP19 في الأبقار المرباة في العراق

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**المستخلص** أجريت الدراسة الحالية لوصف التنوع الجيني لجين اروماتيز السيتوكروم (CYP19) P450 في 34 بقرة (15 محلية و 14 هولشتاين و 5 صلبان) في العراق. أهداف هذه الدراسة هي الكشف عن SNPs (الطفرات) في المنظم p1.1 لجين CYP19 في الأبقار المرباة في العراق باستخدام تقنيات التسلسل. اكتشفت خمسة تشكلات متعددة للتشكلات أحادية النيوكليوتيد (SNP) في جين CYP19 ، وهي G933T و G994C و A1044G و A1062T و C1468A . أوضحت النتائج وجود 3 و 4 و 2 مناطق متعددة الأشكال مما أدى إلى بناء 4 و 5 و 3 أنماط أحادية مختلفة لأبقار الهولشتاين والمحلية والمضربة على التوالي. كان تنوع النمط الاحادي يساوي 0.791 و 0.752 و 0.700 للسلاسل المدروسة على التوالي. بينما كان تنوع النيوكليوتيدات 0.0017 و 0.0022 و 0.0013 على التوالي. إلى جانب ذلك ، اجري تحليل جيني للسلاسل باستخدام هذه التسلسلات لحساب العلاقة التطورية بين الأنواع الحيوانية. تم تسجيل التسلسلات والطفرات التي تم الكشف عنها في قاعدة بيانات GenBank تحت أرقام الانضمام LC490756 و LC490757 و LC491437 و LC491438 و LC491439 و LC491588 و LC491589.

**الكلمات المفتاحية:** جين CYP19 ، الأبقار العراقية، التشكل الوراثي للنيوكليوتيدات المفردة، التنوع الوراثي، شجرة التطور .