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Association of FSHβ Gene (-211 G>T) Polymorphism with Female Infertility in Iraqi Women

Raghad Jabbar Abdul-Sahib[1] Salah Hassan Faraj[2]

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Authors affiliation
.1Directorate of Education Maysan, Ministry of Education, Iraq
2.Department of Biology, College of Science, University of Misan, Iraq
email@ raghad.jabbar@ec.edu.iq

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FSHβ gene; female infertility; single nucleotide polymorphism; genetic variation.

ABSTRACT

Background: Female infertility is a multifactorial disorder influenced by genetic and environmental factors, particularly variations in genes involved in the hypothalamic–pituitary–ovarian axis. The follicle-stimulating hormone beta (*FSHβ*) gene plays an essential role in follicular development and reproductive function. Methods: A case–control study was conducted involving 60 infertile women and 30 healthy controls from Maysan Governorate, Iraq. Genomic DNA was extracted from blood samples, and the -211 G>T polymorphism in the promoter region of the *FSHβ* gene was detected using polymerase chain reaction (PCR) and DNA sequencing. Results: The -211 G>T single nucleotide polymorphism was identified in the promoter region of the *FSHβ* gene. The genotype frequencies (GG, GT, and TT) among infertile women were 87.5%, 6.25%, and 6.25%, respectively. The T allele frequency was higher among infertile women compared with controls. A significant association was observed between the TT genotype and infertility risk ($P < 0.05$). Conclusion: The findings suggest that the -211 G>T polymorphism in the *FSHβ* gene may contribute to susceptibility to female infertility among Iraqi women. However, further studies with larger sample sizes are required to confirm this association and clarify the functional role of this variant

ارتباط تعدد أشكال جين *FSHB* (211 G>T–) بالعقم عند النساء العراقيات

رغد جبار عبدالصاحب[1] وصلاح حسن فرج[2]

يُعد العقم عند الإناث اضطرابًا متعدد العوامل يتأثر بالعوامل الوراثية والبيئية، ولا سيما الاختلافات الجينية في الجينات المشاركة في محور تحت المهاد–الغدة النخامية–المبيض. يلعب جين الوحدة بيتا للهرمون المنبه للجريبات (*FSHβ*) دورا أساسيا في تطور الجريبات ووظيفة الجهاز التناسلي. تهدف هذه الدراسة إلى تقييم العلاقة بين تعدد الأشكال الجيني G>T 211– في المنطقة المحفزة (Promoter region) لجين *FSHβ* وقابلية الإصابة بالعقم لدى النساء العراقيات. الطرائق: أُجريت دراسة حالة–سيطرة شملت 60 امرأة مصابة بالعقم و30 امرأة سليمة كمجموعة سيطرة من محافظة ميسان، العراق. تم استخلاص الحمض النووي (DNA) الجينومي من عينات الدم، وتم الكشف عن تعدد الأشكال الجيني G>T 211– في المنطقة المحفزة لجين *FSHβ* باستخدام تقنية تفاعل البوليميراز المتسلسل (PCR) متبوعا بتسلسل الحمض النووي DNA). (sequencingالنتائج: تم تحديد تعدد الأشكال الجيني G>T 211– في المنطقة المحفزة لجين *FSHβ*. أظهرت تكرارات الأنماط الجينية GG) و GT و(TT اختلافًا بين النساء المصابات بالعقم ومجموعة السيطرة. كما لوحظ ارتفاع تكرار الأليل T لدى النساء المصابات بالعقم مقارنةً بمجموعة السيطرة، وارتبط النمط الجيني TT بزيادة قابلية الإصابة بالعقم و كان هذا الارتباط ذا دلالة إحصائية ($P < 0.05$). الاستنتاج: تشير نتائج الدراسة إلى أن تعدد الأشكال الجيني G>T 211– في جين *FSHβ* قد يسهم في زيادة القابلية للإصابة بالعقم لدى النساء العراقيات. ومع ذلك، هناك حاجة إلى دراسات مستقبلية تشمل أعدادًا أكبر من العينات وتحليلات وظيفية إضافية لتأكيد هذا الارتباط وتوضيح الدور الوظيفي لهذا المتغير الجيني.

INTRODUCTION

Infertility is a global reproductive health problem that affects couples worldwide. Its prevalence, causes, and clinical significance may vary according to geographical location, socioeconomic status, and lifestyle factors. Understanding the risk factors associated with infertility is essential for improving prevention strategies and reproductive outcomes (Deyhoul *et al*., 2017). Infertility is generally defined as the failure to achieve pregnancy after 12 months of regular unprotected intercourse, provided that no obvious reproductive abnormalities are present in either partner (Garolla *et al*., 2021). The human genome contains numerous genetic variations, including single nucleotide polymorphisms (SNPs), which may influence gene expression, protein function, and susceptibility to various diseases (Pei *et al*., 2020).

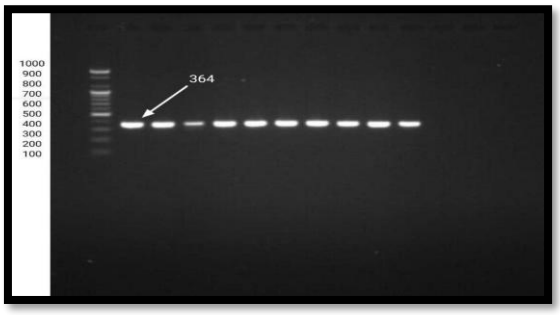
Genetic variations in genes involved in the hypothalamic–pituitary–ovarian (HPO) axis have received considerable attention due to their potential roles in female reproductive disorders. Among these genes, the follicle-stimulating hormone beta (*FSHβ*) gene is considered a key regulator of reproductive function because it encodes the beta subunit of follicle-stimulating hormone (FSH) (Trevisan *et al*., 2019).The *FSHβ* gene encodes the beta subunit of follicle-stimulating hormone, which is essential for the biological activity and receptor interaction of FSH. The FSH beta subunit consists of 111 amino acids and contributes to the regulation of follicular growth and ovarian function (Bianco *et al*., 2021; Bhartiya & Patel, 2021). The *FSHβ* gene is located on chromosome 11p14.1 and contains three exons separated by two introns. Although it is mainly expressed in the pituitary gland, previous studies have reported its expression in other reproductive tissues, including the placenta and uterus (Chu *et al*., 2010; Wang *et al*., 2021). The promoter region of the *FSHβ* gene contains functional genetic variants that may affect transcriptional activity and circulating FSH levels. The -211 G>T polymorphism has been associated with altered FSH secretion, menstrual cycle characteristics, ovarian response during assisted reproductive procedures, and unexplained infertility in women (La Marca *et al*., 2013; Laisk-Podar *et al*., 2015; Hayes *et al*., 2015; Rull *et al*., 2018). Previous studies have reported that this polymorphism may influence reproductive outcomes by modifying *FSHβ* gene expression and hormone regulation (Ruth *et al*., 2016). Previous studies have investigated the relationship between the *FSHβ* -211 G>T polymorphism and female reproductive characteristics in different populations. A study conducted among British women demonstrated that this variant was associated with reproductive physiology, including changes in FSH levels, age at menopause, and susceptibility to reproductive disorders (Rull *et al*., 2018; Ruth *et al*., 2016). Similarly, a study performed on Brazilian women identified the -211 G>T polymorphism in the promoter region of the *FSHβ* gene, with the wild-type G allele being more frequent than the mutant T allele. Women carrying the GT genotype showed differences in hormonal profiles and ovarian response compared with individuals carrying the GG genotype. These findings suggested that the T allele may reduce *FSHβ* transcriptional activity and consequently influence FSH regulation and reproductive outcomes (Grigorova *et al*., 2008). Therefore, the present study aimed to investigate the association between the *FSHβ* gene -211 G>T polymorphism and female infertility among Iraqi women in Maysan Governorate, providing further insight into the genetic factors contributing to reproductive disorders.

MATERIALS AND METHODS

A case–control study was conducted involving 60 infertile women and 30 healthy control women aged between 15 and 45 years. Blood samples were collected from participants in Maysan Governorate, Iraq, during October and December 2021. All participants were residents of Maysan Governorate. The study was approved by the authorized officials of the College of Science, University of Misan, and all procedures were conducted according to the approved ethical guidelines. Blood samples were collected at at private clinicsin Maysan Governorate.

Genomic DNA Extraction and Genotyping

Genomic DNA was extracted from whole blood samples using the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan) according to the manufacturer's instructions. A 364-bp fragment of the *FSHβ* gene promoter region was amplified using the following primers: forward primer (F): 5′-GGAGCCAGATCATGAAATGTT-3′and reverse primer (R): 5′- GACCAATGCTAGCCTGAAGC-3′ (Grigorova *et al*., 2008). PCRamplification was performed in a total volume of 50 µL containing 5 µL of genomic DNA, 25 µL of Master Mix, 4 µL of each primer, and 11 µL of nuclease-free water. The amplification conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 45 s, and extension at 72°C for 45 s. The final extension step was performed at 72°C for 7 min. PCR products were separated by electrophoresis on a 2% agarose gel and visualized under ultraviolet light, as shown in Figure 1.



Figure(1) :Amplification product of the *FSHβ* gene on 2% agarose **gel .**

RESULTS AND DISCUSSION

Molecular diversity

The results of the genetic diversity analysis of the FSHβ gene revealed two genetic variants among the studied sequences. One variant was detected in both infertile women and the control group, whereas another variant was observed among infertile women. A total of one nucleotide substitution was identified in the analyzed region. The studied fragment showed a high content of adenine (A) and guanine (G) bases. The detected substitution occurred in the promoter region at position -211, where guanine (G) was replaced by thymine (T). This substitution represents the -211 G>T polymorphism in the promoter region of the FSHβ gene.

Table (1) Molecular diversity of FSHβ gene in control and infertile women

Samples	Control	Infertility type 1	Infertility type 2	* Total
Number of sequences (N)	4	8	8	20
Number of polymorphic (NH)	1	2	1	2
Number of transitions	1	1	1	1
Nucleotide composition	C: 19.9% T: 23.7% A: 28.9% G: 27.5%	C: 20.7% T: 24.5% A: 29.2% G: 25.6%	C: 20.4% T: 25.4% A: 29.0% G: 25.2%	
GC%	56.6%	56.1%	56.2%	

Multiple Sequence Alignment

Multiple sequence alignment (MSA) analysis of the studied fragment of the FSHβ gene promoter region revealed a single nucleotide variation at position -211 according to the reference sequence (accession number: AH003599.1). The identified substitution involved replacement of guanine (G) with thymine (T), representing the -211 G>T polymorphism.This nucleotide substitution is classified as a transversion because it involves the replacement of a purine nucleotide (G) by a pyrimidine nucleotide (T). The present finding agrees with previous studies that reported the presence of the same polymorphism in the promoter region of the FSHβ gene among different populations. The -211 G>T variant has been detected in studies conducted in British, Brazilian, and other populations, suggesting that this polymorphism may represent a conserved genetic variation associated with reproductive characteristics (Trevisan *et al*., 2019; Rull *et al*., 2018; Huhtaniemi & Rivero-Müller, 2019; Polyzos *et al*., 2021; Anagnostou *et al*., 2021a).

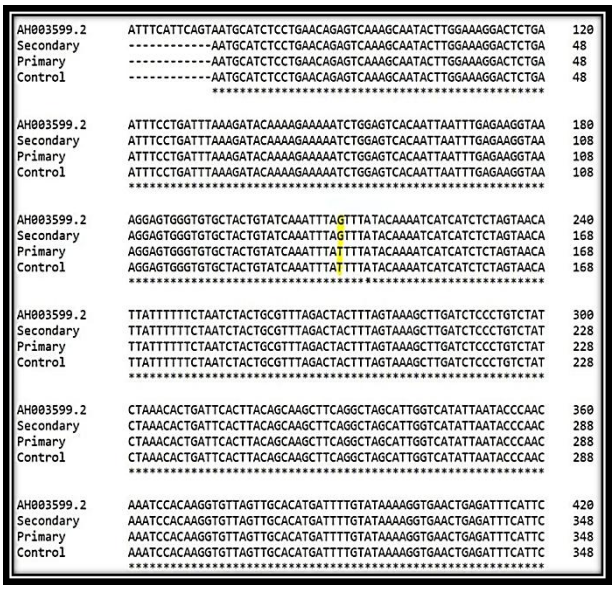


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Figure (2): Comparison of nitrogenous base sequences of the FSHβ gene in infertile women and comparison and accession number (AH003599.2).

Distribution of FSHβ (G>T-211) genotypes in infertile women and control

The genetic structures were extracted using Geneious Software (version 10.1.3). The sequences of the standard (comparative) and infertile samples of type 1 and 2 were compared with the sequences recorded in the GenBank (NCBI). Access Number: (AH003599). Alignment of the women's sequences showed three genetic structures at the studied locus G>T-211 of the follicle-stimulating hormone beta FSHβ gene. The presence of one black curve indicates the wild genotype (GG), i.e., no mutation in either allele. The presence of two red and black curves indicates the heterozygous genotype (GT), i.e., a mutation in one of the alleles, whether the base above the curve changes to G or not. The presence of one red curve and a change of the base above the curve to T indicates the mutant (homozygous) genotype, i.e., a change in either allele (TT). This finding was consistent with the research (Bianco *et al*., 2021). who found that the -211G>T mutation in the FSHβ gene led to the emergence of three genotypes in infertile Italian women. They found that the mutated T allele was associated with elevated LH levels in infertile women with endometriosis. Similarly (Polyzos *et al*., 2021). indicated the rarity of the TT mutated genotype in Asian women compared to European women, based The table(2) shows the numbers and percentages of the distribution of genetic combinations and alleles in the samples of infertile and comparator women. It was observed that the percentage of the

homozygous (wild) genetic combination (GG) in infertile and comparator women was not equal, as it reached (87.5%) in infertile women and (75.0%) in the comparator sample. There was no significant difference, and its value was (P = 0.54) at the probability level (P < 0.05). on their study investigating the relationship between FSHβ gene polymorphisms and ovarian response.

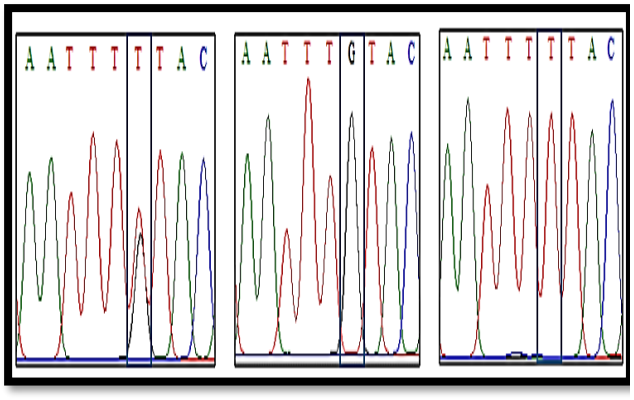

Figure (2): Genetic structures at position -211 G>T of the *FSHβ* gene

The critical ratio (OR) was (2.33), and its percentage as an etiological fraction (EF) causative allele associated with infertility was (0.5). The confidence interval (CI) ranged between (0.156-34.895). Regarding the heterozygous genetic structure of the GT, it was found that its percentage in the comparison sample was higher than in the infertile women, as it reached (6.25%) in the infertile women, while in the comparison samples it reached (0.25%). There was a non-significant difference of value (P = 0.29) at the probability level (P < 0.05), and the critical ratio (OR) was (0.20). Its percentage as a protective allele from infertility, Preventive fraction (PF), reached a value of (0.80), and the confidence interval (CI) ranged between (0.01-4.17). As for the homozygous (mutant) TT genetic structure, its percentage was (6.5%) in infertile women and in the comparison samples, its complete absence was observed, and there was a significant difference of value (P = 0.04) at the probability level (P < 0.05), and the critical ratio (OR) was (0.87), and its percentage as a protective allele against infertility (PF) was (0.13), and the confidence interval (CI) ranged between (0.030-25.284). The result was inconsistent with (Anagnostou *et al*., 2021b). regarding the *FSHβ* gene's nucleotide polymorphism (-211G>T) in infertile Greek women, which showed statistically significant differences related to LH levels. The distribution of the G allele in the infertile women's sample and the control group was 87.5% and 90.6%, respectively. The T allele distribution was 12.5% in the infertile women's sample and 9.4% in the control group, as shown in Figure (4). This is also consistent with the study (Alviggi *et al*.,2018). which found an association between the T allele in the 211G>T- mutation and low FSH and LH levels, and with the findings of (Rull *et al*., 2018). hich linked the T allele in *FSHβ*:c.-211G>T to unexplained infertility.

Table (2): Distribution of the *FSHβ* gene genotypes in infertile and control women.

Genotype	sterile women (%) the number	the control the number (%)	OR	(95%CI)	*P* value
GG	(% 87.5)14	3 (% 75.0)	2.33	0.156-34.895	0.54 NS
P.F	0.5				
GT	(% 6.25) 1	1 (% 25.0)	0.20	0.01-4.17	0.29 NS
E.F	0.80				
TT	(% 6.25) 1	0	0.87	0.030-25.284	*0.04 NS
E.F	0.13				

Odds ratio =OR ، Confidence Intervals =CI ، Preventive fraction =P.F ، Etiological fraction =E.F NS =Not significant at the P<0.05 probability level

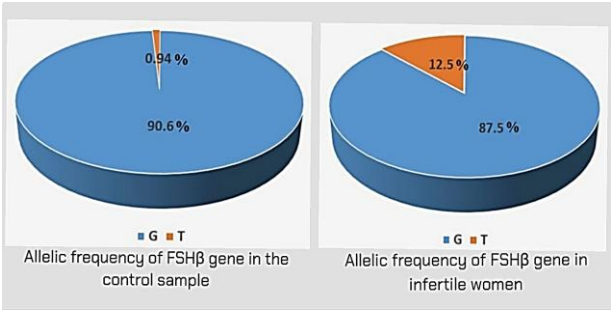

Figure (4) The distribution ratio of the T and G alleles of the *FSHβ* gene in a sample of infertile women and comparisons

Using the Hardy-Weinberg equilibrium law, the results for the *FSHβ* gene showed a statistically significant difference between the infertile women group and the control group, with a value of (P = 0.011*) at a probability level <0.05P. This means that the control group does not follow a Hardy-Weinberg distribution. The observed numbers for the TT, GT, GG1, and 14 genotypes were 0.14, 2.72, and 13.14, respectively, and the predicted numbers were 0.14, 2.72, and 13.14, respectively. The Hardy-Weinberg equilib ium values for the GG1, GT, and TT genotypes were 0.88 and 82.13, respectively, as shown in Table (3).

In addition to genetic factors, female infertility is considered a multifactorial disorder influenced by environmental and lifestyle factors(Hart, 2016).Environmental conditions, nutritional status, body

mass index, psychological stress, exposure to chemical pollutants, and other lifestyle-related factors may affect reproductive function and may interact with genetic variations to influence infertility susceptibility(Bala *et al*., 2021).These interactions between genetic and environmental factors may contribute to differences in reproductive outcomes among populationsp(Low *et al*., 2008).Therefore, future studies should consider the combined effects of genetic variations and environmental factors to provide a more comprehensive understanding of the mechanisms underlying female infertility.

Table (3): *FSHβ* gene structures, their observed and expected numbers, and the Hardy-Weinberg equilibrium value in infertile and control women

Genotype	number of viewers	Expected number	Hardy-Weinberg (%)poise	*P* value
GG	14	13.14	82.13	0.011
GT	3	2.72	16.99	
TT	1	0.14	0.88	

CONCLUSION

The findings of this study suggest that the -211 G>T polymorphism in the *FSHβ* gene may be associated with female infertility among Iraqi women. However, further studies with larger sample sizes and consideration of additional genetic, clinical, and environmental factors are required to confirm this association.

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