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Molecular description of melatonin receptor 1A gene in Iraqi buffalo

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Abstract

The water buffalo has a seasonal reproductive pattern with reduced sexual activity during the longer photoperiod. The goal of this study was to identify the single nucleotide polymorphism (SNP) of melatonin receptor 1A (MTNR1A) gene in Iraqi buffalo cows and 3D structure of its protein and phylogeny with other sequences around the world. The 824 bp fragment of exon II of the MTNR1 A gene was amplified from 190 buffalo cows (4-5 years old) genomic DNA belonging to local breeders in Al-Chibayish Marshes, Southern Iraq. Amplified PCR products underwent custom sequencing at the two ends (5′ and 3′ ends). Five separate polymorphism sites, the 1st included 52 animals with 19 mutations (12 missense), the 2nd included 39 animals with 18 mutations (11 missense), the 3rd included 35 animals with 18 mutations (12 missense), the 4th included 32 animals with 18 mutations (12 missense) and the 5th included 32 animals with 14 mutations (8 missense). These polymorphic sites with accession numbers LC565046, LC565047, LC565709, LC565710 and LC565711 respectively were registered in gene bank. The phylogenetic tree reveals that in some of the Iraqi buffalo, the sequences of MTNR1A gene has identical to the Italian buffalo (GU817415), and the Brazilian buffalo (JN689386). Data revealed a marked difference in the fifth polymorphism sites' 3D protein structure because of the mutations. In conclusion, as a result of mutations, the gene MTNR1A in Iraqi buffalo has polymorphisms; these polymorphisms may be linked to gene function, Therefore, further studies are needed to connect the polymorphisms of this gene with the productive and reproductive traits.

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Introduction

For many countries, particularly in Asia, buffalo is an essential part of the agricultural economy, as it is a very important source of milk and meat (1,2). Iraq is one of the largest buffalo countries in the region (3), as well as one of the world's top countries in the world (4). However, Iraqi buffalo production is rather low (especially milk production) compared to the rest of the producing countries for a number of reasons, including malnutrition or poor management (5-7), as well as the problems resulting from poor veterinary care (8), and the resulting diseases such as mastitis, which directly affects milk production (9), products, whether meat or milk, are also exposed to various bacterial contaminants (10), perhaps one of the most important problems faced by the buffalo breeding sector is the poor reproductive efficiency and low pregnancy rates, which cause great economic losses (11). Recently, evolution in molecular genetics has led to the discovery of molecular markers that are linked to genes that influence development characteristics, for which dominance in development is closely related to genetic selection (12), One of these markers is the gene responsible for the receptors for the hormone melatonin (13). Melatonin plays a significant role in buffalo reproduction (14), since it is capable of reducing oxidative stress (15), by stimulating antioxidant enzymes

(16). Therefrom, it affects the ideal functions of cells and tissues, including the reproductive system, which explains what has hypothalamic neuron receptors that regulate the release of pituitary gonads (17), in the anterior pituitary gonads, and in the adnexal reproductive gonads in both male and female (18,19). Melatonin receptors are categorized into two groups, MTNR1A and MTNR1B (20,21), the first type being one that specifically affects reproduction (22), while previous studies indicated that the melatonin receptor gene polymorphisms are related to productivity and reproductive success in buffalo and sheep (23). Studies on the exon II and its polymorphisms have based mainly on the melatonin receptor gene in chromosome 1 buffalo (24), as it is promoter area size 824 bp (25,26). Due to the lack of knowledge on the melatonin receptor gene in the Iraqi buffalo, as no previous research has listed this gene, this study aimed to characterize the melatonin receptor gene (MTNR1A) polymorphisms in the Iraqi buffalo, the 3D structure of its protein and comparing the phylogenic tree of Iraqi buffalo with other sequences breeds around the world.

Materials and methods

Ethical approve

The study has been approved by the Animal Ethics committee of Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq.

Study location

This study was carried out in the Department of Animal Production, College of Agriculture, University of Basrah, from July 2019 to November 2019 on 190 buffalo cows (4-5 years old) belonging to local breeders in Al-Chibayish Marshes, southern Iraq (°31N47°E).

Sampling

Ten ml of blood was obtained from each animal's caudal vein by using a tube with EDTA, then the samples were kept frozen until the DNA extraction process was performed.

The DNA Extraction

The DNA was extracted from blood by using a commercial genomic DNA kit (Pure-Link Genomic DNA mini-Package, Thermo Fisher Scientific, USA) as recommended by the manufacturer and stored at -20°C until for use (24), then the concentration and purity of DNA was calculated by using Nanodrop (Nano Drop thermo scientific 200/ USA), with 260 / 280 nm ratio (27).

Primer design and PCR amplification

Primer pairs were designed according to Gunwant *et al.* (24) for MTNR1A exon II, forward: GTGAGCCTGGCAGTTGCAGA and Reverse: GGAGAGGGTTTGCGTTTAATTC. The PCR was carried out according to Pandey *et al.* (26), in total volume 25 µl with 12.5 µl of Master mix, 2 µl of DNA template (100 ng/ml), 0.5 µl (10 µM) of each primer and 9.5 µl of water nuclease-free (WNF), the conditions of PCR were done with an initial denaturation at 98 °C for 5 min followed by 1 cycle, then next by 35 cycles of denaturation at 98 °C for 15 sec, Annealing at 55 °C for 30 sec, extension at 72 °C for 30 sec, and final extension at 72°C for 5 min followed by 1 cycle, the PCR product has been detected by using 1.5% Ethidium Bromide 0.5 µg/ml stained agarose gel, equivalent, with 3000 bp DNA ladder. Then PCR product has been purified using QIAquick®, Qiagen kit (Germany) following the manufacturer's protocol.

Sequences analysis

The sequence analysis has been done in first BASE Laboratory in Malaysia, to determine the identity of the resulting sequences BLAST tool has been used, then perform Multiple Sequence Alignment (28), to compare the resulting sequences with *Bubalus bubalis* melatonin receptor type 1A (MTNR1A) gene in the GenBank accession number GU817415 and JN689386 (Depending on the highest matching percentage in BLAST) to set the differences in the resulting polymorphisms.

Construction of phylogenetic tree

The construction of phylogenetic tree has been done by using Mega software (29), to show the convergence the resulting sequences in this study were compared with the sequences of melatonin receptor in buffalo for several different countries, obtained from the GenBank, it is as follows: GU817415 in Italy, JN689386 in Brazil and MF173047 in India.

Three-dimensional protein structure

The Swiss model (Swiss-PdbViewer/Switzerland) has been used to detected 3D protein structure (30).

Results

Amplifying PCR

The findings of NanoDrop's DNA analysis show that the ratio 260/280 was 1.75 - 1.85, The size of the PCR product in *Bubalus bubalis* was 824bp (Figure 1).

The Analysis of Sequences

Five separate polymorphisms were obtained by comparing the findings with the *Bubalus bubalis* accession numbers (GU817415 Italian polymorphism and JN689386 Brazilian polymorphism), all of which were registered at GenBank under the accession numbers LC565046, LC565047, LC565709, LC565710, and LC565711. The percent identification with the *Bubalus bubalis* reference gene (Italian polymorphism) was 97.69 percent, 97.82

percent, 97.82 percent, 97.82 percent, and 98.30 percent respectively, as for the Brazilian polymorphism, the matching ratio was 96.51, 96.87, 95.90, 96.02 and 96.39 respectively, 100 percent mismatch occurred due to various mutations occurring in each polymorphism. On the other hand, some mutations occurred in more than one polymorphism, not to mention that some of them corresponded to the Italian polymorphism, the other with the Brazilian polymorphism, and some matched with both (Figure 2), all mutations, their types and positions are listed in the tables 1-5, some mutations were silent (they did not code for a new amino acid), while others were missense (caused by the encoding of new amino acid).

Phylogenetic tree analysis

The phylogenetic tree diagram (Figure 3) shows that the sequences of the MTNR1A gene in some of the Iraqi buffalo are similar to the Italian buffalo (accession number GU817415), while the other sequences were similar to the Brazilian buffalo (accession number JN689386), the homogeneity rate was very high despite these variations resulting from mutations.

3D protein structure

Despite the similarity of the 3D protein structure for all the polymorphisms that resulted in the analysis, there are differences in the expected 3D structure between them (Figure 4 - 8), as a result of the various mutations, this can be clearly seen in Figure 2, as there is no perfect match between all the polymorphisms.

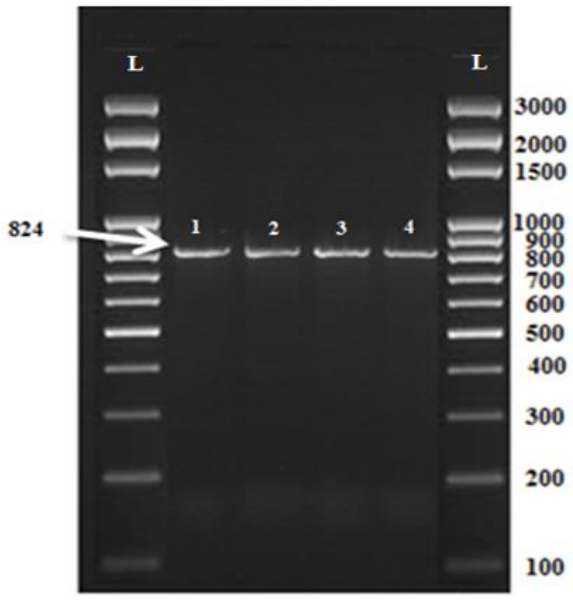

Figure 1: Gel electrophoresis of PCR product for MTNR1A gene (L: 3kb, DNA marker; 1, 2, 3 and 4: PCR product).

Table 1: Polymorphisms of the studied gene (Polymorphism 1, LC565046, 52 animals)

No.	GU817415	JN689386	Polymorphisms	Position	Mutation	
					Type	Amino acid
1	C	T	T	51	Silent	
2	A	G	G	76	Missense	Serine to Glycine
3	C	G	A	113	Missense	Proline to Glutamine
4	C	C	G	128	Missense	Proline to Arginine
5	T	T	G	156	Silent	
6	T	A	A	194	Missense	Glutamine to leucine
7	A	G	G	240	Silent	
8	G	A	A	286	Missense	Aspartic to Asparagine
9	C	C	G	349	Missense	Histidine to Aspartic
10	A	A	T	472	Missense	Serine to Cysteine
11	G	C	A	516	Silent	
12	G	G	C	564	Silent	
13	C	C	T	620	Missense	Threonine to Isoleucine
14	C	C	G	682	Missense	Glutamine to Glutamic
15	A	A	G	714	Silent	
16	T	T	A	765	Missense	Aspartic to Glutamic
17	G	A	A	775	Missense	Glycine to Arginine
18	A	A	T	803	Missense	Asparagine to Isoleucine
19	C	T	T	822	Silent	

Table 2: Polymorphisms of the studied gene (Polymorphism 2, LC565047, 39 animals)

No.	GU817415	JN689386	Polymorphisms	Position	Mutation	
					Type	Amino acid
1	T	G	C	9	Silent	
2	G	G	T	38	Missense	Glycine to Valine
3	G	G	C	87	Missense	Tryptophan to Cysteine
4	G	C	T	135	Silent	
5	C	T	T	203	Missense	Proline to Leucine
6	A	G	G	240	Silent	
7	T	C	C	302	Missense	Valine to Alanine
8	T	T	A	365	Missense	Leucine to Histidine
9	C	G	G	442	Missense	Leucine to Valine
10	C	A	T	491	Missense	Threonine to Methionine
11	T	C	C	540	Silent	
12	C	C	T	597	Silent	
13	C	G	G	598	Missense	Leucine to Valine
14	C	C	G	682	Missense	Glutamine to Glutamic
15	T	A	C	693	Silent	
16	T	T	C	744	Silent	
17	T	T	A	765	Missense	Aspartic to Glutamic
18	G	A	A	775	Missense	Glycine to Arginine

Figure 2: The Multiple Sequence Alignment for each of the Italian and Brazilian polymorphisms, as well as the polymorphisms obtained in the study. 1, 2, 3, 4, and 5: Iraqi polymorphisms, LC565711, LC565710, LC565709, LC565047 and LC565046 respectively. 6: Italian polymorphism, GU817415. 7: Brazilian polymorphism, JN689386.

Table 3: Polymorphisms of the studied gene (Polymorphism 3, LC565709, 35 animals)

No.	GU817415	JN689386	Polymorphisms	Position	Mutation	
					Type	Amino acid
1	G	T	C	91	Missense	Glutamic to Glutamine
2	T	T	G	105	Silent	
3	C	A	A	153	Silent	
4	A	A	G	173	Missense	Asparagine to Serine
5	C	T	T	224	Missense	Alanine to Valine
6	C	C	A	285	Silent	
7	A	T	T	329	Missense	Glutamic to Valine
8	T	T	A	393	Silent	
9	T	T	C	454	Missense	Serine to Proline
10	C	C	G	487	Missense	Glutamine to Glutamic
11	G	T	C	527	Missense	Cysteine to Serine
12	C	C	A	663	Silent	
13	C	C	G	682	Missense	Glutamine to Glutamic
14	G	C	C	706	Missense	Glutamic to Glutamine
15	G	G	C	751	Missense	Valine to Glutamine
16	T	T	A	752	Missense	Valine to Glutamine
17	T	T	A	765	Missense	Aspartic to Glutamic
18	A	A	G	789	Silent	

Table 4: Polymorphisms of the studied gene (Polymorphism 4, LC565710, 32 animals)

No.	GU817415	JN689386	Polymorphisms	Position	Mutation	
					Type	Amino acid
1	G	T	T	15	Missense	Glutamic to Aspartic
2	A	G	T	30	Missense	Arginine to Serine
3	C	C	A	42	Silent	
4	G	A	A	70	Missense	Valine to Isoleucine
5	T	T	C	129	Silent	
6	G	G	A	176	Missense	Cysteine to Tyrosine
7	C	G	G	244	Missense	Proline to Alanine
8	C	C	T	312	Silent	
9	G	G	A	363	Silent	
10	G	A	A	416	Missense	Arginine to Histidine
11	A	A	T	486	Missense	Glutamine to Histidine
12	T	T	C	542	Missense	Phenylalanine to Serine
13	T	C	C	594	Silent	
14	G	G	C	636	Silent	
15	C	C	G	682	Missense	Glutamine to Glutamic
16	T	T	A	765	Missense	Aspartic to Glutamic
17	A	A	C	802	Missense	Asparagine to Histidine
18	C	C	A	817	Missense	Leucine to Isoleucine

Table 5: Polymorphisms of the studied gene (Polymorphism 5, LC565711, 32 animals)

No.	GU817415	JN689386	Polymorphisms	Position	Mutation	
					Type	Amino acid
1	T	T	C	3	Silent	
2	G	T	T	15	Missense	Glutamic to Aspartic
3	T	T	C	53	Missense	Valine to Alanine
4	G	T	T	86	Missense	Tryptophan to Leucine
5	G	G	C	141	Missense	Glutamic to Aspartic
6	G	C	C	186	Silent	
7	G	T	T	254	Missense	Arginine to Leucine
8	G	G	A	318	Silent	
9	A	T	G	396	Silent	
10	T	T	A	489	Silent	
11	A	A	C	654	Missense	Leucine to Phenylalanine
12	C	C	G	682	Missense	Glutamine to Glutamic
13	T	T	A	765	Missense	Aspartic to Glutamic
14	C	T	A	822	Silent	

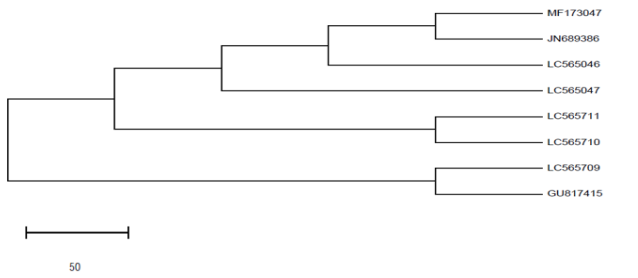

Figure 3: Phylogenetic tree of MTNR1A gene in Iraqi buffalo with different buffalo (LC565046, LC565047, LC565709, LC565710, and LC565711: MTNR1A gene in Iraqi buffalo), (GU817415: MTNR1A gene in Italy buffalo). (JN689386: MTNR1A gene in Brazilian buffalo), (MF173047: MTNR1A gene in Indian buffalo).

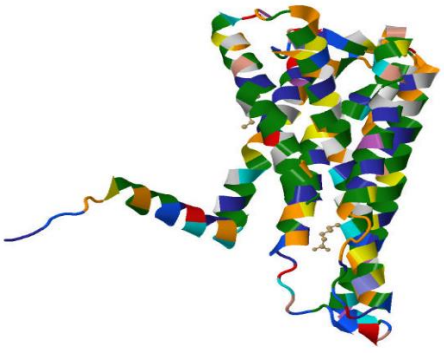

Figure 4: 3D protein structure of MTNR1A gene in Iraqi buffalo, LC565046 polymorphism.

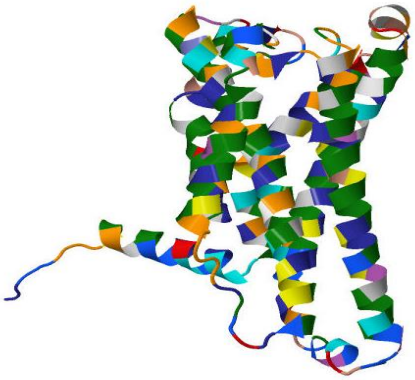

Figure 5: 3D protein structure of MTNR1A gene in Iraqi buffalo, LC565047 polymorphism.

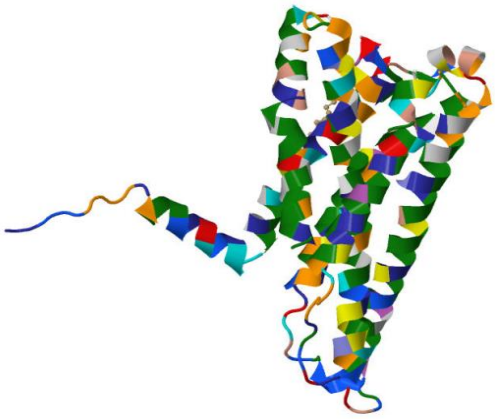

Figure 6: 3D protein structure of MTNR1A gene in Iraqi buffalo, LC565709 polymorphism.

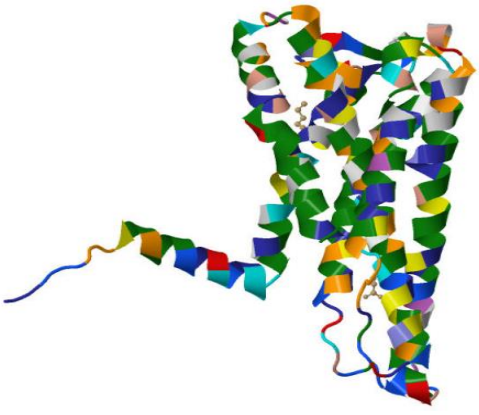

Figure 7: 3D protein structure of MTNR1A gene in Iraqi buffalo, LC565710 polymorphism.

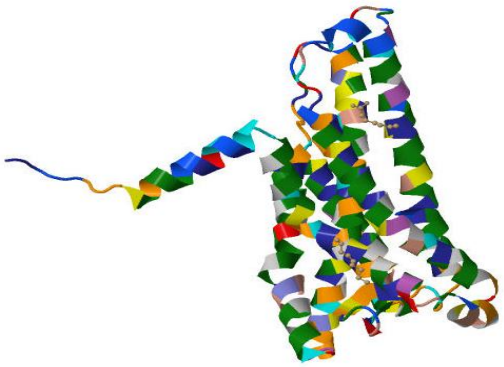

Figure 8: 3D protein structure of MTNR1A gene in Iraqi buffalo, LC565711 polymorphism.

Discussion

The result of the size of PCR product was entirely equivalent to the main part of the exon II, this is consistent with what Miziara (19) pointed out. The resulting genetic polymorphisms in the study are consistent with other polymorphism studies of the melatonin receptor (MTNR1A) gene (31), and genetic polymorphism of Iraqi buffalo genes (32).

The occurrence of various silent mutations can contribute to a change in protein structure and function, as well as their effect on protein folding, missense mutations can cause instability in protein function and the effect on interaction with other biological molecules (33). The occurrence of a number of mutations in more than one polymorphism may be due to the fact that these animals are of the same origin (34), but it is important to note that both the Italian and Brazilian polymorphisms share the same mutations with the polymorphisms obtained in the current study.

The variations in the gene may account for the different development and reproductive success between animals. MTNR1A gene's polymorphisms can influence the protein through the impact on the amount of melatonin that affects the amount of prolactin (35). So, the MTNR1A gene's polymorphisms can be considered as a reproductive genetic marker (36,37), and therefor this genetic variation can effectively contribute to the reproductive performance of the Iraqi buffalo (38).

The analysis of the phylogenetic tree agrees with the results of the study, where we can note that the Iraqi buffalo was closer to the Italian polymorphism, despite the close convergence with the Brazilian and Indian polymorphisms, this may give another impression about the genetic diversity of the Iraqi buffalo (39).

On the other hand, the difference in the 3D structure of protein is due to the occurrence of silent and missense mutations, the 3D structure of the protein is an effective

indicator for understanding the effect of mutations, this is due to its impact on the processes of fastening and dismantling as well as an assembly (40), so these structural changes may affect the function of the protein (41), either negatively or positively, which can contribute to a clear discrepancy in the productive performance of animals.

Conclusion

Based on the results of the present study MTNR1A gene in Iraqi buffalo has polymorphisms as a result of mutations, these polymorphisms may be related to gene function, therefore further studies are required to link this gene's polymorphisms to the productive and reproductive traits.

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Conflict of interest

The authors of this manuscript stated there is no conflict of interest regarding the writing process or data analysis.

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الوصف الجزيئي لجين مستقبل الميلاتونين أ١ في الجاموس العراقي

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الخلاصة

يمتلك جاموس الماء نمطًا تناسليًا موسميًا مع انخفاض النشاط الجنسي خلال فترة الضوء الأطول. كان الهدف من هذه الدراسة هو التعرف على تعدد أشكال النوكليوتيدات الفردي في جين مستقبلات الميلاتونين أ١ (MTNR1A) في أبقار الجاموس العراقي والتركيب ثلاثي الأبعاد لبروتينه وشجرة النشوء مع تتابعات أخرى حول العالم. تم تضخيم ٨٢٤ زوج قاعدي من الاكسون الثاني للحمض النووي لجين MTNR1 A لـ ١٩٠ بقرة جاموس (٤-٥ سنوات) التابع لمربي محليين في أهوار الجبايش، جنوب العراق. خضعت منتجات PCR المضخمة لتسلسل مخصص عند الطرفين (٥′ و ٣ نهايات). تم الحصول على خمسة مواقع منفصلة لتعدد الأشكال، الأول شمل ٥٢ حيوانا مع ١٩ طفرة (١٢ محسوسة)، والثاني شمل ٣٩ حيوانًا مع ١٨ طفرة (١١ محسوسة)، والثالث شمل ٣٥ حيوانًا مع ١٨ طفرة (١٢ محسوسة)، والرابع شمل ٣٢ حيوانا مع ١٨ طفرة (١٢ محسوسة) والخامسة تضم ٣٢ حيوانًا مع ١٤ طفرة (٨ محسوسة). تم تسجيل هذه المواقع متعددة الأشكال بأرقام الانضمام LC565046 و LC565047 و LC565709 و LC565710 و LC565711 على التوالي في بنك الجينات. تكشف شجرة النشوء والتطور أن تسلسلات جين MTNR1A في بعض الجاموس العراقي متطابق مع الجاموس الإيطالي (رقم الانضمام GU817415) والجاموس البرازيلي (رقم الانضمام JN689386). كشفت البيانات عن اختلاف ملحوظ في بنية البروتين ثلاثية الأبعاد لمواقع تعدد الأشكال الخمسة بسبب الطفرات. في الختام، نتيجة للطفرات، لذلك فإن جين MTNR1A في الجاموس العراقي له أشكال متعددة، ويمكن ربط هذه الأشكال المتعددة بوظيفة الجين، لذلك هناك حاجة إلى مزيد من الدراسات لربط تعدد الأشكال لهذا الجين بالسمات الإنتاجية والتناسلية.