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Investigation of Single Nucleotide Polymorphisms within the *FSHR* Gene and Biological Network Analysis of Non-Coding RNAs in Polycystic Ovary Syndrome Patients

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Abstract

BACKGROUND/AIMS: Polycystic ovary syndrome (PCOS) is associated with numerous health issues. The study aims to examine the allelic frequencies of the *follicle stimulation hormone receptor (FSHR)* gene, specifically for the oogenesis-regulating variants p.Ser680Asn (c.2039C>T; rs6166) and p.Ala307Thr (c.919G>A; rs6165). Further in biological network analysis, the aim is to investigate the relationship of the *FSHR* gene with non-coding RNAs, symptoms of PCOS, and drugs used for PCOS treatment.

MATERIALS AND METHODS: One hundred and twenty whole blood samples were collected from non-PCOS and PCOS females. Real-time polymerase chain reaction was applied in the assessment of single nucleotide polymorphisms (SNPs) in the *FSHR* gene. The relationship of this gene with miRNAs and lncRNAs and the association of FSHR with drugs, symptoms of PCOS, and other associated diseases were investigated.

RESULTS: The outcome of this project did not show significance in the investigated FSHR polymorphisms between PCOS patients and the control group. Biological network analysis showed that this gene was associated with a number of miRNAs and lncRNAs. Furthermore, this gene was associated with a number of PCOS symptoms besides other women's health anomalies.

CONCLUSION: The SNPs of the FSHR, p.Ser680Asn (c.2039C>T; rs6166) and p.Ala307Thr (c.919G>A; rs6165), did not seem to have an association with the pathophysiology of PCOS. Therefore, it remains a possibility that FSHR polymorphisms involved in symptoms or progression of PCOS. However, these SNPs have been associated with many symptoms. Thus, further analysis must be performed to investigate these in larger populations.

Keywords: Polycystic ovary syndrome, polymorphism, SNP

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifactorial and complex disorder where patients may have metabolic abnormalities and obesity.[1,2] PCOS is a multifaceted condition influenced by a combination of genetic[3] and environmental factors.[4,5] Single nucleotide polymorphisms (SNPs) may affect hormonal regulation, influencing normal oocyte development, in which hormonal imbalances can lead to the development of PCOS.[6] Around 80% of females with PCOS produce high levels of androgens.[7] PCOS has been linked to ovarian

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hormone abnormalities resulting in anovulation, hyperinsulinemia, and hyperandrogenemia. Additionally, studies prove that females with PCOS are at an increased risk of endometrial cancer.[8]

Since studies have shown a possible genetic and epigenetic regulation in PCOS development, investigation of molecular mechanisms regulating PCOS has become a hot topic. follicle stimulation hormone (FSH) mediates its function through the follicle stimulation hormone receptor (FSHR),[9] and it is responsible for development of follicles, regulation of steroid synthesis and maturation of oocytes.[10] Previously published studies genotyped the FSHR Ala307Thr and FSHR Ser680Asn and analysed the association with PCOS, showing a statistically significant correlation between FSHR Ala307Thr and FSHR Ser680Asn polymorphisms and PCOS patients.[11] Another study in China revealed a clear correlation between Ser680Asn and PCOS;[12] Ala307Thr is statistically correlated with PCOS in Italian patients.[13] On the contrary, no such association was reported for Ala307Thr and Ser680Asn between PCOS patients and controls in the Turkish population.[14] Similarly, no association between Ser680Asn polymorphism and PCOS patients was reported in the Han ethnic population in China,[15] and in the Netherlands.[16]

Since the evaluation of SNPs has proven important in identifying regulatory functions in disease development, such as PCOS, especially in genes like FSHR where discrepancies have been reported, this practice is crucial. This study aimed to examine the allelic frequencies of the *FSHR* gene, specifically for the oogenesis-regulating variants p.Ser680Asn (c.2039C>T; rs6166) and p.Ala307Thr (c.919G>A; rs6165). Further biological network analysis was aimed at investigating the relationship of the *FSHR* gene with non-coding RNAs, the symptoms of PCOS, and drugs used for PCOS treatment.

MATERIALS AND METHODS

Sample Collection and Genotype Analysis

In this project, samples from patients undergoing gynaecological assessment were taken at the Near East University Hospital Scientific Research Ethics Committee (approval number: YDU/2019/67-784, date: 28.03.2019) granted the ethical approval, and each patient signed the informed consent. The inclusion criteria for both groups included subjects ages 18-35 years with a normal body mass index (MRI). The control group included patients undergoing routine check-up in the gynaecology clinic who have a normal menstrual cycles with normal ovarian morphology. Patients with PCOS were diagnosed according to the Rotterdam criteria by an expert gynecologist. The exclusion criteria included patients with secondary causes of PCOS, such as those diagnosed due to hormonal therapy. Additionally, subjects with syndromes such as Down syndrome were excluded from the study. One hundred and twenty subjects were divided into two groups consisting of 65 PCOS patients and 55 subjects with no PCOS diagnosis. All the subjects are of Turkish origin.

DNA extraction was performed using a commercial kit (Pure Link Genomic DNA Mini Kit, Thermo Fisher Scientific) following the manufacturer's instructions. The NanoDrop ND-200 (Thermo Scientific, Pittsburgh, United States of America) was utilized to determine the quantity and quality of the DNA obtained. The real-time polymerase chain reaction (PCR) followed by high resolution melting (HRM) analyses were applied to examine the *FSHR*, p.Ser680Asn (c.2039C>T; rs6166) and p.Ala307Thr (c.919G>A; rs6165), following manufacturer's protocol (LightCycler SYBR Green 480) with a final primer concentration of 2.5 µM. The primer sequences were obtained from a previously published study.[17] The results were analyzed using dedicated software.

Statistical Analysis

Version 25 of the SPSS statistical program was used to analyze the data. Fisher's exact test was used to analyze the heterozygosity of the SNPs within the *FSHR* gene in both PCOS and control groups. Statistical significance was determined as a p value < 0.05, and the HWE test was conducted for the PCOS group of FSHR p.Ala307Thr (c.919G>A; rs6165).

Biological Network Analysis

The relationship of *FSHR* gene variations with the PCOS symptoms and related women's health issues was studied.[18] The relationship between the *FSHR* gene, along with the other genes within the GnRH signalling pathway, was examined to analyse the drug association using the DGIdb site.[19] Finally, the relationship between the *FSHR* gene and microRNAs and lncRNAs was investigated.[20,21]

RESULTS

The primary goal of the project was to examine the two SNPs of *FSHR* implicated in PCOS. The whole blood samples used for this project consisted of 65 PCOS patients and 55 females without PCOS. In both groups, the mean age was 20 years, and the MRI was 17.

The SNP analysis of the *FSHR* gene, p.Ser680Asn (c.2039C>T; rs6166) and p.Ala307Thr (c.919G>A; rs6165), was performed using real-time PCR and HRM, in which the generated amplicon was progressively melted. In the PCOS group, the heterozygosity of *FSHR* Ala307Thr was 96.6%, while in the control group, it was 100% (Figure 1). Consequently, no statistically significant difference was found between the two groups (p=0.498). Due to the absence of PCR amplification, six samples from the PCOS category and four samples from the control category were ruled out from the project. For *FSHR* Ser680Asn, both the PCOS and control groups exhibited 100% heterozygosity. Due to the absence of PCR amplification, six samples from the PCOS group and one sample from the control group were ruled out from the project. The chi-square test for HWE in the PCOS group of FSHR p.Ala307Thr (c.919G>A; rs6165) was 51,271, and none (0.0%) have expected frequencies less than 5. The minimum expected frequency is 14.8.

In the second part of this study, a number of biological network analyses were performed. The association of *FSHR* gene, along with the FSHB, CGA, and GNAS, which also function in the GnRH signalling pathway, was shown to interact with a number of drugs, such as cisplatin, mifepristone, and urofollitropin (Figure 2). The SNPs investigated were associated with a number of PCOS symptoms as well as related women's health issues (Figure 3). Additionally, *FSHR* was shown to interact with a number of miRNAs and lncRNAs (Figure 4).

DISCUSSION

PCOS is a common and complex endocrine disorder where the pathophysiology is believed to be significantly influenced by both genetic and environmental factors.[22] In this investigation, real-time PCR and HRM analysis were used to investigate the association of *FSHR* Ala307Thr and *FSHR* Ser680Asn with PCOS. The study's findings did not show any significance in heterozygosity status for two FSHR polymorphisms investigated in the PCOS and control groups. Thus, this study suggests that the p.Ala307Thr and p.Ser680Asn *FSHR* gene

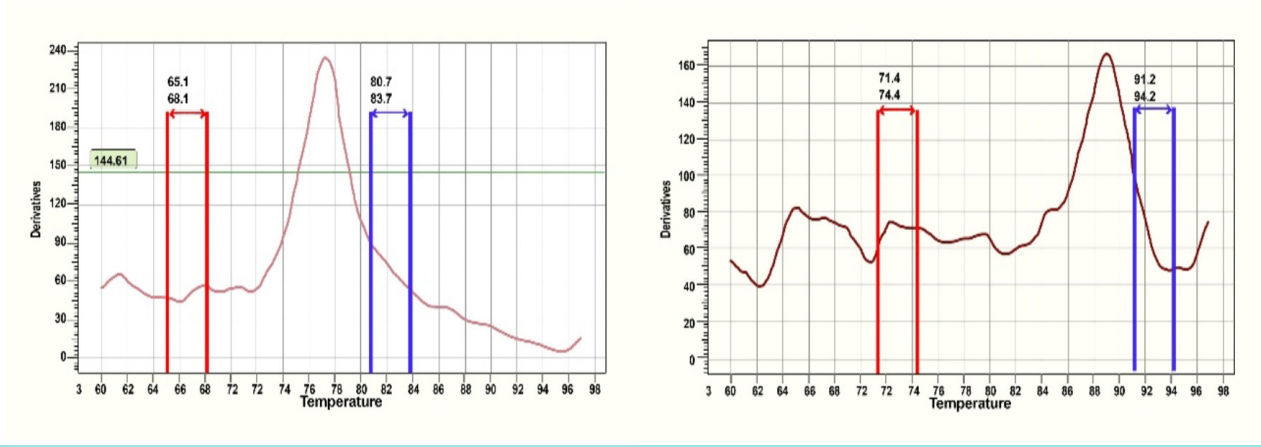

Figure 1. (a) HRM image showing the amplification of mutant type allele p.Ala 307Thr(c.919 G<A; rs6165). (b) HRM image showing the amplification of wild type allele p.Ala307Thr.

HRM: High resolution melting.

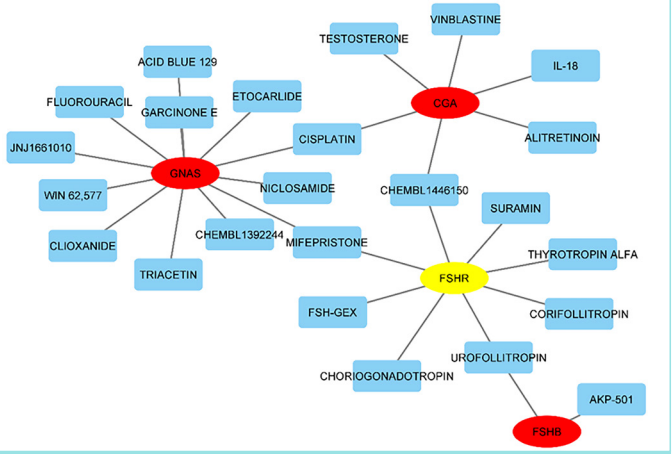

Figure 2. The association of drugs on selected genes.

FSHR: Follicle stimulation hormone receptor.

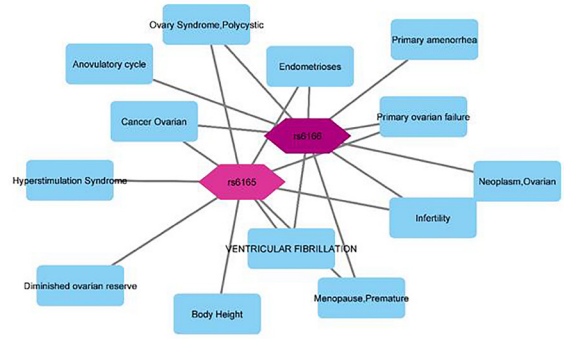

Figure 3. The association of FSHR SNPs with PCOS symptoms and related women's health abnormalities.

FSHR: Follicle stimulation hormone receptor, SNPs: Single nucleotide polymorphisms, PCOS: Polycystic ovary syndrome.

polymorphisms may not have a significant impact on PCOS in the studied group. In accordance with our findings, several studies also showed that there was no significant association between PCOS and FSHR Ala307Thr and FSHR Ser680Asn.[14-16] In conflict with our results, a number of studies indicated a statistically significant link between FSHR Ala307Thr[13] and FSHR Ser680Asn[21] polymorphisms in females diagnosed with PCOS,[14,15] in different populations. These contrasts among different studies may be due to variations in ethnicity or the sample size.

Biological network analysis was shown to be a valuable tool in health sciences to understand the possible interactions of gene variations, diseases, and their symptoms. An association between drug response or resistance and several genes has been reported, including FSHR. In the Caucasian populations, a number of genes including FSHR were reported as potential candidates to predict drug response and resistance for PCOS treatment. It was reported that PCOS patients with G/G *FSHR* polymorphism (rs6166) has lower chance in restoring ovulation under clomiphene citrate treatment.[23] The results of this study showed that *FSHR* SNPs are particularly associated with a number of PCOS symptoms. A previously published study reported that there is no association between *FSHR* (rs6166, rs6165, rs2349415) and PCOS symptoms, though an association was reported with SNVs *ESR2* rs4986938 and *LHCGR* rs2293275.[24] It is possible that, with an increased sample size or in a different ethnic group, outcomes would differ. Thus, to better understand the involvement of FSHR polymorphisms, a large cohort study should be organized. Numerous studies have utilized bioinformatics tools to investigate gene expression levels and their correlation with miRNA and lncRNAs. Since non-coding RNAs have a critical role in the modulation of gene expression, the biological network analyses have played an important role in identifying the underlying mechanisms of the molecular regulation as well as the possible biomarkers. A recently published study investigated the lncRNA-miRNA-mRNA interaction networks *via* coding-non-coding gene co-expression networks and ceRNA network analysis showing that *FSHR* was down-regulated in PCOS patients and that there was both positive and negative correlation of lncRNAs with the six genes, including *FSHR*25.

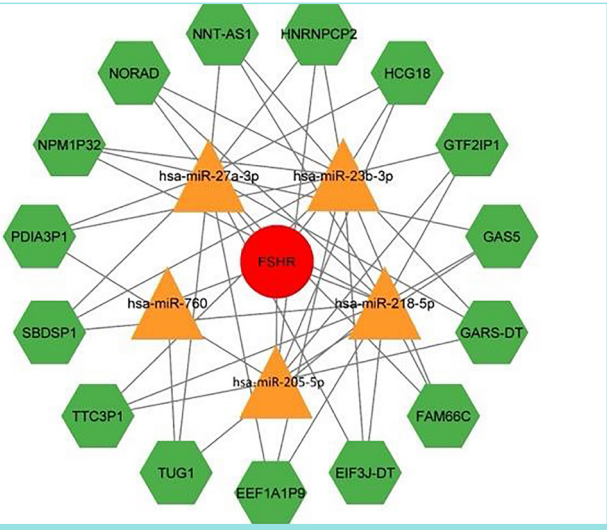

Figure 4. The relationship between *FSHR* gene and miRNAs and lncRNAs.

FSHR: Follicle stimulation hormone receptor.

CONCLUSION

In conclusion, this study found no significant difference in the heterozygosity of the *FSHR* gene polymorphisms, p.Ser680Asn (c.2039C>T; rs6166) and p.Ala307Thr (c.919G>A; rs6165), between the PCOS and control groups in the Turkish population. Biological network analysis was employed to investigate the possible association between PCOS symptoms and gene variations. Additionally, the association of *FSHR* with non-coding RNAs was investigated. This study postulates a foundation for future research, focusing on the regulation of the *FSHR* gene by non-coding RNAs and the potential identification of PCOS biomarkers.

MAIN POINTS

- No association of FSHR p. Ala307Thr (c.919G>A; rs6165) and p. Ser680Asn (c.2039C>T; rs6166) was observed in PCOS patients of Turkish origin.
- Biological network analysis of the *FSHR* gene in association with PCOS showed associations with miRNA and lncRNA.
- Biological network analysis for *FSHR* gene showed an association between PCOS-related symptoms and women's health abnormalities.

ETHICS

Ethics Committee Approval: Ethics committee approval was received for this study from Near East University Hospital Scientific Research Ethics Committee (approval number: YDU/2019/67-784, date: 28.03.2019).

Informed Consent: The patients signed the informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.Ö., A.C.Ö., Concept: P.T., Design: P.T., Data Collection and/or Processing: B.Ö., A.C.Ö., Analysis and/or Interpretation: S.M., B.O.-H., Literature Search: S.M., P.T., Writing: S.M., B.O.-H., P.T.

DISCLOSURES

Conflict of Interest: The authors declare no conflicts of interest.

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