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Genetic Study of the Progesterone Receptor for Infertile Iraqi Women

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Abstract: The genetic causes of infertility in women are almost completely unknown. One of the genes associated with a higher risk of female infertility is the human progesterone receptor gene. Multiple polymorphisms have been discovered in this gene; however, PROGINS stands out as a variation with a 306-bp Alu-insertion in intron G and two-factor mutations in exon 4 and 5 (V660L and H770H, respectively), which both occur in the PGR polymorphism. For the purpose of determining the prevalence and pattern of the PROGINS 306 bp Alu insertion in intron G polymorphism in Iraqi women with unexplained infertility, it was an associated risk factor; we are doing this research. PGR gene polymorphism (Alu insertion in intron G) was evaluated in 70 patients with idiopathic infertility and 60 healthy fertile women as controls in this research, which looked at the genotype frequency polymorphism of PROGINs. According to the findings, the proportion of patients with unexplained infertility who had polymorphism genotypes (G1/G2 and G2/G2) was substantially greater than that of the control group (33%). (16 percent) We find that the PROGINS polymorphism of the PGR gene has a strong link with unexplained infertility in Iraqi women.

Keywords: PGR, gene, PROGINS, Estrogen, hormones, pregnancy, Iraq.

INTRODUCTION

Infertility and reproductive-related disorders effect millions hundreds of women throughout the globe today (Wetendorf and DeMayo,2014). The disease of the reproductive system that leads to no pregnancy after 12 months of unprotected reguler intercourse is referred to as infertility by the World Health Organization. According to Bakhtiyar and coworkers (2019), infertility affects an estimated 80 million couples globally. The rate of infertility varies from (6.9-9.3) % to 3.5-16.7) % in developing countries. Infertility accounts for 30% of the total males, whereas female infertility accounts for 70%. Infertility is caused by the following factors: Ovulatory factor accounts for 25% of infertility, tubal factor for 15%, and uterine factor for 5%. Approximately 25% of infertility is due to unexplained infertility. Oocyte maturation, fertilization failure, embryonic arrest, and preimplantation embryo mortality have all been linked to many genes (Sang, *et al*., 2021).

Estrogen and progesterone are hormones produced by the ovaries, and the uterus depends on their corresponding receptors to communicate with each other (Wetendorf and DeMayo 2014). Ovarian and adrenal glands produce progesterone (also known as P4), a steroid hormone; the term "pregnancy hormone" is often used to describe it (Medina-Laver, *et al*., 2021). Non-reproductive tissues, such as the central nervous system and the mammary gland in preparation for breastfeeding, as well as bones and the cardiovascular system, all benefit from progesterone presence; it is thought that P4 is involved in regulating a wide range of processes that occur in the female reproductive system and pregnancy all depend on progesterone, its play a vital role in the development of the breast, ovulation, the embryonic development, implantation, fertilization and parturition (Ondruska, *et al*., 2020, Medina-Laver, *et al*., 2021). Luteal phase defect is often treated with progesterone in clinics (Yu, *et al*., 2018). Steroid hormones and their receptors control gene expression and cell proliferation in eukaryotes (Ondruska, *et al*., 2020). The aim of this study is to analysis genetic varaition of PROGINs gene as its imported role in

MATERIAL AND METHOD

Patients:

Seventy blood samples were collected from unexplained infertility women who attended to Al-Imameen Al-Kazimin medical city and Al-Karkh Maternity Hospital from June 2021 to September 2021. The patient age range was (17-45) years, and have been trying to conceive for at least a year. All women enrolled in this study had ovulatory cycles lasting 25 to 34 days, as demonstrated by urine ovulation testing, as well as normal luteal phase endometrial biopsy results; the blood tests showed normal levels of the hormones FSH and TSH on day three, and adequate levels of the hormone prolactin. They had a normal intrauterine cavity. Six women had only mild to moderate signs of endometriosis. No anti-sperm antibodies were found by immunobead testing in seminal plasma

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from male partners. Sixty fertile women with at least one full-term kid and no history of infertility participated as a control group of fertile women. It was approved by the Ministry of Health in Iraq. Blood Specimens were collected from venous from each subject's group; 3 mL of whole blood were placed in a tube containing EDTA (Ethylene Diamine Tetra Acetic Acid). Body mass index has been measured; by dividing body mass in kilograms by the square of the height in meters conferring to the equation.

$$\text{BMI} = \frac{\text{Mass(kg)}}{\text{Hieght}(\text{m}^2)}$$

DNA extraction and PCR

DNA was extracted from blood samples using the Geneaid DNA kit (Korea). Polymerase Chain Reaction was performed to detect the PROGINS Polymorphism**.** The primers that used in this study was depend on (Costa, *et al*., 2013) Table (1).

Table 1: Sequence of primers used in this study

Name of primer	**Sequence of primer** (5′- 3′)	**Size of product (bp)**
Foeward	5'-GGC AGA AAG CAA AAT AAA AAG A-3'	306 bp
Revers	5'-AAA GTA TTT TCT TGC TAA ATG TC-3'	

The PCR reactions were done in a 25 µl reaction mixture including (12.5 µL) Master Mix, 2 µL from each forward and reverse primers, 3µL of DNA template (100 ng/mL), and 5.5 µL nuclease-free water.

The PCR condition (denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 51°C for the 30s, and 72°C for 30s, after that extension at 72°C for 10 min followed by a final extension at 72°C for 10 min and hold at four °C). PCR amplifications were achieved in an Applied Biosystem 96 thermocycler. PCR amplifications were achieved in an Applied Biosystem 96 thermocycler.

RESULTS AND DISCUSSION

Patients Characteristics

The study was performed on 70 Iraqi women who had been diagnosed with unexplained infertility, as well as 60 Iraqi women who had at least one full-term child and no history of infertility as a control group (Tables 2). The study results showed that the mean age was (27.53 ± 0.65) years for the infertility group and (31.53 ± 1.07) years for the control group, and the mean body mass index (BMI) for the patient group was (27.91 ± 0.45). While BMI was (27.31 ± 0.54) for the control group. No significant association between Iraqi women's unexplained (p = 0.151 and p = 0.447 depending on the age and BMI, respectively). Also, the results refer to no significant differences depending on location and type of Infertility patients with unexplained infertility, and in the control group, our results revealed that the number and percentage of patients with infertility were higher in urban locations in comparison with rural locations 58 (82.86%) with p-value 0.667 NS.

Table 2: Demographic Characteristics of patients

Characteristics	**Patients**	**Control**	***P-value***
Age	27.53 ± 0.65	31.30 ± 1.07	0.151
BMI	27.91 ± 0.45	27.31 ± 0.54	0.447
Location			
Urban	58 (82.86)	52 (86.67)	0.667 NS
Rural	12 (17.14)	8 (13.13)	0.667 NS
Type of Infertility			
Primary Infertility	59	-----	-----
Secondary Infertility	11	------	------

THE MOLECULAR RESULTS

Two different PCR products were obtained from the PROGINs gene amplification; the first was called G1 and represented the wild-type allele (149 bp) without the Alu insertion, while the second was called G2 and represented the mutant allele (455) that was created by inserting 306 bp into the progesterone receptor's intron G. Patient's homozygous for wild type (G1/G1) or polymorphic are those who have two identical bands. In contrast, patients heterozygous for each allele type are those who have two distinct bands (G1/G2), Figure 1(A and B).

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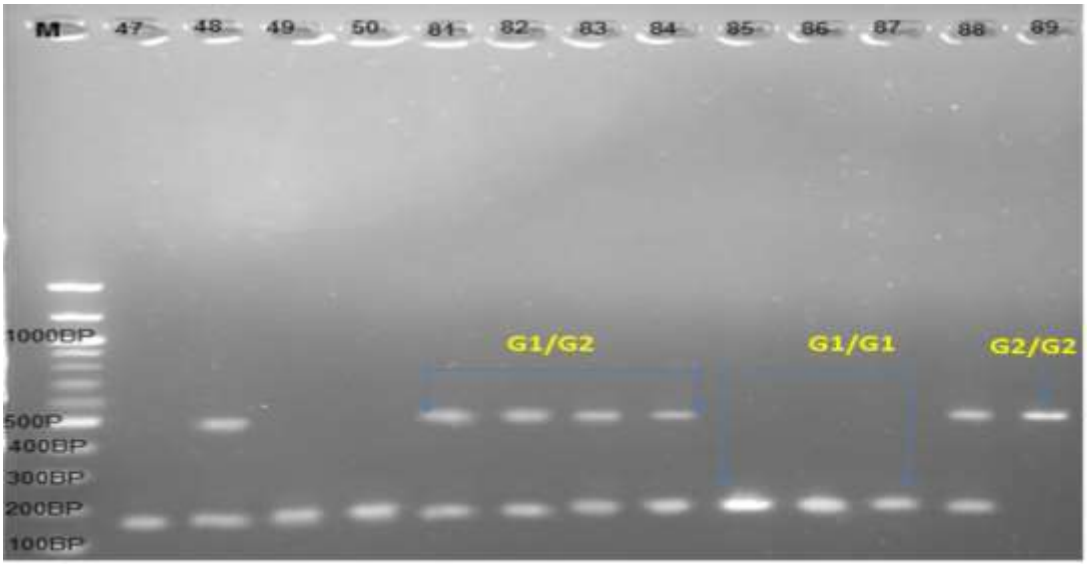

Figure 1: A: PROGINS polymorpism on agarose gel (1.5%) stained with red-safety M: DNA ladder (100 bp), G1/G1 Homozygous wild type (149 bp), G1/G2 Polymorphic heterozygous (149 bp and 455 bp results from 149 bp + Alu insersion of 306 bp), G2/G2 polymorphic homozygous.

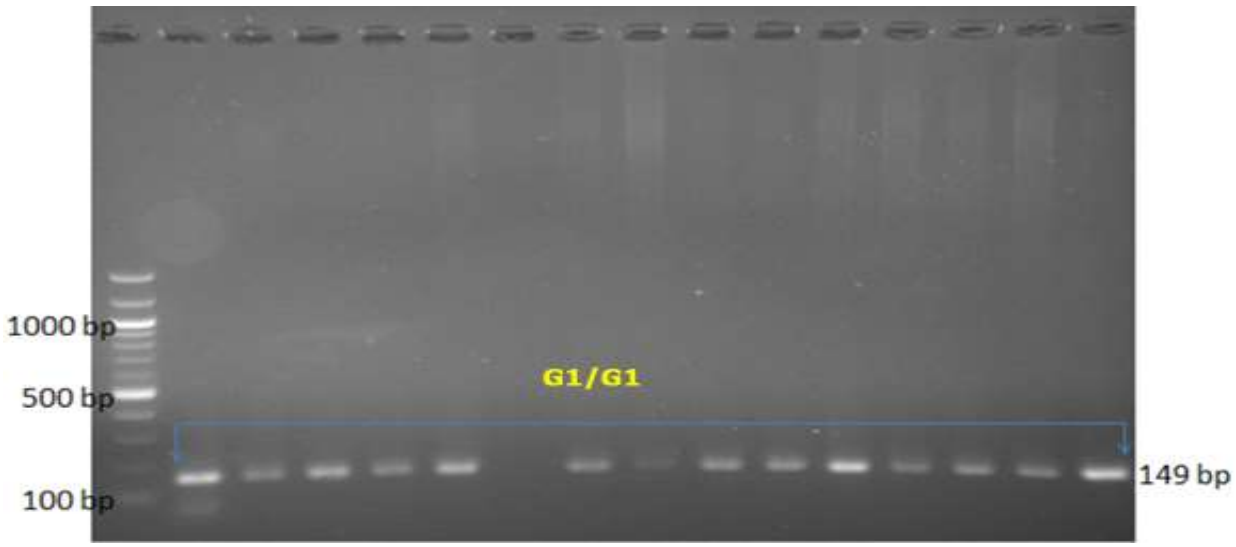

Figure 1: B: PCR products of PGR gene on agarose gel (1.5%) stained with red-safety M: DNA ladder (100 bp), G1/G1 Homozygous wild type (149 bp) in the control group.

The analysis data of DNA sequencing for the amplified products of the PGR gene in Iraqi infertility and control groups were done as illustrated in (Figure 2: A and B), which show the alignments of DNA sequences for the PGR gene in the Iraqi women infertility group women without Alu insersion in comparision with Homo sapiens chromosome 11, GRCh38.p13 using the Blast web site in the NCBI database.

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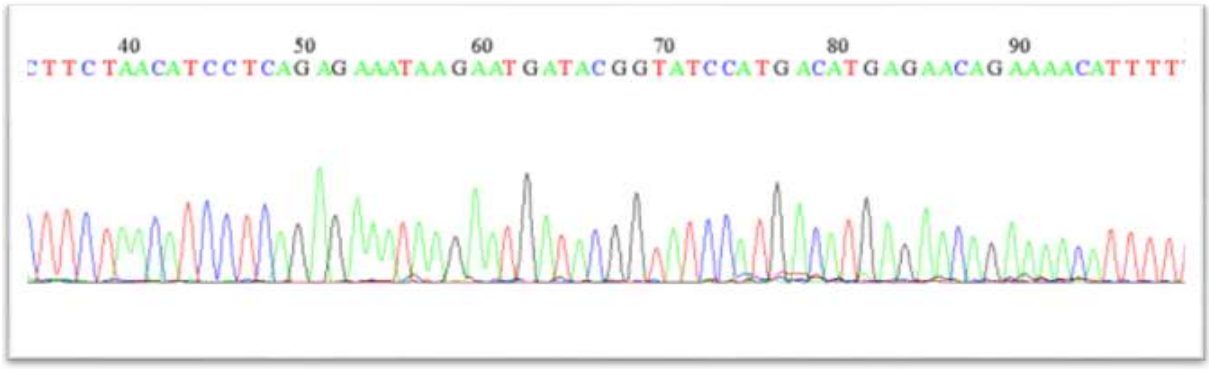
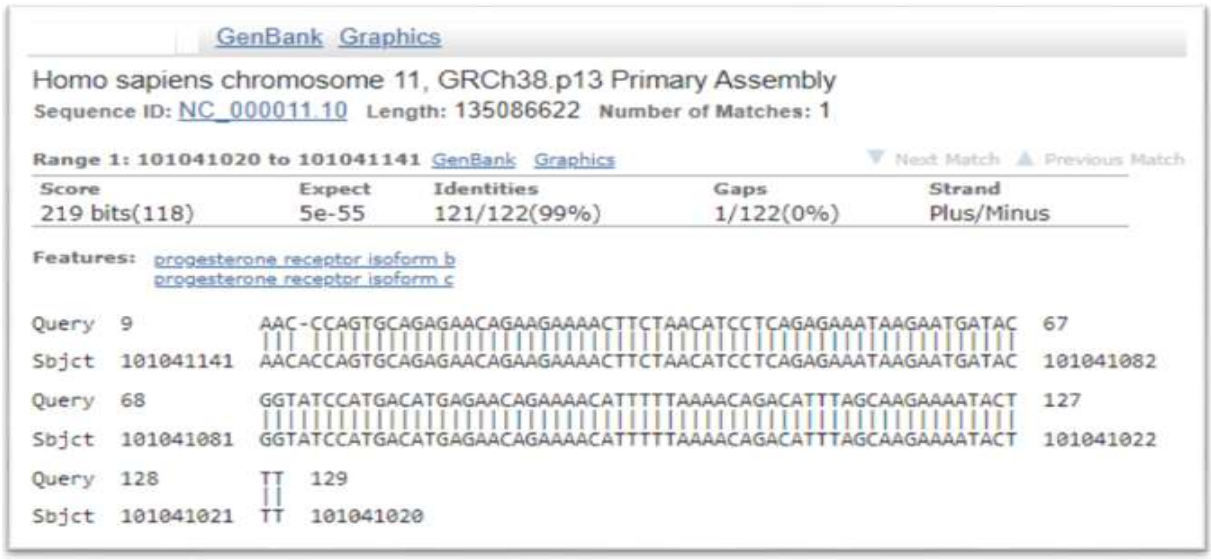


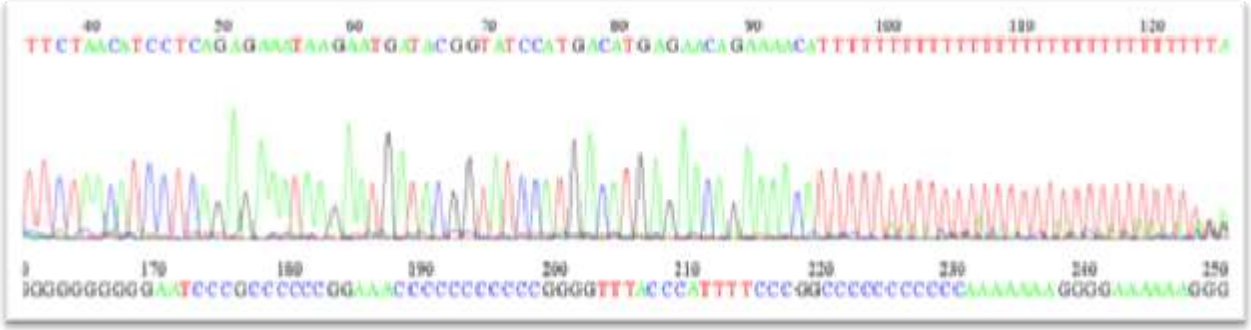
A



B

Figure 2: **A**: The sequence of PGR gene in Iraqi infertility women without Alu insersion **B**: Alignments of DNA sequences from Iraqi infertile women with similar gene (NCBI Reference Sequence: NC_000011.10)

The sequences and the of PGR gene in Iraqi infertility women with the partial gene for PGR Alu element DNA (insersion) are showed in (Figure 3. A and B), Alignments in cmparisino with (NCBI Reference Sequence I D: Z49816.1).



A

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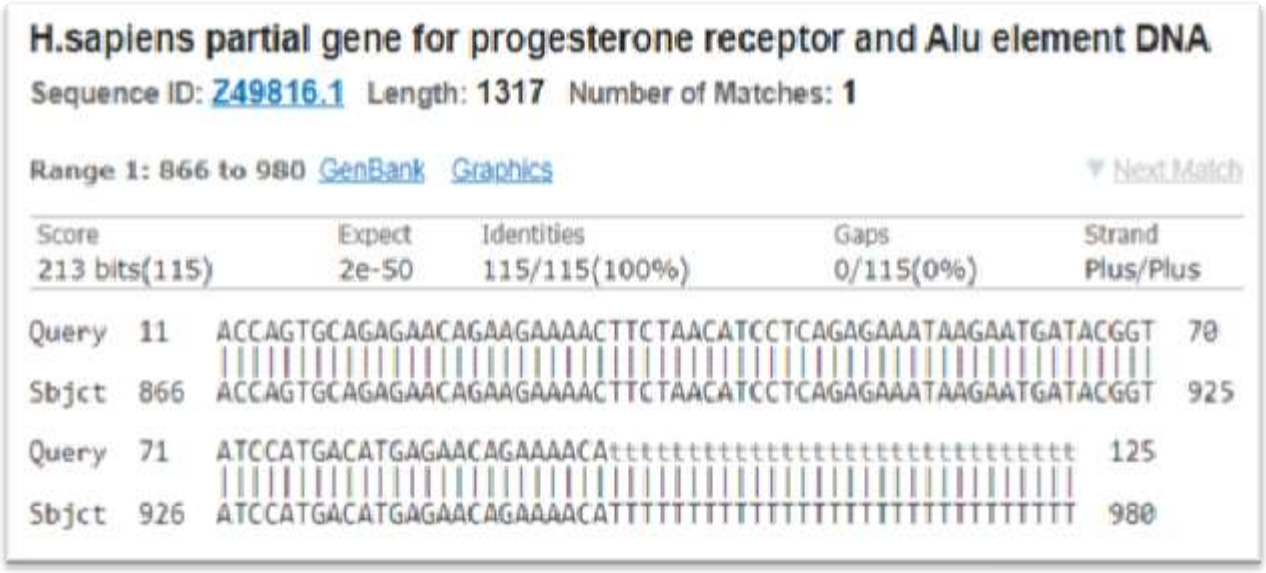

B

Figure 3: **A:** The sequence of PGR gene in Iraqi infertility women with partial gene for PGR Alu element DNA (insersion) **B:** Alignments of DNA sequences of PGR Alu element DNA from Iraqi infertile women with similar gene (NCBI Reference Sequence ID: Z49816.1).

The Genotype and Allelic Frequences of the PGR Gene

The compression of the prevalence and the percentage of progesterone receptor polymorphism (Alu insertion at intron G) in unexplained infertility and fertile women are summarized in (Table 3). When analyzing the genotype distribution of the PROGINS polymorphism group with unexplained infertility (N= 70), we obtained 58.57 % (41/70), 28.57% (20/70), and 12.86% (9/70) for the G1G1, G1G2 and G2G2 genotype frequencies respectively, the G1G2 genotype with OR=1.07, (95% CI: 0.75-1.56, P=0.0095), and 41.43% (29/70) of unexplained infertility group had polymorphic genotype for PROGINS (G1G2+G2G2). In the control group (N = 60), the genotype frequencies were 86.67% (52/60), 10.0% (6/60), and 3.33% (2/60) for G1G1, G1G2, and G2G2, respectively, while the genotype for PROGINS (G1G2+G2G2) had 13.33% (8/60). Compared to the control group, the genotype distributions of the combined polymorphism genotypes (G1G2+G2G2) were three times greater in the group suffering from unexplained infertility (P = 0.0007). Allele frequencies were also found to be significantly different across populations. It had been revealed that PROGINS polymorphism was associated with a high risk for unexplained women infertility. These findings indicate that PROGINS polymorphism has an impact on unexplained women infertile. The results of the current study were identical to the findings of a previously published study which concluded impaired levels of progesterone receptors in patients with unexplained women infertility. (Petousis, *et al*., 2018), Also, our study was in agreement with a previous study conducted by (Pisarska, *et al*., 2003). They published a significant relationship between progesterone receptor polymorphism and high prevalence of unexplained women infertility.

Table 3: Genotype and allelic frequencies of PGR gene in patients and control groups

PROGINS Genotype	**Patients**		**Control**		***P*-value**	**O.R. (C.I.)**
	No	**%**	**No**	**%**		
G1G1	41	58.57	52	86.67	0.0083**	1.00
G1G2	20	28.57	6	10.00	0.0095 **	1.07(0.75-1.56)
G2G2	9	12.86	2	3.33	0.071 NS	0.662 (0.29-1.15)
(G1G2+G2G2)	29	41.43	8	13.33	0.0007**	
Total	70	100%	60	100%	--	--
Allele	**Frequency**					
G 1	0.73		0.92		--	--
G 2	0.27		0.08		--	--

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** (P≤0.01).

The patient's ages were divided into three categories, as shown in (Table 4) The G1G2genotype frequency came in second place, distributed as the following: 10 patients (20-29), nine patients (30-39) patients, and one patient for (40-45) years, and in the last place, G2G2 genotype was distributed as the following: 4 patients (20-29 years old)) year and five patients (30-39) years old. Our data illustrated that the distribution of the genotypes of PROGINS frequencies in unexplained infertility patients and control depend on age group; the distribution of polymorphism in patients were (30%, 14.3%) for G1G1, G2G2 genotype, respectively, it were higher in age group (20-29), no significant age-related differences (P>0.05). Patient unexplained infertility had a higher PROGINS polymorphism genotype percentage when compared to a control group of women between the ages of 21 and 30 years old, which may be due to this being women's reproductive age. The rise in the average age of childbearing during the last two decades is a major factor in infertility. Increased risk of chromosomal abnormalities and miscarriage occurs when the mother's reproductive capacity diminishes, and the ovary becomes less effective (Toprak, *et al*., 2019).

Table 4: Distribution of PROGINS Polymorphism according to age in patient and control groups

PROGIN polymorphism	**Patients age group**				**Chi-Square Tests Significance (2-sided)** ***P-value***
	20-29 No./%	**30-39 No./%**	**40-45 No./%**	**Total No./%**	
G1G1	21/30	19/27	1/1.4	41/58.4	0.940
G1G2	10/14.3	9/12.9	1/1.4	20/28.6	
G2G2	4/5.9	5/7.1	0/0	9/13	
Total	34	34	2	70	
Allele	**Frequency**				
G1	0.76	0.72	0.75		
G2	0.4	0.28	0.25		
Control group					
G1G1	27/45	25/41.6	1/1.6	54/89.9	0.715
G1G2	1/1.7	3/5	1/0	4/6.7	
G2G2	1/1.7	1/1.7	0/0	2/3.4	
Total	29	29	2	60	
Allele	**Frequency**				
G1	0.94	0.9	0.75		
G2	0.06	0.1	0.25		

The PROGINS genotypes appeared in 35(50%), 15(21.4%), and 9(13%) in an urban area compared with 6(8.5%), 5(7.1%),0/0%) for G1G1, G1G2, and G2G2 genotype frequencies respectively in rural area were as shown in (Table 5), no significant differences between the frequency of genotype PGR of unexplained infertility (p= 0.221) and the demographic location. Also, no significant differences (P=0.22141) were observed for genotypes G1G1 and G1G2 among urban locations. This may be due to those living in urban areas being more exposed to environmental pollution factors compared to rural areas; it is known from previous studies that the proven effect of pollutants and chemicals and their impact on the endocrine system and the occurrence of mutations and variations in genetic material (Darbre 2015). Till now, there are no studies that proved the relationship between the area and frequency polymorphism of genotype and allele of the PGR gene.

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Table 5: Distribution of PROGINS Polymorphism according to location in patients' group

PROGINS Genotype	Patient Location			Chi-Square Tests Significance (2-sided) *P-value*
	Urban No./%	Rural No./%	Total No./%	
G1G1	35/50	6/8.5	41/58.5	0.22141
G1G2	15/21.4	5/7.1	20/28.5	
G2G2	9/13	0/0	9/13	
Total	59	11	70	
Allele	**Frequency**			
G1	0.72	0.77		
G2	0.28	0.23		

The present study demonstrated the distribution of PROGIN Polymorphis according to BMI in patients and control groups. The results showed no significant association of PROGINS genotype in infertility patients depending on BMI (Table 6).

Table 6: Distribution of PROGINS Polymorphism according to BMI in patients and control groups

PROGINS Genotype	Patient BMI kg/m^2			Chi-Square Tests Significance (2-sided) *P-value*
	Overweight (23-27.4) No./%	Obesity class 1 (27.5-32.4) No./%	Total No./%	
G1G1	19/27.1	22/31.4	41/58.5	0.165
G1G2	5/7.1	15/ 21.4	20/28.5	
G2G2	2/3	7/10	9/13	
Total	26%	44%	70/100	
Allele	**Frequency**			
G1	0.82	0.67		
G2	0.18	0.33		
Control BMI kg/m^2				
G1G1	26/43.3	28/46.6		0.482
G1G1	3/5	1/1.7		
G2G2	1/1.7	1/1.7		
Total	30/50%	30/50		
Allele	**Frequency**			
G1	0.90	0.95		
G2	0.10	0.05		

The present study indicate to the association between the type of infertility and PROGINS polymorphism; it was found to be a significant association (P- <0.05) in primary infertility (as shown in table 8) when compared with secondary in-patient infertility. Higher percentage of 30 (42.8%) and 20 (28.6%) of G1G1 and G1G2 genotypes, respectively, among primary infertility when compared with secondary infertility; this may be due to the high percentage of infertile women whose participate in this study were with primary infertility.

Table 7: Distribution of patient PROGINS Genotype according to patient infertility type

PROGINS Genotype	Infertility type (No./percentage)			Chi-Square Tests Significance (2-sided) *P-value*
	Primary No./%	Secondary No./%	Total No./%	
G1G1	30/42.8	11/15.8	41/58.6	0.010 *
G1G2	20/28.6	0/0	20/28.6	
G2G2	9/12.8	0/0	9/12.8	
Total	59	11	70/100	
Allele	**Frequency**			
G1	0.67	0.90		
G2	0.33	0.10		

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Women with unexplained infertility are identified when the fallopian tubes are patent with a normal ovulation process and the male partner with normal seminal fluid analysis (Du, *et al*., 2011). Thin endometria, as seen on hysteroscopy in these infertile women, is indicative of poor growth and development in the fetus. The main hormones that control female human reproduction are progesterone, estrogen, and follicle-stimulating hormones (FSH) (Sen, *et al*., 2013).

For maintaining implantation and promoting uterine development, progesterone is critical. Almost all of the progesterone's beneficial effects on the body are caused by its receptors (Mojarrad, *et al*., 2013)

There are two protein isoforms of the progesterone receptor gene: PR-A and PR-B, which are situated on the long arm of chromosome 11, bands 22-23 (11q22-23) in humans (Donadio, *et al*., 2006, Xiao, *et al*., 2020). Endometrial progesterone's antiproliferative effects are mediated by isoform A, whereas activation of isoform B in the absence of a type-A receptor results in epithelial proliferation. PROGINS is a collection of three distinct genetic variants that are exclusively seen in humans. A 306-bp Alu insertion in intron G between exons 7 and 8 of the PGR gene is what makes the PROGINS polymorphism stand out (Donaldson, *et al*., 2002). Progesterone's ability to bind to the hormone receptor may be impaired as a consequence of this insert, resulting in a reduction in the final activity mediated by progesterone (Donadio, *et al*., 2006, Ylmaz, *et al*., 2009).

In Iraqi women, the frequency of PROGINS alleles was shown to be strongly linked with unexplained infertility, according to the current study's clinical-pathological variable analysis.

It has been found that the endometrial expression of progesterone receptors is impaired in women with unexplained infertility. Progesterone has a major role and important functions, such as the proliferation of epithelial cells, stromal decidualization, and embryo attachment in the endometrium, which is performed by interacting with the nuclear progesterone receptor located on endometrial cells representeded in the study. Petousis and colleagues (2018). In luteal phase insufficiency and local overproduction of estrogen, pro-inflammatory pathways are activated as a result of progesterone receptor resistance to endogenous progesterone. Endometriosis is one of the most common reasons of infertility in women with low luteal phases who delay treatment. Pregnancy is dependent on the synchronization of the processes of endometrial maturation and implantation, which is critical to its success.

CONCLUSION

The current results revealed that the genotype of the PROGINS genotype gene may be have a role in Iraqi infertility women. The Alu insertion allele was shown to be associated with an increased incidence of infertility in women. This study indicated to the significance association of unexplained infertility in the urban. No association was found between PROGINS polymorphism depending on the age and BMI factors in infertility women. Other study with a large number of infirtility pateints are need to confirm the current results.

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