*Egypt. J. Vet. Sci.* **Vol. 55,** No. 2, pp. 381-391 (2024)

**Egyptian Journal of Veterinary Sciences**

**https://ejvs.journals.ekb.eg/**

# Novel Nucleotides Polymorphism in CXCR2 Gene Associated with Mastitis In River Buffalo, Egyptian Breed

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**Abstract**

ONE of the major sources of economic loss in dairy herds is Mastitis. The present study aimed at analyzing SNPs in the CXCR2 gene in river buffalo (Egyptian breed) and their association with mastitis. DNA from blood was extracted from thirty buffaloes, 15 healthy and 15 with mastitis and CXCR2 gene (1923bp) was amplified. The nucleotide analyses revealed the presence of 17 single nucleotide polymorphisms (SNPs) (11 mutual and 6 non-mutual) of which 16 SNPs were novel. Three mutual SNPs were associated with mastitis buffalo with genotypes CT and TT at both c.1-g.139(C>T) and c.546(C>T); GG at c.3+g.229 (A>G) were susceptible to mastitis. Buffalo with CC at both c.1-g.139 and c.546; AG and AA at c.3+g.229 were resistant to mastitis. On the other hand, six non-mutual SNPs were associated with mastitis. Healthy buffaloes were TT and CC homozygous at SNP c.1-g.53 (T>A) and c.127 (C>A), respectively. Mastitis buffalo were CC, TT, GG, CC homozygous at SNPs c.1-g.103 (A>C), c.779(C>T), c.784 (A>G), c.790 (A>C), respectively. Overall, the results showed that CXCR2 can be a valuable candidate gene for the prediction of disease resistance in buffalo.

**Keywords:** *Bubalus bubalis*; SNPs; disease; mastitis

## Introduction

Egyptian buffalo belong to the river type (*Bubalus bubalis*). Its population exceeds 3.7 million heads [1]. Buffalo plays an economically important role in Egypt. It is a main source of milk and meat. Recently Egyptian buffalo was identified as a unique breed [2] contrary to a previous thought that it belongs to the Mediterranean River buffalo [3].

Mastitis causes a reduction in milk yield and a change in the milk composition. It is predominantly caused by pathogens that contribute to a significant economic loss of dairy herds. Dysfunctional mammary defence can adversely affect the health and milk production of the animal [4].

The innate immune system eliminates pathogens causing mastitis such as Escherichia coli and *Staphylococcus aureus* [5, 6] by initiating the proper inflammatory response. Improvement of mastitis through selection is based on somatic cell count suggested by Shook [7]. Somatic cell counts (SCC) can be used indirectly to evaluate susceptibility to mastitis and are routinely used in most countries [8]. Several genetic relationships of alternative mastitis SCC with milk yield, and composition of udder-type traits in cattle were investigated [9, 10].

Genetic variations in genes related to mammary innate immune response can influence disease susceptibility. Single nucleotide polymorphism (SNPs) in genes involved with the innate immune response of the mammary gland such as TLR4 [11], CARD15 [12], BoLA-DRB [13, 14], and lactoferrin [15] can be of interest as markers for mastitis.

CXCR2 and CXCR1 present on neutrophil surfaces interact with Interleukin-8 (IL-8) (which is an important ELR CXC chemokine) [16]. They recognize chemokines causing neutrophil activation that end in phagocytosis of the pathogen [17]. Several studies reported a significant association of

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(Received 09/07/2023, accepted 03/10/2023)
DOI: 10.21608/EJVS.2023.219160.1528

CXCR1 SNPs with resistance to mastitis [18, 19, 20, 21] and can influence somatic cell count [22] in cattle. Significant association between CXCR1 c.337A> G and c.365C>T and somatic cell scores (SCS) in Chinese native cattle was reported by Zhou et al. [23].

On the other hand, studies on the association of CXCR2 and mastitis in bovine are limited. CXCR2 has been investigated in Vrindavani cattle by Dige et al., [24, 25, 26, 27]. They reported a lack of association between CXCR2 variations and mastitis. In buffalo, Wani et al. [28] investigated. They detected only a single SNP that was not associated with mastitis. However, in a previous investigation on the Egyptian buffalo breed, we reported an association between c.127 with mastitis [29] that encouraged us to investigate the gene further.

The present study aimed to investigate the association between SNPs in the CXCR2 gene (including the 5'UTR, promoter, 5' and 3' segment of the intron, the full coding region, and the 3'UTR) with mastitis in Egyptian buffalo. Genetic improvement of buffalo is of great importance regarding reproductive performance and disease resistance.

## Experimental

In the present study thirty purebred Egyptian dairy buffalo (15 healthy and 15 with mastitis) maintained at the Livestock Research Centre at Mahalet Mousa, Kafr El Sheikh Governorate, were investigated, based on somatic cell count (SCC) [30,31] and California mastitis test (CMT) [32,33] of their milk samples.

### A) DNA extraction

The farm veterinarian collected blood samples from buffaloes on EDTA. Genomic DNA was extracted according to Miller et al. [34] and was stored at 4ºC or -20ºC. DNA purity was checked by measuring its optical density using NanoDrop1000, Thermo Scientific spectrophotometer. For PCR, DNA concentrations were adjusted to 50 ng/µl.

### B) Polymerase Chain Reaction (PCR), purification and sequencing

Three primer pairs were designed based on Bubalus bubalis XM_006046377.2. Primer1 covers 5'UTR, exon1, and 5' segment of the intron. Primer 2 covers the 3' segment of the intron, 5'part of exon 2, and primer 3 covers 3' of exon 2 (overlapping with the 5' segment) and 3'UTR (Table 1).

PCR reactions were performed by adding 25.5 µl of nuclease-free water, 5 µL of 10X DreamTaq™ DNA polymerase buffer, 5µL (100 µM) dNTPs, 5 µL of each primer (20 µM), 0.5 µl (5U /µl) of DreamTaq™ DNA polymerase (Fermentas, Waltham, USA) and 4µl genomic DNA (50 ng/µl). The reaction mixture was run for 35 cycles in a Q-Cycler, (HVD life science, Wien, Austria). Thermal cycling consisted of initial denaturation at 95ºC for 5 min, followed by 35 cycles of denaturation at 95ºC for 1min, annealing for 2min at temp 66°C (for primers 1 and 2) and 67°C (for primer3) followed by extension at 72ºC for 2 min. The run was terminated by a final extension at 72°C for 10 min.

The amplified products were separated by electrophoresis using 1.5% agarose gel at 100 volts for 1h, stained with ethidium bromide (Applichem, Darmstadt, Germany), and photographed using Gel documentation system -In Genius- (Syngene bioimaging, Cambridge, UK). The PCR amplicons were purified using MEGA quick-spin TM total fragment DNA purification Kit (iNtRON Biotechnology, Gyeonggi-do, South Korea) and were two ways sequenced by Macrogen-company, Seoul, South Korea). Sequence specificity was verified using BLAST (Basic Local Alignment Search Tool) search program available [35]. Polymorphic sites were determined by visual examination of the sequence's charts. For multiple alignments analysis, we used CLUSTAL–Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/).

### C) Protein analysis

The protein sequence was predicted using EMBOSS Sixpack (https://www.ebi.ac.uk/Tools/st/emboss_sixpack/) and the possible SNPs-based amino acids substitutions were evaluated. The protein domains were investigated using SignalP-5.0 software (http://www.cbs.dtu.dk/services/SignalP/) to predict the cleavage sites and signal peptides. SMART analysis (http://smart.embl-heidelberg.de/) was used to detect the protein domains of genes. To illustrate the structure of the CXCR2 chemokine receptor we used the 'TransMembrane Protein Re-Presentation in 2 Dimensions' tool (TMRPres2D) Version 0.9. PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2/) was used to predict the significance of amino acid changes on protein function.

### D) SNPs and association with mastitis

The genotype frequencies were tested for deviation from Hardy-Weinberg equilibrium (HWE) by using the Fisher Exact test. The univariate logistic regression model was performed for SNPs that were significantly associated with the disease. The odds Ratio test (OR) was calculated with a 95% confidence interval. Statistical analysis was

performed using R statistical program and the P-value was corrected using the Bonferroni method [36].

**E) SCC association with mastitis**

The association between Somatic Cell Count (SCC) and CXCR2 genotypes in buffalo was investigated using R software. SCC of buffalo presented as SCC/µl were transformed to somatic cell scores (SCS) to confer linearity to the data and by adjusting the daily yield to the lactation period according to the formula: SCS = log2 [(SCC /100000) +3] [37].

## Results

The CXCR2 gene in healthy and mastitis buffaloes was investigated. The analysed DNA nucleotide sequence (1923bp) covered 305bp of 5' upstream sequence, 83bp exon1, 275bp 5' the intron, 47bp 3' the intron, 1089bp exon2, and 124bp 3' UTR (ACCESSION: MN394470). Nucleotide polymorphic sites (SNPs) in individuals of healthy and in mastitis buffaloes were identified and analysed. Supplement 1 presents the analysed DNA sequence and the identified SNPs positions.

*Association between SNPs and mastitis*

Seventeen SNPs were detected in this study of which 16 SNPs were novel. Six SNPs were non-mutual i.e. were present either in healthy or diseased buffaloes and eleven were mutual (present in both healthy and mastitis buffalo at different levels). Healthy buffaloes with non-mutual SNPs were TT and CC homozygous at SNP c.1-g.53 (T>A) and c.127 (C>A), respectively. Whereas mastitis buffaloes were CC, TT, GG, CC homozygous at SNPs c.1-g.103 (A>C), c.779(C>T), c.784 (A>G), c.790 (A>C), respectively.

The allelic and genotypic frequencies of the eleven mutual SNPs are presented in Tables 2 and 3 respectively. Three SNPs: c.1-g.139 C>T in 5'UTR, c.3+g.229 G>A in 5' of the intron and c.546 C>T in exon2 were statistically significant ($P<0.05$) between healthy and diseased buffalo, after the Hardy – Weinberg equilibrium analysis and Bonferroni test.

SNPs significantly associated with mastitis were further analysed using Odd Ratio and Somatic cell score tests (Table 4). Results showed that buffaloes with genotypes CT and TT at both c.1-g.139 and c.546; GG at c.3+g.229 were susceptible to mastitis, whereas buffaloes with genotypes CC at c.1-g.139 and c.546; AG and AA at c.3+g.229 were resistant to mastitis.

**Protein analysis**

CXCR2 coding region was analysed for the presence of signal peptide using the SignalP-5.0 program. The results showed a low SignalP-5.0 Likelihood 0.0006 indicating the absence of signal peptide indicating that CXCR2 is a non-secretory protein.

Analysis of the coding region using the SMART program revealed the structure of the CXCR2 chemokine receptor which comprises seven transmembrane receptors present in codons 55-77, 89-108, 133-152, 164-186, 221-242, 254-270, and 304-320. The structure of the transmembrane domains comprised of a short acidic N-terminal end, 7 helical transmembrane domains with 3 intracellular and 3 extracellular loops, and an intracellular C-terminus (Fig.1). The N-terminal end binds to chemokine(s) and is important for ligand specificity. C-terminal end usually couples to G-proteins, which is important for receptor signalling following ligand binding.

Four of the six SNPs detected in the coding region were located on transmembrane receptors: sSNP c.546C > T on transmembrane receptor 4, whereas nsSNPs c.779C >T, c.784A >G and c.790A >C were located on transmembrane receptor 6 (Fig. 1). Using PolyPhen-2 program, nsSNP c.784A>Gp.Val262Ile was predicted to be benign. Whereas nsSNPs c.779C >Tp.val260Ala and c.790A >Cp.Leu264Met were predicted to be probably damaging.

## Discussion

CXCR2 and CXCR1 genes have the same actions toward neutrophil activation by recognizing chemokines and are closely located on chromosome 2. Human diseases were linked to polymorphic variations in these two genes which increased the interest in their association with mastitis in farm animals [38, 39].

In cattle, CXCR1 was more investigated than CXCR2. CXCR1 SNPs in cattle were significantly associated with mastitis resistance [18- 21]. Significant association between CXCR1 c.337A> G and c.365C>T and somatic cell scores (SCS) in Chinese native cattle were also reported [23].

The first reported association of SNPs in CXCR2 with mastitis in cattle was by Yougerman et al. [40], however, the annotation of CXCR2 was later corrected to CXCR1 [41]. The association of CXCR2 SNPs and mastitis in cattle was controversial. Three SNPs in CXCR2 were reported to be associated with mastitis in Holstein dairy cows and Simmental dairy cows [42]. However, Dige et al. [24- 27] investigated the nucleotide variations in CXCR2 coding region in cattle (Vrindavani breed), they reported 5 SNPs with no association to mastitis.

In a study on CXCR2 expression in cows, Alhussein et al. [43] reported that CXCR2 expression increased significantly ($P < 0.05$) only in mastitis cows. In a recent insilico study on the evaluation of nsSNPs in exons of several genes of Vechur and crossbred cattle, nsSNPs V122A in CXCR2 present in both Vechur and crossbred cattle was found to be deleterious and affected protein function. Whereas K327R is present in Vechur cows only, affected protein functions. It was reported that missense variants resulting from nsSNPs, change the tertiary structure of the protein, its functions and its stability, which results in deleterious phenotypes [44].

In buffalo, CXCR2 gene investigations were very limited. To the best of our knowledge, two reports published on the association of CXCR2 with mastitis in buffalo. El Nahas et al. [29] reported CXCR2 SNP c.127 to be associated with mastitis in the Egyptian breed. Whereas Wani et al. [28] reported a single SNP at 459bp at the 6th base of CXCR2 exon2 which was not associated with mastitis in the Murrah breed. This SNP was not detected in Egyptian buffalo.

The SNP present in the regulatory region (c.1-g.139) in Egyptian buffalo with mastitis may influence mRNA and protein synthesis since SNPs in the UTR were related to deregulation in gene expression at both transcriptional and post-transcriptional levels [45]. In human SNPs in UTRs have been reported to change the mRNA structure of genes associated with diseases in humans [46].

Synonymous SNPs c.127A>Cp43Arg; c.546C>Tp.182Pro associated with mastitis, reported in this study, may not be silent concerning function [47] since they can affect protein and RNA folding [48,49]. Synonymous SNPs are increasingly being demonstrated to be important in human diseases, evolution, and biotechnology [50, 51, 52]. The two Amino acids substitutions (c.779C>T>p.Val260Ala and c.790A>C>p.Leu264Met) located on transmembrane receptor 6 in Egyptian buffalo were predicted to be probably damaging Mutations in different amino acids on CXCR1 transmembrane 6 were essential for CXCR1 G protein activation and receptor function [53]. It was suggested that separate mutation of receptors causes dramatic increases in agonist-independent receptor activity [54].

## Conclusions

The present results report 16 novel SNPs in the buffalo Egyptian breed. It provides preliminary evidence about the impact of CXCR2 mutations in buffalo mastitis. This investigation reveals the possible use of certain genotypes to select buffaloes resistant to mastitis. To confirm this result large numbers of animals should be investigated.

*Acknowledgement*
The authors thank Ahmed H. El Kassas for helping with the PCR.

*Conflicts of interest*
The authors declare that they have no competing interests.

*Funding Sources*
This study was funded by the National Research Centre, Egypt Project No.11020106 (2016-2019). The NRC administered the project through which the research was conducted. The fund covered the required supplies.

**TABLE 1. CXCR2 primer pairs**

| Primer | 5'-sequence-3' | Annealing Temperature | Target length |
|---|---|---|---|
| **1** | F1: ACAACTGGAATGCAGGGAGA<br>R1: GGGGAAGGAGAGCCTCACTA | 66°C | 701 bp |
| **2** | F2: GGCTAGAATCTGGGGAGGTT<br>R2: GCACGACAGCAAAGATGA | 66°C | 832 bp |
| **3** | F3: GAGGACATGGGTGCCAATAC<br>R3: ATGGCCTCAGCAACTTCC | 67°C | 613bp |

**TABLE 2. Allelic association between CXCR2 mutual SNPs of healthy and mastitis buffalo.**

| SNPs position | Allele frequency (%) | | P value of allelic comparison | Bonferroni correction P |
|---|---|---|---|---|
| **c.1-g.303A>C** | **A** | **C** | 0.4795 | 1.0000 |
| Healthy | 54 | 46 | | |
| Diseased | 48 | 52 | | |
| **c.1-g.299G>T** | **G** | **T** | 0.2408 | 1.0000 |
| Healthy | 81 | 19 | | |
| Diseased | 88 | 12 | | |
| **c.1-g.139C>T** | **C** | **T** | 0.0001 | 0.0008* |
| Healthy | 93 | 7 | | |
| Diseased | 71 | 29 | | |
| **c.1-g.138C>T** | **C** | **T** | 0.4752 | 1.0000 |
| Healthy | 54 | 46 | | |
| Diseased | 60 | 40 | | |
| **c.1-g.100C>A** | **C** | **A** | 0.0138 | 0.1104 |
| Healthy | 67 | 33 | | |
| Diseased | 83 | 17 | | |
| **c.3+g.79T>A** | **T** | **A** | 0.4756 | 1.0000 |
| Healthy | 78 | 22 | | |
| Diseased | 83 | 17 | | |
| **c.3+g.212C>A** | **C** | **A** | 0.0183 | 0.1464 |
| Healthy | 74 | 26 | | |
| Diseased | 88 | 12 | | |
| **c.3+g.229G>A** | **G** | **A** | 0.0001 | 0.0008* |
| Healthy | 54 | 46 | | |
| Diseased | 83 | 17 | | |
| **c.546C>T** | C | T | 0.0032* | 0.0096* |
| Healthy | 94 | 6 | | |
| Diseased | 79 | 21 | | |
| **c.562A>C** | A | C | 0.0279 | 0.0837 |
| Healthy | 88 | 12 | | |
| Diseased | 75 | 25 | | |
| **c.1092+g.62 A>G** | A | G | 0.0893 | 0.2679 |
| Healthy | 55 | 45 | | |
| Diseased | 42 | 58 | | |

**TABLE 3. Genotypic association between CXCR2 mutual SNPs of healthy and mastitis buffalo.**

| SNPs position | Genotype frequency (%) | | | P value of genotype comparison | Bonferroni correction P |
|---|---|---|---|---|---|
| c.1-g.303A>C | AA | AC | CC | 0.91448817 | 1.000 |
| Healthy | 29 | 50 | 21 | | |
| Diseased | 27 | 50 | 23 | | |
| c.1-g.299**G>T** | GG | GT | TT | 0.20452709 | 1.000 |
| Healthy | 66 | 30 | 4 | | |
| Diseased | 77 | 21 | 2 | | |
| **c.1-g.139**C>T | CC | CT | TT | 0.00000007* | 0.00000056* |
| Healthy | 87 | 12 | 1 | | |
| Diseased | 51 | 40 | 9 | | |
| c.1-g.138C>T | CC | CT | TT | 0.47978698 | 1.000 |
| Healthy | 29 | 50 | 21 | | |
| Diseased | 36 | 48 | 16 | | |
| c.1-g.100A>C | CC | AC | AA | 0. 0119793 | 0.09583440 |
| Healthy | 45 | 44 | 11 | | |
| Diseased | 69 | 28 | 3 | | |
| c.3+g.79T>A | TT | TA | AA | 0.45133465 | 1.000 |
| Healthy | 60 | 35 | 5 | | |
| Diseased | 69 | 28 | 3 | | |
| c.3+g.212**C>A** | CC | AC | AA | 0. 0172407 | 0.13792560 |
| Healthy | 54 | 39 | 7 | | |
| Diseased | 77 | 21 | 2 | | |
| **c.3+g.229G>A** | GG | AG | AA | 0.00000001* | 0.00000008* |
| Healthy | 29 | 50 | 21 | | |
| Diseased | 69 | 28 | 3 | | |
| **c.546C>T** | CC | CT | TT | 0.00012* | 0.00036* |
| Healthy | 87 | 12 | 1 | | |
| Diseased | 62 | 33 | 5 | | |
| c.562A>C | AA | AC | CC | 0.0252 | 0.07560 |
| Healthy | 77 | 21 | 2 | | |
| Diseased | 56 | 38 | 6 | | |
| c.1092+g.62A>G | AA | AG | GG | 0.0624 | 0.18720 |
| Healthy | 30 | 50 | 20 | | |
| Diseased | 17 | 49 | 34 | | |

**TABLE 4. Association of buffalo genotypes and mastitis using Odd Ratio test and Somatic cell Score**

| SNPs position | Genotype | P value | OR | SCS mean |
|---|---|---|---|---|
| c.1-g.139C>T | CC | 0.0001* | 0.1555a | 1.957 |
| | CT | 0.0001* | 4.8889[b] | 2.263 |
| | TT | 0.0320* | 9.7912[b] | 2.070 |
| c.3+g.229G>A | GG | 0.0001* | 5.4494[b] | 2.150 |
| | AG | 0.0016* | 0.3889 [a] | 1.903 |
| | AA | 0.0007* | 0.1163 [a] | 1.971 |
| c.546C>T | CC | 0.0001* | 0.2438 [a] | 1.954 |
| | CT | 0.0006* | 3.6119[b] | 2.199 |
| | TT | 0.1352 | 5.2105[b] | 2.036 |

**OR**<1.00= buffalo resistant to mastitis[a]; **OR**>1.00 = buffalo susceptible to mastitis[b]
SCS>2= buffalo susceptible to mastitis, SCS<2=buffalo resistant to mastitis.

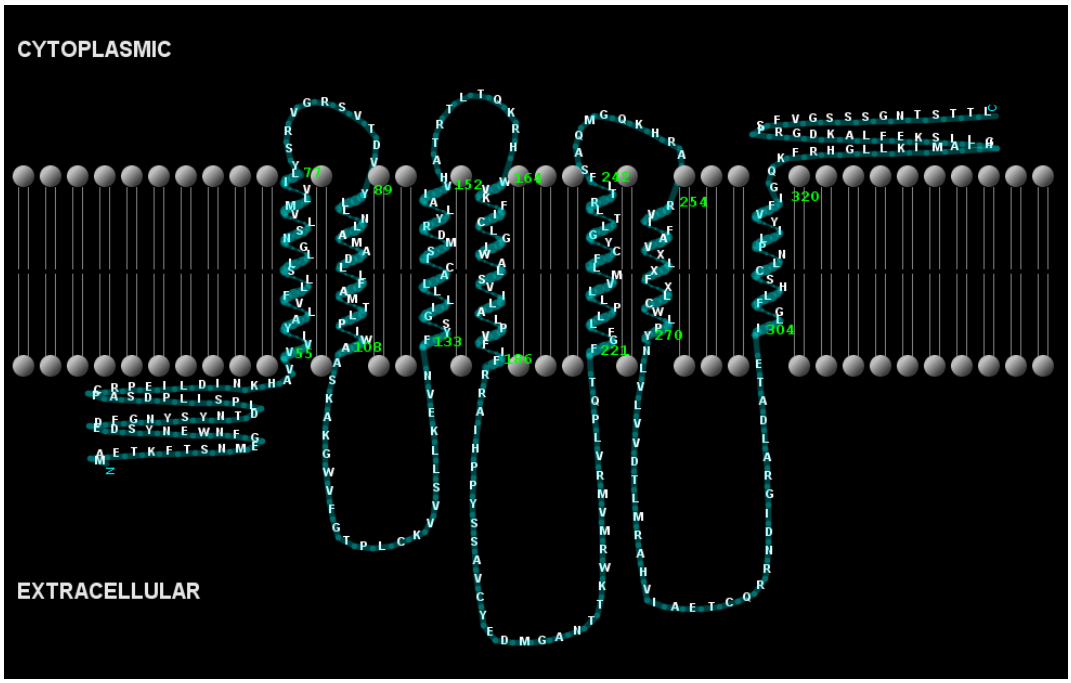


**Fig. 1. Two-dimensional diagram of buffalo of *CXCR2* using TMRPres2D showing seven transmembrane domains (TM1–TM7) connected by three extracellular loops and three intracellular (cytoplasmic) loops. The three nSNPs c.779C>Tp.$^{val}$260$^{Ala}$; c.784A>Gp.$^{Val}$262$^{Ile}$ and c.790A>Cp.$^{Leu}$264$^{Met}$ are located in Transmembrane 6 and represented by x.**

ggaagcagggagaaacaggcagggcagctgaaagagctcggggctgctgagctcaaggcaaagacaagaaatctgtgccatg**M**cgg**K**ccaactacttcctt
tgggtgggcctcagtttcctcatctgggaaatggaaagattggctaggaaaccccttcccaaaatgaactctgccagttcataaaatgacgactaaagagctgacctg
ggaattaattattcaaactatcaaaagcatcccgcac**YY**tcaggggctccaccccagaattgagcaatataat**M**ca**M**cactcacttcctcactgtgTTCTCA
GCACCGCCGCCCTTTCTTCAG**W**CAGACACATCTGCCTTTCCTGGAGGTGTCCCACAGGTGACAAGC
CCAGAATC**ATG***GTAAGTCTTGAAACACCCACCCCTCCCACCTTCCAGACACTTCTCTGTTCTCTGTCCCC*
*CTTGACACCCAGGCTCCAT**W**TGTCCCCTCCATCTATCCCCACACCTCTCAGTCTCTCCATTCTCTCTTTAG*
*ACCACTCTGTCCTCAAAGGGAGAGCCTCTGCTCCTCTTCCATACCCCTCTCTCCAGCAAGCACCTAGGGC*
*AACTCACTGCC**M**ACCCTCTTCTCTTCCC**R**CTCCCAGCCAGCTCTCCCTGCCTTGGCTAGAATTGAGTCTG*
*AACCA*
[gap100bp] Expand Ns
*ATCTGGGGAGGTTTTACTATGCAGTAACCCTGATCTCTCTTCTTTAG***GCTGAAACAAAATTCACTTCAA**
**ATATGGAAGGATTTAACTGGGAAAATTACAGCGATGAAGATTTTGGCAATTACAGTTACAACA**
**CTGACCTGCCCTCTATTCTACCAGACTCTGCCCCATGT**M**GGCCAGAAATTCTGGATATCAATA**
**AGCATGCTGTGGTCGTCATCTATGCCCTGGTCTTCTTGCTAAGCCTCCTGGGAAACTCCCTGG**
**TGATGCTGGTCATCTTATACAGCCGGGTCGGTCGCTCTGTCACTGATGTCTACCTGCTGAACC**
**TGGCCATGGCTGACCTGCTCTTCGCCATGACCTTGCCTATCTGGGCCGCCTCCAAGGCAAAG**
**GGCTGGGTCTTCGGCACACCCCTGTGCAAGGTGGTCTCACTCCTGAAGGAAGTGAACTTCTA**
**CAGCGGTATTCTACTGCTGGCCTGCATCAGCATGGACCGCTACCTGGCCATTGTCCATGCCA**
**CACGCACGCTGACCCAGAAGCGGCACTGGGTCAAGTTCATATGTTTAGGCATCTGGGCCCTG**
**TCCGTGATCCTGGCCCTGCC**Y**GTCTTCATCTTCCGC**M**GGGCCATCCACCCACCCTATTCCAG**
**TGCAGTCTGCTATGAGGACATGGGTGCCAATACAACGAAATGGCGAATGGTGATGAGGGTCC**
**TACCCCAGACCTTTGGCTTCCTCCTGCCCCTGCTGGTCATGCTGTTCTGCTACGGACTCACCC**
**TGCGCACGCTGTTTTCAGCCCAAATGGGGCAGAAGCACCGGGCCATGCGGGTCATCTTTGCT**
**GTCGTGCTCGTCTTCCTGCTCTGCTGGCTGCCCTACAACCTGGTCCTGGTCGTGGACACCCT**
**CATGAGGGCCCATGTGATCGCCGAGACCTGTCAGCGCCGCAACGACATTGGCCGGGCCCTG**
**GATGCCACCGAGATCCTGGGCTTCCTGCACAGCTGCCTCAACCCTCTCATCTACGTCTTCATT**
**GGCCAGAAGTTTCGCCACGGACTCCTCAAGATCATGGCCATCCATGGCCTGATCAGCAAGGA**
**GTTCTTGGCCAAGGATGGCAGGCCTTCCTTTGTTGGCTCTTCTTCAGGGAACACGTCTACTAC**
**CCTC**TGA**gactccacaccgggctccttgcttcccctcagcatcatcccaagccccatgtcctctggcc**R**ttcacagtctcatgtcaaggctgctccct**
**gttgtggttacaggaagttgctggaaggccata**

**Supplement 1**. Nucleotide sequences of full CXCR2 gene of buffalo showing positions of all detected SNPs. The 5' UTR & 3' downstream sequences are in small letters; coding region is in bold and capital letters. The intron is in capital letters & Italic. [gap 100bp] Expand Ns indicates the uncovered segment of the intron. Start (ATG) and stop codon (TGA) and SNPs are bold and underlined R: A>G, W: C>A, Y: C>T, M: A>C, K: T>G.

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# نوكليوتيدات جديدة متعددة الأشكال لجين CXCR2 وإرتباطها بالتهاب الضرع في الجاموس النهرى ، السلالة المصرية.

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التهاب الضرع هو المصدر الرئيسي للخسارة الاقتصادية في قطعان الجاموس الحلابة. هدفت الدراسة إلى تحليل تعدد الأشكال في جين CXCR2 في الجاموس النهرى (السلالة المصرية) وإرتباطها بالتهاب الضرع. تم إستخراج الحمض النووي من ثلاثين جاموسة ، 15 منها سليمة و 15 مصابة بالتهاب الضرع. تم تضخيم جين CXCR2 (1923 bp). كشف تحليل النيوكليوتيدات عن وجود 17 نيوكليوتيدة مفردة متعددة الأشكال (11 متبادلة و 6 غير متبادلة) منها 16 نيوكليوتيدة مفردة متعددة الأشكال جديدة. إرتبطت ثلاثة نيوكليوتيدات متعددة الأشكال متبادلة بإلتهاب الضرع حيث وجد أن الأنماط الجينية CT و TT في كل من c.1-g.139 ( C> T) و c.546(C> T) فى GG ؛ c.3 + g.229 (A> G) عرضة للإصابة بالتهاب الضرع. في حين الأنماط الجينية CC في كل من c.1-g.139 و c.546 ؛ AG و AA فى c.3 + g.229 مقاومة لالتهاب الضرع. إرتبطت ستة أشكال متعددة النيوكليوتيدات غير متبادلة بالتهاب الضرع. كانت الجواميس السليمة من TT و CC متجانسة عند النيوكليوتيدة المفردة المتعددة الأشكال ( c.1-g.53 (T> A و c.127 ( C>A) ، على التوالي. في حين كان الجاموس المصاب بإلتهاب الضرع متماثل اللواقح CC، TT، GG, CC في النيوكليوتيدة المفردة متعددة الأشكال c.1-g.103(A>C)، c.784 (A>G) , c.790(A>C) c.779(C>T) , على التوالي. بشكل عام ، أظهرت النتائج أن CXCR2 يمكن أن يكون جينًا مرشحًا مهماً للتنبؤ بمقاومة المرض في الجاموس المصري.

**الكلمات الدالة:** الجاموس - تعدد أشكال النوكليوتيدات المفردة - التهاب الضرع