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Identification of mutations in the CYP19 gene in patients with polycystic ovary syndrome in Thi-Qar city

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Abstract

This study was conducted to diagnose the possible relationship between genetic variations in the CYP19 gene with the progression of polycystic ovary disease in Thi- Qar city. This study included ten samples, and a genetic fragment with a length of 202 base pairs was amplified in the 3′ untranslatable region (3′UTR) of the CYP19 gene. These samples were subjected to experiments to determine the sequence from both directions of the amplified fragment. Sequence identification experiments found that there was one previously undiagnosed point mutation, g.27933770C>G, while these experiments found that there were two previously known point mutations, that is, previously discovered in the same amplified fragment, namely rs1201251894 and rs1163221780. Of the total of three-point mutations discovered, the current results indicate the presence of two of those point mutations observed in all the study samples, namely the new g.27933770C>G and the previously known rs1201251894, while the mutation rs1163221780 was observed in only three samples, which are samples No. 3 and 5 and 10.

As a result, this observation may contribute to revealing a specific intervention of these discovered mutations, or one of them, in the development of polycystic ovary disease in Thi -Qar. This research is the first to describe the presence of an unprecedented point mutation distributed in all samples subject to sequencing experiments for the CYP19 genetic segment in all patients with polycystic ovary syndrome. This study recommends investigating the pattern and mechanisms of this unprecedented mutation in its possible relationship with polycystic ovary syndrome within this geographical region.

Keywords: PCOS; Cyp19; Infertility; Genetic; Point mutations; Polycystic ovary syndrome.

1. Introduction

Infertility: Infertility is defined as the inability of the couple is anti-pregnancy to achieve pregnancy in one year (Roupa et al., 2009; Turchi, 2015). Infertility is still a widespread global condition. And affects between 8 and 12% of couples of childbearing ages worldwide (Inhorn and Patrizio, 2015).

Primary infertility is diagnosed in couples who have no surviving children, while secondary infertility is diagnosed when the female is pregnant at least once, but is unable to tolerate subsequent pregnancies (Whitehouse and Hollos, 2014). Female infertility can be due to different conditions such as ovulation disorders, uterine or cervical abnormalities, Fallopian tube damage or blockage, endometriosis, primary ovarian insufficiency, insufficiency, pelvic adhesions, and the use of cytotoxic chemotherapy to treat cancer (Abrao et al.,2013). Moreover, it can be disturbances in a number of hormones that play a crucial role in the development of infertility cases. These hormonal disorders may include one or more sex-related hormones, including prolactin (PRL), thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH) Luteinizing hormone (LH), estrogen, progesterone and testosterone (Veeresh et al., 2015).

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The main causes of infertility in females are tubal and peritoneal factors (25-35%) factors, ovulatory factors (30-40%), endometritis (1-10%) (Dutta and Chakravarty, 2003) Among the factors of ovulation is polycystic ovarian syndrome is one of the most important causes of infertility. PCOS affects 4% to 12% of women in childbearing age (Knochenhauer et al., 1998).

This syndrome can result from an abnormal function of the hypothalamus-pituitary-ovarian (HPO) axis. This is associated with important reproductive diseases, including infertility, secondary amenorrhea and increased pregnancy loss.

2. Material and methods

2.1. DNA isolation

DNA samples were extracted from peripheral blood from 10 samples from patients with polycystic ovary syndrome using a special kit for this purpose and according to the details attached to that kit (Mammalian DNA extraction kit, Geneaid Biotech, Taiwan). The concentration and purity of the isolated DNA were measured using a Nanodrop device (BioDrop µLITE, BioDrop co., UK), while the integrity of the extracted DNA was tested by electrophoresis in 0.8% agarose gel using ethidium fluoride dye (at a concentration of 0.7 micrograms per milliliter) in a TAE buffer. (Containing 40 mM Tris acetate and 2 mM EDTA at pH 8.3), using a standard 1Kb ladder (Cat # D-1040, Bioneer, Daejeon, South Korea). The isolated DNA was used as a template in amplification experiments (PCR).

2.2. PCR amplification

One PCR fragment was chosen to perform this amplification, which is supposed to cover a specific site within the CYP19 gene, which is 202 base pairs within the genome (Table 1).

Table 1 Details of the pair of specialized primers chosen to amplify a specific site within the CYP19 gene.

Initiator code	Sequence (5′-3′)	Amplification segment size	Serial number AH001446.2	Source
CYP19-F	CTGGAACACTAGAGAAGGCTGGTCAGTGC	202 bp	(7135–7164)	Yang *et al.* 2015
CYP19-R	GTTCTCTGGTGTGAACAGGAGCAGATCAC		(7307–7336)	

Lyophilized primers were purchased from the Korean company Bioneer. Gene amplification experiments were conducted using a mixture specialized for this purpose (AccuPower PCR premix, Cat # K-2012, Bioneer, Daejeon, South Korea). Every 20 microliters of this mixture contains one international unit of the specialized enzyme (Top DNA polymerase), 250 micromolar of nucleotide triphosphates, 10 micromolar of tris-Cl with a pH of 9, 30 micromolar of KCl, and 1.5 mmol MgCl2). The reaction mixture was supplemented by adding 10 picomole of each primer and 50 ng of genomic DNA.

The following program was implemented in a thermal rotator (MyGenieTM 96/384 Thermal Block, Bioneer, Daejeon, South Korea). Replication was initiated by initial denaturation at 94°C for five minutes, followed by thirty cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds, and enzymatic elongation at 72°C for 30 seconds. The final enzymatic elongation was completed at 72°C for five minutes (Yang et al., 2015). The success of the amplification process was confirmed by electrophoresis in an agarose gel stained with ethidium bromide in TBE buffer (composed of tric-Cl, borate, and EDTA at pH 8.3), using a special standard ladder for PCR reactions (Cat # D-1010). , Bioneer, Daejeon, South Korea 100 bp DNA ladder,). It was ensured that the resulting PCR packages were specialized and clean so that they could be successfully sent to sequence identification reactions.

2.3. Reading the sequence reactions of the amplification products

The sequences of the PCR products were read from both ends according to the working methods of the respective company (Macrogen Inc. Geumchen, Seoul, South Korea). Only pure chromatograms were analyzed from the resulting sequence files, in order to ensure that the mutations resulting from the reading were not due to a technical defect due to what was in the amplification products or in the sequence reading. When DNA sequences observed in local samples were compared with DNA sequences derived from reference sequences found on the NCBI website, the exact location and other details of the derived amplification fragments were identified, identified, and extracted accordingly.

2.4. Analysis of read sequencing reactions:

Several processing operations were carried out for the sequencing results of the amplification products and different samples, which included cleaning, alignment, and analysis with the specific sequences present in the reference regions on the NCBI website using the BioEdit program designated for this purpose (DNASTAR, Madison, WI, USA). Variations observed in each sample subjected to sequencing reactions were numbered in the PCR amplified fragments relative to their assigned locations within the human reference genome.

3. Results and discussion

Of the 10 exons contained in the CYP19 gene (Mendelson et al., 1990), the region selected here, that is, 202 base pairs long, focused on the partial amplification of a non-coding region located after the coding region of the tenth exon of the CYP19 gene. This region is known as the non-coding region. Translatable 3′ UTR. The PCR product sequences of the ten samples were read according to the procedures prepared by the relevant company (Macrogen Inc. Geumchen, Seoul, South Korea). Only pure chromatograms from the resulting sequence files were analyzed, in order to ensure that the resulting mutations Of the reading, it is not due to a technical defect due to what is in the amplification products or in the reading of the sequences. When comparing the DNA sequences observed in the studied samples with the DNA sequences derived from the reference sequences for humans and found on the NCBI website (GenBank acc. AH001446.2), the exact location is Other details of the derived amplification plots were identified, defined and extracted accordingly (Figure 1).

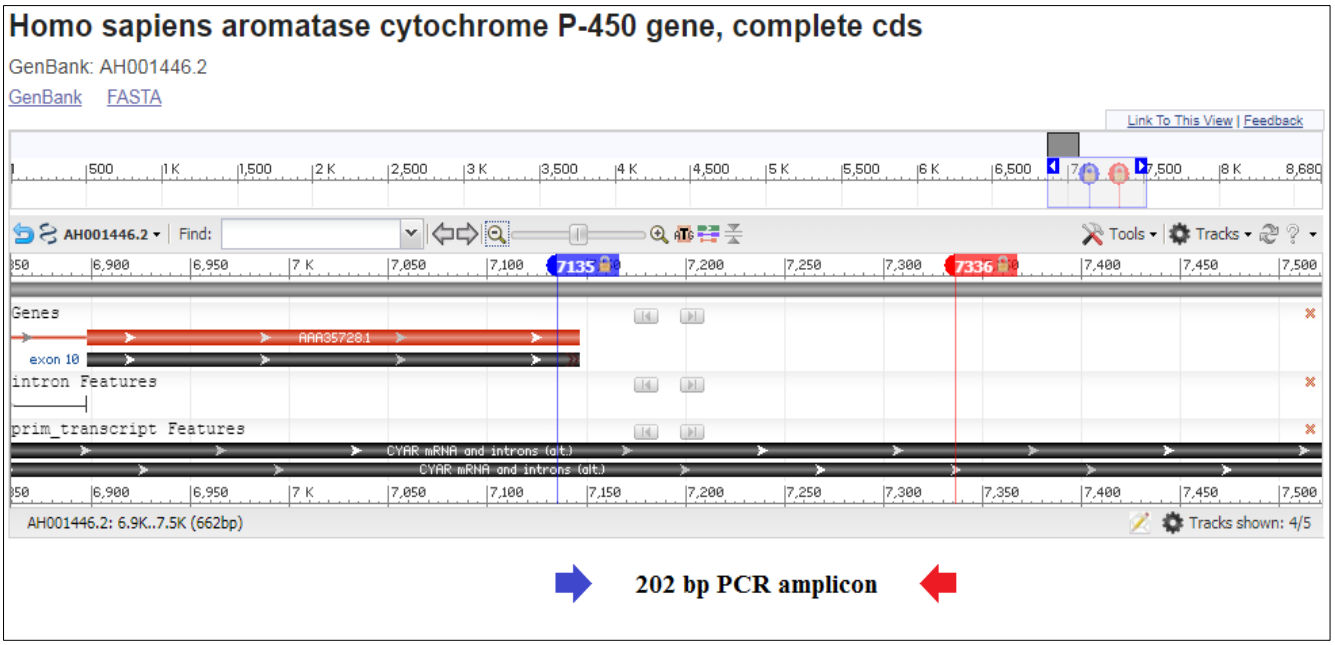

Figure 1 Exact location of the PCR amplified fragment within the 3′ UTR in the sequences of the CYP19 gene (acc no. AH001446.2). The blue arrow indicates the starting point of the amplification segment and the red arrow indicates the end point of this segment.

After knowing the location and sequence of the relevant fragment within the 3′ UTR in the sequences of the CYP19 gene, the details of these sequences were clarified (Table 2).

Table 2 Location and length of the 202 base pair PCR fragment used to amplify the 3′ UTR fragment in CYP19 gene sequences. The amplified sequence spanned from 7135 to 7336 within the NCBI reference sequence (GenBank acc. no. AH001446.2).

Amplified widget	**Reference site sequences (5′-3′)**	**Height**
CYP19 DNA sequences	CTGGAACACTAGAGAAGGCTGGTCAGTACCCACTCTGGAGCATTTCTCATC AGTAGTTCACATACAAATCATCCATCCTTGCCAATAGTGTCATCCTCACAG TGAACACTCAGTGGCCCATGGCATTTTATAGGCATACCTCCTATGGGTTGT CACCAAGCTAGGTGCTATTGGTCATCTGCTCCTGTTCACACCAGAGAAC*	202 bp

* Note: The back primer sequence is placed in a complementary and opposite manner to the sequence shown in Table 1 in order to match the forward primer sequence.

The alignment results for the 202 base pair fragment whose sequences were known indicated the presence of three point mutations that were diversely distributed in the ten samples compared to the reference sequences of the CYP19 gene (Figure 2).

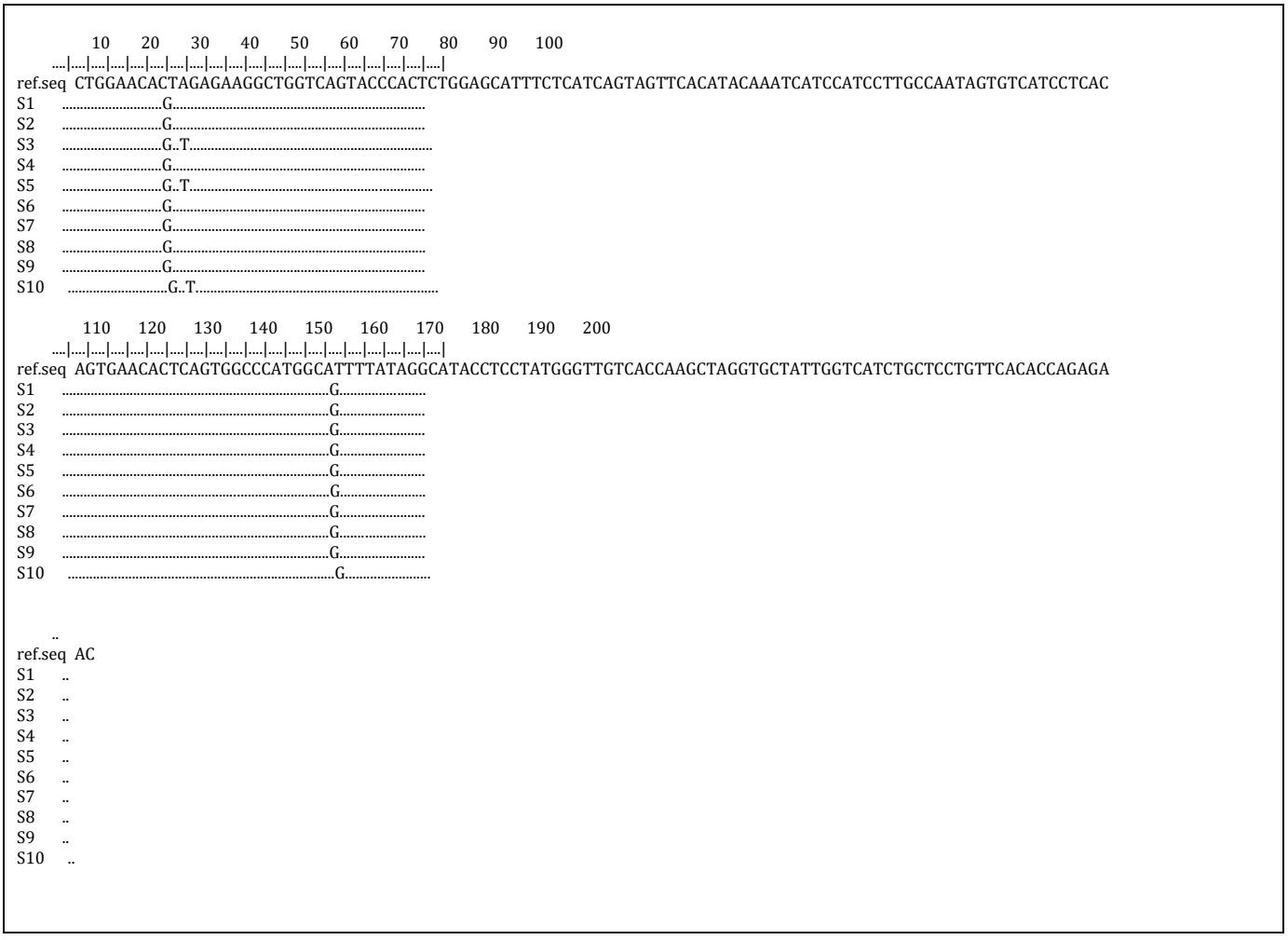

Figure 2 Observed DNA alignment sequences for ten people with polycystic ovary syndrome compared to reference sequences for the 202-base-pair segment of the 3′ UTR within the CYP19 gene. Each point mutation was indicated according to its location in the PCR product. The symbol "ref" indicates the NCBI reference sequences, while the symbols S1 to S10 indicate the numbers of the ten samples subjected to sequence identification experiments.

The sequencing chromatogram indicated the pattern and details of each point mutation detected (Figure 3).

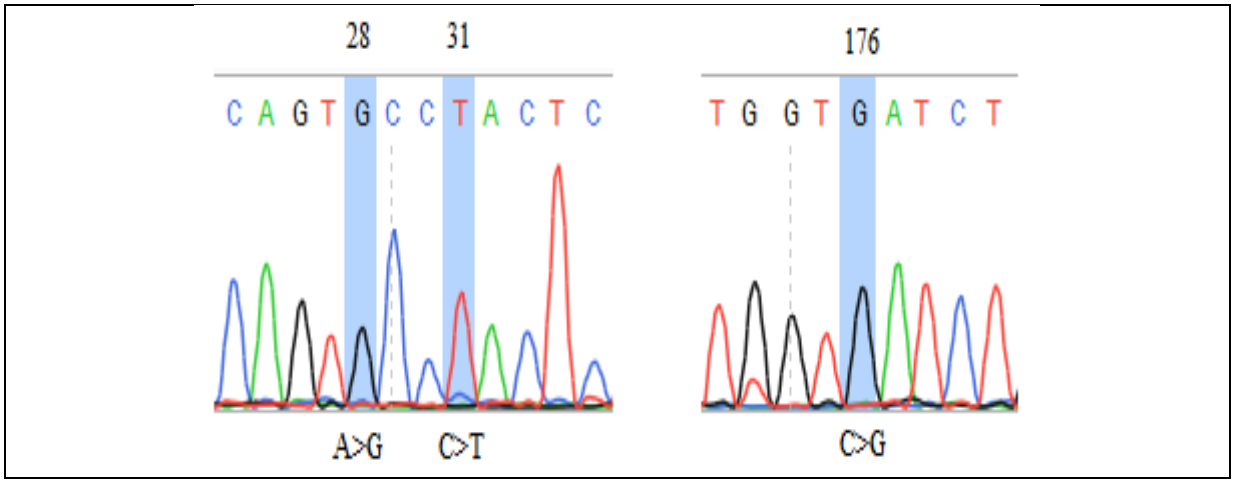

Figure 3 Chromatogram pattern of the 202-length genetic fragment of the 3′ UTR region within the CYP19 gene sequences. Each point mutation was indicated according to its location in the PCR product. The symbol ">" indicates the nature of the change in the nitrogenous bases of the point mutation in question.

3.1. Verifying the authenticity of the satisfied point mutations:

To determine the locations of the observed mutations in relation to the tables of mutations discovered within the 3′UTR site of the CYP19 gene and stored globally on the dbSNP website (https://www.ncbi.nlm.nih.gov/projects/SNP/), a series of procedures were carried out to determine The exact location of each mutation revealed. To find the nature of each observed mutation, each mutation discovered in this research was represented according to the data in the CYP19 gene stored in the NCBI dbSNP site within the fifteenth chromosome (15q21.2) (according to Chen et al., 1988). The dbSNP mechanism indicated the presence of two previously discovered mutations out of a total of three mutations, namely rs1201251894 and rs1163221780 (Figure 4). While the precedence of the third mutation g.27933770C>G has been confirmed here. Using the same tool, the position of each of these three point mutations was determined.

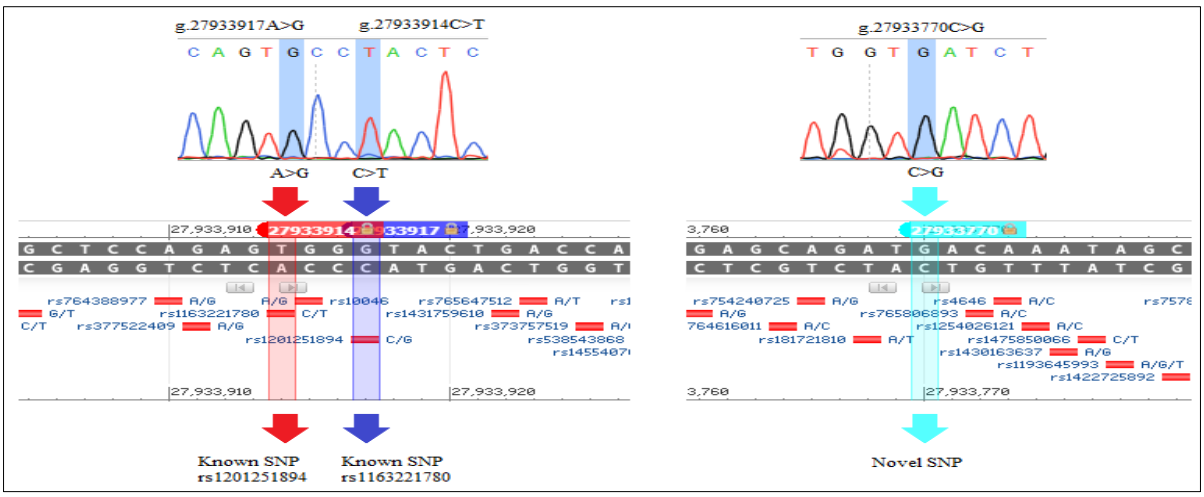

Figure 4 Determining the authenticity of the three point mutations discovered in the amplified 202 base pair genetic fragment of the CYP19 gene using the dbSNP mechanism. Each different color indicates one of the three mutations detected. One unprecedented mutation has been observed, g.27933770C>G (right).

To summarize the results observed in this report, which were extracted from the 202 base pair length fragment, the exact location of the observed variations was mentioned according to their locations in the NCBI reference sequences (Table 3).

Table 3 Description of point mutations observed in the 202 base pair segment of the CYP19 gene (serial number NT_010194.18). Note here the footnote for all three observed mutations.

N0.	mutation	Sample number	Location in the amplification plot	Location in reference sequences NT_010194.18	The nature of genetic variation	Location of the mutation within the gene	Mutation summary
1	A>G	All samples	28	27933917	Genetically identical	3′ UTR*	Already known mutation rs1201251894
2	C>T	Sample No. 3, 5 and 10	31	27933914	Genetically heterogeneous	3′ UTR	Already known mutation rs1163221780
3	C>G	All samples	176	27933770	Genetically identical	3′ UTR	previously unknown mutation (unprecedented mutation)

The CYP19 gene encodes an enzyme called cytochrome P450 aromatase (Tchoudakova and Callard , 1998), which is a specific enzyme for estrogen biosynthesis (Desta et al., 2009). The fact that some genetic variants of this gene fragment are associated with the progression of PCOS has been confirmed (Kado et al., 2002). Therefore, genetic variations in the CYP19 gene may confer variable aromatase activity, which may be associated with several variants of PCOS around the world. For this reason, the current study focused on this relationship for the Thi- Qar region. As shown in the results section, this study detected three mutations, namely g.27933917A>G, g.27933914C>T, and g.27933770C>G. The novelty of the latter mutation was confirmed in the present study. It was found that both mutations g.27933917A>G and g.27933770C>G were distributed in all samples examined, while it was found that the mutation g.27933914C>T was present only in samples No. 3, 5 and 10. However, the pattern of distribution of these point mutations may indicate a possible role for these mutations in the mechanism of development of polycystic ovary syndrome.

The only point surrounding this research that affects its scientific impact is the limited number of samples studied, which was limited to only ten samples. In any case, such restriction in collecting samples is linked to the many controls imposed on the researcher, which leads to a certain limitation in samples, in addition to the economic aspects related to cost, which must not be overlooked in this regard, all of which contributed to reducing the number of samples studied in this research. However, the current research provided a clear observation about the pattern of genetic variation in the 202 base pair segment of the CYP19 gene. This observation arose from the discovery of an unprecedented mutation in all the samples under study. This mutation detected here was found in all study samples. The universal presence of this mutation in all research samples must carry with it a possible relationship between this mutation and polycystic ovary syndrome within this geographical region. It is worth noting that polycystic ovary syndrome is not the only pathological condition associated with genetic variation for this amplified fragment (Kado et al., 2002). Many studies have demonstrated a significant relationship between some genetic variants of this same fragment with the development of other serious disease conditions, such as breast cancer (Ma et al., 2010, ; Zhang et al., 2009 ; Talbott et al., 2008).

Despite the small number of samples included in the research, the importance of the presence of such a mutation in all of these samples has added vital evidence to the serious relationship between the development of polycystic ovary syndrome and the exchange of cytosine to quinine at position 27933770 within the unprecedented mutation in the CYP19 gene sequences. Here, many computer analyzes are available to predict what the mutation will do to the structure of the resulting protein, its function, and its relationship with other proteins (Al-Shuhaib et al., 2018), which of course reflects on the pathological mechanism by which the product of the CYP19 gene intervenes in causing polycystic ovary disease. Unfortunately, this mutation occurred in the untranslatable site of the 3′UTR, so definitive prediction of the effect of this mutation is very difficult (Al-Shuhaib et al.,2017).

Although there is very little information related to these mutations discovered here, this discovery includes a special intervention for this unprecedented mutation in the progression of polycystic ovary disease in the population studied. For this reason, this research recommends expanding the scope of the study to include more samples over a larger

geographic range in Iraq to discover new mutations that may be important like this unprecedented mutation. The research recommends investigating the relationship of genotype variation with the precise pathological characteristics of the development of polycystic ovary syndrome and its severity from samples taken from the patients to be included in the research, in order to find out more details about the nature of this relationship, which may be fruitful in early detection of the possibility of developing polycystic ovary syndrome before it occurs from a standpoint. Clinical.

4. Conclusion

The universal presence of this mutation in all research samples must carry with it a possible relationship between this mutation and polycystic ovary syndrome within this geographical region. It is worth noting that polycystic ovary syndrome is not the only pathological condition associated with genetic variation for this amplified fragment. The results of this research help the scientific community specialized in this field to understand how this disease occurs.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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