Mutacije v CYP21A2 genu: povezava p.V281L mutacije s sindromom policističnih jajčnikov (PCOS)

Mutations of the CYP21A2 gene: Association of p.V281L mutation with polycystic ovarian syndrome (PCOS)

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Izvleček

Namen: *Gen CYP21A2 kodira encim 21–hidroksilazo, ki je odgovoren za proizvodnjo steroidnih hormonov, ki so temeljni posredniki pri doseganju spolne zrelosti in zanositvi. Pri bolnikih se okvarjenost gena izrazi v različnih obdobjih življenja z različno stopnjo vpliva na življenje. Namen študije je bil primerjati genetske profile žensk z nepojasnjenimi težavami pri zanositvi, z genetskimi profili zdravih kontrol. Hkrati je bila opravljena analiza povezave med mutacijami v genu CYP21A2 in različnimi kliničnimi in laboratorijskimi parametri.*

Metode: *V našo retrospektivno študijo smo vključili 300 žensk, ki jim je bila postavljena diagnoza neplodnost. Pri*

Abstract

Purpose: *The CYP21A2 gene encodes the enzyme 21–hydroxilase, which is responsible for the production of steroids. These hormones are key mediators of sexual development and conception. Patients with 21–hydroxilase deficiency tend to be affected in different stages of life. The purpose of this study was to compare the genetic profiles of women with unexplained infertility problems with the genetic profiles of healthy controls. Furthermore, were analyzed associations between mutations of the CYP21A2 gene and various clinical and laboratory parameters.*

Methods: *We enrolled 300 women, diagnosed with unexplained infertility problems, into this retrospective study.*

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preiskovankah smo določili pomembne klinične kazalce in pridobili laboratorijske vrednosti glede na fazo menstrualnega cikla. V kontrolno skupino smo vključili 100 vzorcev. Iz periferne venske krvi smo izolirali DNA in jo s pomočjo PCR pomnožili.

Rezultati: *Med testiranimi vzorci jih je bilo 11,6% s pomembnimi hormonskimi odkloni (HD) (estrogeni in/ali progestini in/ali celotnim testosteronom) in 5,3% s sindromom policističnih jajčnikov (PCOS). Ugotovili smo statistično pomembno povezavo med mutacijo p.V281L in prisotnostjo pomembnih HD (x^2 = 6,99, p = 0,01) in prisotnostjo PCOS (x^2 = 16,78, p = 0,00). Medtem ko nismo ugotovili statistično pomembnih razlik v frekvencah mutacij med preiskovanci in kontrolami. Prav tako nismo ugotovili statistično pomembne povezave med mutacijami v genu CYP21A2 in vrednostmi laboratorijskih kazalcev.*

Zaključki: *Rezultati kažejo na statistično pomembno povezavo mutacije p.V281L v genu CYP21A2 s frekvenco PCOS in s pogostostjo pomembnih HD.*

For each subject, we recorded important clinical and laboratory parameters during different phases of the menstrual cycle. In the control group, we enrolled 100 samples. Each subject provided a blood sample, which was used to isolate DNA for subsequent polymerase chain reaction (PCR).

Results: *In total, 11.6% of the test subjects exhibited significant hormonal deviations (HD) (estrogens and/or progestins and/or total testosterone) and 5.3% were diagnosed with polycystic ovarian syndrome (PCOS). We identified a significant association between the p.V281L mutation and the frequency of test subjects with significant HD (x^2=6.99, p=0.01). A similar association was also observed between p.V281L mutation and the frequency of test subjects with PCOS (x^2=16.78, p=0.00). However, we did not establish any associations between the frequency of mutations in test subjects when compared with controls. In addition, we did not find any significance in the frequency of CYP21A2 gene mutations and any of the laboratory parameters tested.*

Conclusions: *Our results identify a significant association of the p.V281L mutation in the CYP21A2 gene with the frequencies of both PCOS and significant HD.*

INTRODUCTION

Infertility is defined as the inability to conceive after one year of regular unprotected sexual activity. Despite the fact that previous research has provided us with information relating to hundreds of genes associated with infertility, we have yet to establish definitive links relating to the genetic determinants of human infertility. The World Health Organization (WHO) estimates that almost half of infertile couples will have an underlying genetic abnormality (1). A multitude of specifically regulated mechanisms have been identified that act as specific determinants of fertility. Unsurprisingly, the high frequency of infertile

couples results in improper urogenital organ formation, incorrect gamete maturation and faulty sexual behaviour (1, 2).

The hypothalamo-pituitary-adrenal (HPA) axis regulates the production of sex hormones, cortisol and adrenal steroid hormones. The adrenal glands are stimulated by adrenocorticotropic hormone (ACTH), which is produced from the pituitary, circulates in the blood and stimulates steroid production from the adrenal glands (1, 3, 4). Aldosterone regulates the balance of electrolytes and acidity while cortisol is involved in the regulation of carbohydrate, fat and protein. Dehydroepiandrosterone and androstenedione are the predominant circulating male sex hormones and are only present in females at low concentrations (2, 5). The main sex hormones in the ovaries are estrogens and progesterone. Estrogens mediate the proliferation of endometrial mucosa and sex maturation while progesterone is involved in uterine maturation, zygote implantation and lactation. Both of these hormones are steroids by definition, as are aldosterone and cortisol (1, 2, 6). The reproductive cycle in the female begins with the maturation of oocytes. During a woman's menstrual cycle, the ovaries eject one oocyte into the abdominal cavity where it is engulfed by the Fallopian tube. If successful fertilization occurs, the embryo implants itself into the uterus, leading to placental and fetal development (1, 2, 6, 7).

Mutation of the CYP21A2 gene leads to the reduced production of aldosterone and cortisol and the increased production of testosterone and dihydrotestosterone (2, 8). Due to the excess of androgens, it is possible that the hypothalamus might become desensitized. This condition leads to the hypothalamus increasing the synthesis of gonadotropin releasing hormone (GRH). In the developing fetus, an excess of androgens may disrupt the HPA, resulting in ovarian hyperandrogenism. Furthermore, the resultant increase in the tonic secretion of androgens leads to the inhibition of ovarian follicle maturation, resulting in the disruption of ovulatory cycles (1, 6, 9).

Ovarian hyperandrogenism is one of the basic features and clinical manifestations of polycystic ovary syndrome (PCOS). This is one of the most common endocrinopathies worldwide, affects 5–7% of women of reproductive age women, and is a complex genetic trait; strong heritability accounts for almost 70% of the development of this disorder (10, 11). As the name suggests, PCOS causes the formation of multiple, yet harmless, ovarian cysts which may cause hormonal imbalance. However, if caught early and treated correctly, the symptoms of PCOS can be managed and long–term problems can be avoided.

Hormones are chemical substances produced by glands and triggering numerous different processes, such as growth and energy production. Sometimes, one hormone triggers the release of another hormone. PCOS involves significant hormonal balance. Even a minor change in one hormone can trigger changes which result in consequential changes in other hormonal changes. As stated earlier, women produce small amounts of male androgens. However, in PCOS, women begin to produce more androgens than normal; this can cause a woman to grow excess facial and body hair, prevent ovulation and lead to acne. Insulin resistance can also be a problem in women with PCOS. When the body does not use insulin efficiently, blood sugar levels increase. Over time, this increases the risk of diabetes.

The cause of PCOS is not yet fully understood; however, genetics are thought to be a key factor. PCOS is a condition that appears to run in families, so the chances of having PCOS is higher if other women in a family have PCOS or have irregular periods or diabetes. Furthermore, PCOS can be passed down from either the maternal or paternal side (12).

Numerous studies have attempted to identify candidate genes that may be responsible for PCOS. These studies have been able to identify associations between candidate genes and PCOS but such studies also have some limitations in that they rely on the understanding of candidate genes and related diseases; they can produce false-positive results when the ethnic backgrounds of cases and controls are not well matched and only a few of these studies have yielded sufficiently robust results that could be replicated in different populations or by different research groups (13). Our current understanding is that PCOS candidate genes can be classified into four groups: genes involved in the biosynthesis and action of androgens (Group 1); genes related to metabolism (Group 2), genes correlated with inflammatory cytokines (Group

3) and other candidate genes (Group 4) (14).
The CYP21A2 gene is a gene involved in steroidogenesis and is considered to be an essential candidate of PCOS. A key part of steroidogenesis is the conversion of 17–hydroxyprogesterone into 11–deoxycortisol, a process catalysed by the 21–hydroxylase enzyme encoded by CYP21A2. Dysfunction of the 21–hydroxylase enzyme is responsible for 90% of disrupted steroid hormone production and is responsible for most cases of congenital adrenal hyperplasia (15). Deficiency of the 21–hydroxylase enzyme is associated with increased levels of serum 17–hydroxyprogesterone. This is a common finding in women with functional hyperandrogenism or PCOS: an increased serum 17–hydroxyprogesterone response to adrenocorticotropin (ACTH) stimulation (15).
In order to identify the modifying loci responsible for unexplained fertility problems, we performed CYP21A2 mutation analysis for four candidate modifier loci on genomic DNA samples obtained from 300 women diagnosed with unexplained fertility problems. The genetic profiles of women with unexplained fertility problems were then compared with the genetic profiles with healthy controls. We also analysed the associations between the most common mutations of the CYP21A2 gene (p.P30L, c.290–13A/C>G, p.I172N and p.V281L) and various clinical and laboratory parameters.

MATERIALS AND METHODS

Study sample

This retrospective study included 300 females with unexplained infertility problems and 100 female healthy controls without a history of infertility. Females with unexplained infertility problems were referred to the Laboratory of Medical Genetics, Department of Reproductive Medicine and Gynecologic Endocrinology, Medical Clinical Centre Maribor, Maribor, Slovenia, for routine karyotyping. All of these women were childless, unrelated individuals of reproductive age, and had not received hormonal therapy for at least three months prior to the study commencing. The subjects were of Caucasian origin as residents of different geographical areas of Slovenia. Their personal and clinical data of interest was obtained from a questionnaire designed specifically for this study (including age, ethnicity, weight, height and family reproductive history) and existing medical records were collected between 2005 and 2012. Female healthy controls were blood donors with an unremarkable reproductive history and were recruited through the Department of Transfusiology, Medical Clinical Centre Maribor, Maribor, Slovenia. This study was approved by the hospital ethical committee (Reference No. 117/03/11) and informed consent was obtained from all participating individuals.

Clinical and biochemical parameters
A peripheral venous blood was collected during the phases of the menstrual cycle to interpret the value of hormones that may be relevant to fertility. Biochemical analysis of serum samples was performed using the Architect i1000 system (Abbott Diagnostics, Illinois, USA) using chemiluminescent Chemiflex technology according to the manufacturer's protocols. A range of data were collated for each subject: the concentration of serum prolactin (nmol/L), the concentration of serum dihydroepiandrosteron (nmol/L), the concentration of free testosterone in serum (nmol/L), the concentration of Anti-Muller hormone levels in serum (nmol/L) and body mass index (BMI). Subjects were also investigated for the presence of thyroid disease, autoimmunodisease or PCOS.
During the follicular phase of the menstrual cycle were also determined the serum concentrations of follicle-stimulating hormone in serum (nmol/L), luteinizing hormone (nmol/L) and 1,2–estradiol (nmol/L). During the luteal phase of the menstrual cycle, we also determined the concentration of serum progesterone (nmol/L).

Sample collection

Peripheral venous blood was collected in standard vacutainer tubes, containing EDTA anticoagulant. Genomic DNA was extracted from blood leukocytes using a simple method which involved salting out the cellular proteins by dehydration and precipitation with a saturated NaCl solution (29).

Polymerase chain reaction for the most common mutations in the CYP21A2 gene

Allele–specific polymerase chain reaction (PCR) was used to screen p.P30L, c.290–13A/C>G, p.I172N and p.V281L, the most common mutations in the CYP21A2 gene. Allele–specific PCR is used to determine specific single nucleotide variants (SNVs) and is based on specific hybridization of the initial oligonucleotides to a target DNA, thus enabling the multiplication of either the mutated or normal allele. During the initial phase of the initial oligonucleotide, a double helix is formed between the initial oligonucleotide and the denatured target DNA. The 3 'end (–OH group) is a site recognized by the DNA polymerase to begin the synthesis of another complementary chain. When the last 3' base does not match the substrate, it is much more difficult or even impossible, for the DNA polymerase to continue making the DNA strand. Multiplication of the PCR fragment does not stop if the initial oligonucleotide is not completely complementary to the target DNA due to the presence of genetic modification. If each sample is multiplied by a pair of oligonucleotides for a normal and mutant sequence, then allele-specific PCR also allows the detection of heterozygotes. One to two days were required to complete the test. Allele-specific PCR is a highly sensitive molecular genetic test that detects a mutation if it is present at least in 1–5%. Furthermore, the test does not require special equipment. It is important to emphasize that allele-specific PCR is a target-specific molecular genetic test that does not allow the detection of other mutations that may be present in the DNA sample; only the targeted mutation will be detected. Allele-specific PCR amplification of all DNA samples involved in this study was carried out in a total volume of 5 µL containing 1 µM of each primer for the mutant sequence (Table 1),

Table 1: *List of polymerase chain reaction (PCR) primers used to detect the four most common mutations in the CYP21A2 gene.*

Mutation	Position	PCR primer	PCR primer sequence
p.P30L	exon 1	CAH-30LT-P-F CAH-I1-A-R1	5'-AAGCTCCGGAGCCTCCACCTACT-3 5'-AGCATAGCAAGAACCCATCTGTT-3'
IVS2-13C>G	intron 2	CAH-I2-A2-F CAH-In2-M-G-R	5'-CTAACTACATATCTGGTGGGGAGAAAGC-3' 5'-CAGCTTGTCTGCAGGAGGCGC-3'
p.I172N	exon 4	CAH-172NA-F CAH-I172N-A-RL	5'-TCTCTCCTCACCTGCAGCATGAA-3' 5'-TTCATGTCGTCCTGCCAGAAAAGCAG-3'
p.V281L	exon 7	CAH-I6-A-F CAH-281LT-P-R	5'-CAGCACAAGGTGGGGACTGGAC-3' 5'-GGTCCACTGCAGCCATGTGAAA-3'

1x Multiplex PCR kit (; Qiagen GmbH, Hilden, Germany) and 50 ng of genomic DNA. A negative control, containing the same reaction mixture but excluding the genomic DNA template, was included in every experiment. An initial denaturation step at 95°C for 15 minutes was followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s and extension at 72°C for 1 min. Each PCR product was then separated by electrophoresis on a 3% agarose gel for 15 min at 160 V, stained with SYBR Green I and visualized and photographed under ultra-violet illumination for documentation and mutation scoring (Figure 1).

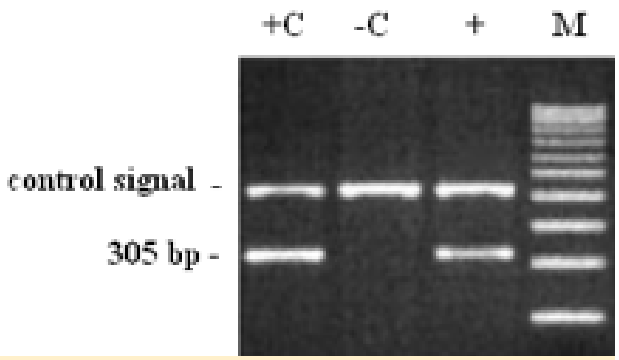

Figure 1. *305 bp PCR products of p.V281L mutation in the CYP21A2 gene; lane +C: positive control (sample with p.V281L mutation), lane -C: negative control (sample without p.V281L mutation), lane +: mutation is present, M: marker.*

Statistical Analyses

SPSS–21 (Statistical Package for the Social Science, version 21 software program (IBM Corporation, Armonk, NY, USA)) was used for all statistical analysis. Data are expressed as an arithmetic mean ± standard deviation (SD). Relationships between variables were analysed by analysis of variance (ANOVA). The distribution of mutations was compared using the chi–square test (x^2) where $p \leq 0.05$ was considered as statistically significant.

RESULTS

This study involved 300 infertile female patients with average and median age of 30 years (range 20-40 years) and 100 healthy female blood donors (the control group) with average and median age of 42 years (range 27-66 years). Significant clinical parameters, biochemical parameters and the simultaneous presence of a thyroid disease, an autoimmune disease, PCOS, conditions related to the concentration of sex hormones and conditions related to the concentration of progesterone were determined and presented in Table 2 and Table 3.

Table 2: *Laboratory data for the probands.*

Parameter (nmol/L)	N of probands	Minimum	Maximum	Mean	SD
FSH (nmol/L)	164	1.80	73.40	6.85	2.71
LH (nmol/L)	161	1.10	55.20	4.53	4.80
Prolactine (nmol/L)	160	4.60	141.30	18.06	14.35
1,2-estradiol (nmol/L)	143	<0.04	6.10	0.30	0.83
AMH (nmol/L)	65	0.14	88.10	3.26	10.84
Whole testosterone (nmol/L)	30	0.4	5.20	2.65	1.21
Unbound testosterone (nmol/L)	24	1.00	24.00	5.08	5.37
DHEA (nmol/L)	23	2.40	12.30	6.09	2.61

SD = standard deviation; FSH = follicle stimulating hormone; LH = luteinising hormone; AMH = Anti-Muller hormone; DHEA = dihydroepiandrosterone.

Table 2 shows that the laboratory parameter with the highest average was prolactin (18.06 nmol/L, SD=14.35) , while the averages of 1,2–estradiol (0.30 nmol/L, SD=0.83) and whole testosterone (2.65 nmol/L, SD=1.21) were the lowest.

Table 3: *Number and proportion of probands with irregularities.*

Laboratory parameter/clinical indicators	N (%)
Hor	37 (11.6)
Est	20 (6.30)
Prog	3 (0.9)
TD	17 (5.5)
AD	5 (1.6)
PCOS	17 (5.3)

Hor = deviation of estrogens and/or 17á -OH progesterone and/or testosterone in serum; Est = deviation of estrogens in serum; Prog = deviation of 17á-OH progesterone in serum; TD = thyroid disease; AD = autoimmune disease; PCOS = polycystic ovary syndrome.

Similarly, it can be seen from Table 3, that 11.6% of probands had irregularities within the concentration of hormones. At the same time, only 0.9% of probands had deviation of 17-OHP in serum.

Next, we compared probands and controls in terms of the frequencies of p.P30L, c.290–13A/C>G, p.I172N and p.V281L, the most common mutations in the CYP21A2 gene. Table 4 shows that there were no significant differences in the mutation frequencies for all four of these most common mutations (x^2 = 2.25, p = 0.32).

Table 4: *p.P30L, c.290-13A/C>G, p.I172N and p.V281L mutation frequencies in probands and controls.*

Mutation in CYP21A2	Probands (n=300) (%)	Controls (n=100) (%)	χ^2 *	p-value*
c.290-13A/C>G	2 (0.67)	1 (1.00)	0.33	0.56
p.I172N	2 (0.67)	3 (3.00)	0.20	0.66
p.P30L	0 (0.00)	0 (0.00)	0.00	0.00
p.V281L	1 (0.33)	0 (0.00)	1.41	0.49
∑	5 (1.67)	4 (4.00)	2.25	0.32

* chi-square (χ^2) test

Next, we used ANOVA to test the possible impact of p.P30L, c.290–13A/C>G, p.I172N and p.V281L mutations in the CYP21A2 gene upon laboratory parameters. ANOVA showed no effect of these mutations on laboratory parameters for probands. We then investigated the potential impact of p.P30L, c.290–13A/C>G, p.I172N and p.V281L upon clinical parameters. For this purpose, we used association analysis. Clinical parameters of the probands with p.P30L, c.290–13A/C>G, p.I172N and p.V281L

mutations were compared with probands without p.P30L, c.290–13A/C>G, p.I172N and p.V281L mutations (Table 5).

Table 5: *The impact of p.P30L, c.290-13A/C>G, p.I172N and p.V281L mutations upon clinical parameters.*

Clinical parameter	IVS2	I172N	p.P30L	p.V281L
Hor	$\chi^2 = 0.58$	$\chi^2 = 0.58$	$\chi^2 = 0.00$	$\chi^2 = 6.99$
	p = 0.45	p = 0.45	p = 0.00	p = 0.01
Est	$\chi^2 = 0.30$	$\chi^2 = 0.30$	$\chi^2 = 0.00$	$\chi^2 = 0.07$
	p = 0.58	p = 0.58	p = 0.00	p = 0.79
Prog	$\chi^2 = 0.04$	$\chi^2 = 0.04$	$\chi^2 = 0.00$	$\chi^2 = 0.01$
	p = 0.85	p = 0.85	p = 0.00	p = 0.92
TD	$\chi^2 = 0.26$	$\chi^2 = 0.26$	$\chi^2 = 0.00$	$\chi^2 = 0.06$
	p = 0.61	p = 0.61	p = 0.00	p = 0.80
AD	$\chi^2 = 0.08$	$\chi^2 = 0.08$	$\chi^2 = 0.00$	$\chi^2 = 0.02$
	p = 0.78	p = 0.78	p = 0.00	p = 0.89
PCOS	$\chi^2 = 0.24$	$\chi^2 = 0.24$	$\chi^2 = 0.00$	$\chi^2 = 16.78$
	p = 0.62	p = 0.62	p = 0.00	p = 0.00

Hor = deviation of estrogens and/or 17á-OH progesterone and/or testosterone in serum; Est = deviation of estrogens in serum; Prog = deviation of 17á-OH progesterone in serum; TD = thyroid disease, AD = autoimmune disease; PCOS = polycystic ovary syndrome.

DISCUSSION

Most of the candidate genes associated with PCOS are involved in the biosynthesis and activity of androgens. Some of these genes are also related to metabolism or are correlated with inflammatory cytokines. One of the basic characteristic and clinical manifestations of PCOS is hyperandrogenism. One important candidate PCOS gene related to steroidogenesis is CYP21A2, which encodes the 21–OH enzyme responsible for the catalysis of one of the two steps of steroidogenesis (14).

In our study, we performed CYP21A2 mutation analysis for p.P30L, c.290–13A/C>G, p.I172N and p.V281L candidate modifier loci on genomic DNA samples obtained from 300 females, diagnosed with unexplained fertility problems. The genetic profiles of females with unexplained fertility problems were compared with those from healthy controls. We also analyzed the associations between p.P30L, c.290–13A/C>G, p.I172N and p.V281L, the most common mutations in the CYP21A2 gene, and various clinical and laboratory parameters. We did not find any statistical differences between the frequency of these mutations in infertile females and the healthy female blood donors which were used as controls. However, it is important to point out that although we did not identify any statistically significant associations, we cannot exclude the potential importance of p.P30L, c.290–13A/C>G, p.I172N and p.V281L CYP21A2 mutations in infertility. Furthermore, in samples without these four most important CYP21A2 mutations, we cannot exclude the presence of other mutation variants in the CYP21A2 gene.

After reviewing the available medical documentation, we examined the hormonal profiles of our subjects during different stages of the menstrual cycle and determined the presence of any diagnosed concomitant illnesses. We did not find any statistically significant association between mutation frequency, hormonal deviations and concomitant illnesses. Nevertheless, we can conclude that hormonal status is not affected by the presence of a single mutation in the CYP21A2 gene.

On the other hand, we identified two important associations. First, the association between the p.V281L CYP21A2 mutation and the deviation of estrogens and/or 17–OHP and/or testosterone in the serum (p=0.01). Second, the association between the p.V281L CYP21A2 mutation and the frequency of PCOS (p=0.00). Previous research reported an increased serum 17–hydroxyprogesterone response to ACTH simulation in women with PCOS (16, 17). Several studies of European populations have investigated CYP21A2 gene mutations and their potential association with PCOS. Two studies showed that children with premature pubarche, and adolescent girls with hyperandrogenism, were heterozygous for mutations in CYP21A2 (18, 19). Five further studies reported two of the most prevalent genetic defects, the c. 655A/C>G (IVS2–13A/C>G) and c. 1683G>T (p.Val281Leu) mutations (20-24). Other research studies have investigated the contribution of CY-

P21A2 heterozygosity to the pathogenesis of PCOS. However, such studies found that the frequency of CYP21A2 heterozygous mutations in PCOS women and controls did not differ (25), or found that there was no clear concordance between the CYP21A2 genotype and the functional origin of excessive androgen levels (26, 27). This could be partly explained by the different methodologies used by different researchers, and by the diversity of the clinical phenotype among different patients. Although we found two statistically significant associations, we cannot confirm an actual association due to the small number of subjects studied. Further research is now necessary to clarify whether the CYP21A2 and p.V281L associated mutations play a key role in the development of PCOS.

CONCLUSION

From our findings, we concluded that there were no significant differences in the prevalence of CYP21A2 mutations in females diagnosed with unexplained fertility problems when compared with the genetic profiles of healthy controls without a history of infertility. However, our results also demonstrated a significant association between the p.V281L CYP21A2 mutation and (1) the frequency of significant hormone deviations and (2) PCOS. Moreover, we believe that the p.V281L mutation requires further research with a large number of probands in order to fully elucidate its association with PCOS.

In the future, broad population genome-wide association study (GWAS), using multiple gene markers and extensive clinical information, will be important in helping clinicians to not only treat disease but also to predict the risk of concomitant disease occurrence (28). Replication studies for the newly discovered variants in larger cohorts should also be performed in samples from different genetic backgrounds and new techniques such as next generation sequencing (NGS) should be deployed. NGS in particular, may identify rare variants and provide a significant advancement in our understanding of the genetic basis of this PCOS. Collectively, this information should contribute to a better understanding of the pathogenesis of PCOS and lead to the development of risk prediction models and new therapeutic strategies for the care of PCOS patients.

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