

An Association Study between INSR/NsiI (rs2059806) and INSR/PmII (rs1799817) SNPs in Women with Polycystic Ovary Syndrome from West Azerbaijan Province, Iran

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Abstract

Background: It has been demonstrated that insulin signaling pathway related genes have important roles in polycystic ovary syndrome (PCOS) risk. The goal of present investigation was to assess the potential association between INSR/NsiI (rs2059806) and INSR/PmII (rs1799817) SNPs and PCOS.

Methods: 50 women with PCOS and 47 normal controls entered the study. NsiI and PmII SNPs in the INSR gene were determined by RFLP-PCR.

Results: INSR/NsiI (rs2059806) SNP GG, GA, AA, G and A genotypic and allelic frequencies were 45(90%), 5(10%), 0(0%), 95(95%) and 5(5%) in cases and 41 (87.2%), 6(12.8%), 0(0%), 88(93.6%) and 6(6.38%) in controls, respectively. INSR/PmII (rs1799817) SNPs resulted in three genotypes of CC, CT, and TT with C and T alleles. The frequencies of PmII (rs1799817) SNPs in the INSR gene were 37(37%) and 63(63%) in cases, also 39(41.49%) and 55 (58.51%) in controls regarding T and C alleles. The frequencies of PmII (rs1799817) SNPs in the INSR gene were 4(8%), 29(58%), and 17(34%) in cases, also 5(10.64%), 29(61.7%), and 13(27.66%) in controls regarding TT, TC, and CC genotypes, respectively.

Conclusion: The present study as the first investigation of its own kind in Iranian Azeri Turkish women, reported no association between NsiI (rs2059806) and PmII (rs1799817) SNPs in the INSR gene and PCOS risk.

Keywords: Insulin/genetics, Polycystic ovary syndrome, Polymorphism, Receptor.

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Introduction

Polycystic Ovary Syndrome (PCOS) as the most common abnormality of endocrine system is defined with hyperandrogenism and anovulation (1). The pathophysiology of PCOS is poorly understood (1, 2). The role of several candidate genes and gene polymorphisms has been studied in PCOS regarding calcium homeostasis-dependent process, steroidogenesis, and pathways of insulin signaling (2). The insulin receptor (INSR) gene is mapped to chromosome 19p13.3-p13.2 (3). The INSR gene has several genetic poly-

morphisms and was recognized to be associated with PCOS and insulin resistance (IR) (3-8). The relationship between INSR gene variants and PCOS is still controversial (9). Ranjzad et al. (2010) showed that there are no statistically significant differences between the PCOS women and controls regarding the studied markers in the vitamin D receptor (VDR), parathyroid hormone (PTH), insulin receptor (INSR), and adiponectin (ADIPOQ) genes for VDR/Tru9I (rs757343), PTH/DraII (rs6256), INSR/NsiI (rs2059806), INSR/

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PmlI(rs1799817), and ADIPOQ/BsmI (rs1501299) single nucleotide polymorphisms (SNPs) (10). The INSR gene has a central role in hyperinsulinemia and IR predisposition (9-11). IR leads to reproductive problems in PCOS women (12-14). Considering diverse ethnicities in Iran, an attempt was made in this study to determine the frequency of INSR gene polymorphisms in PCOS women and normal controls.

Methods

This study was performed in Urmia University of Medical Sciences. During the period of 2011-2013, 50 PCOS women and 47 controls entered the study. The ethics committee of Urmia University of Medical Sciences approved this investigation. PCOS women and controls were genetically unrelated, whereas matched regarding ethnicity, geographical region and age. All subjects underwent various tests in ART Reproductive Center and Infertility Clinic by related specialists. Same physicians evaluated medical history, physical and clinical examinations for all individuals. PCOS women were diagnosed based on the finding of three or more of the criteria as proposed by the Rotterdam criteria (15) and National Institute of Child Health and Human Development (NICHD) (16). Confounding features such as oligomenorrhea, amenorrhea, hyperandrogenism (non-classic congenital adrenal hyperplasia), and hyperprolactinaemia as well as participants taking drugs affecting calcium homeostasis were excluded from the analysis (10). All subjects signed informed consents. Genomic DNA was isolated from 3-5 ml of whole blood using "salting out" method (17). NsiI (rs2059806) and PmlI (rs1799817) SNPs were genotyped by RFLP-PCR. PCR was carried out in 25 μ l solution using 100 ng of DNA, 1x reaction buffer, 10 pmol of each primer (INSR/NsiI (rs2059806): 5'-cggctcttg taagggtactg-3' and 5'-gaatt cacattccaagaca-3'; INSR/PmlI (rs1799817): 5'-cc aaggatgctgtgtagataa g-3' and 5'-tcaggaaagccagccc atgtc-3'), 200 μ mol of each dNTPs, 0.5 unit of Taq DNA polymerase, and 1.5 mmol MgCl₂ under 35 cycles of PCR program (93°C 45 s, 56°C 30 s, 72°C 45 s) (10). The PCR leads to the formation of a 324 and 317 bp amplicon in the cases of INSR/NsiI (rs2059806) and INSR/PmlI (rs1799817) SNPs, respectively. 10 μ l of PCR products were digested by 10 units of NsiI and PmlI restriction enzymes (Fermentas, Stockholm, Sweden) by incubating the samples at 37°C for 4 hr in two separate reactions. When an A allele was present in

NsiI SNP (rs2059806) within the exon 8 (A/G) of the INSR gene, the NsiI enzyme yielded 239 and 89 bp fragments on the agarose gel. The PCR products remained uncut (324 bp) in the presence of the NsiI enzyme, confirming the presence of a G allele in this locus. When a C allele was present in PmlI SNP (rs1799817) within the exon 17 (T/C) of the INSR gene, the PmlI enzyme yielded 274 and 43 bp fragments on the agarose gel. The PCR products remained un-cut (317 bp) in the presence of the PmlI enzyme, confirming the presence of a T allele in this locus. NsiI/PmlI based RFLPs were analyzed by electrophoresis on 2.5% agarose gel containing ethidium bromide stain. UV transilluminator was used for monitoring presence or absence of tested alleles. Genotypic and allelic frequencies were counted directly. Hardy-Weinberg equilibrium (HWE) test was performed for determination of deviation of genotype distribution.

Results

Genotypic and allelic frequencies of NsiI SNP within the exon 8 (A/G) and PmlI SNP within the exon 17 (T/C) of the INSR gene in studied groups are summarized in table 1. Our controls were consistent with HWE regarding NsiI SNP within the exon 8 (A/G) and PmlI SNP within the exon 17 (T/C) of the INSR gene. INSR NsiI-SNP (rs2059806) resulted in three genotypes of GG, GA, and AA with G and A alleles. INSR NsiI-SNP GG, GA, AA, G and A genotypic and allelic frequencies were 45(90%), 5(10%), 0(0%), 95(95%) and 5(5%) in cases and 41(87.2%), 6(12.8%), 0(0%), 88(93.6%) and 6(6.38%) in controls, respectively. INSR PmlI-

Table 1. Genotypic and allelic frequencies of NsiI SNP within the exon 8 (A/G) and PmlI SNP within the exon 17 (T/C) of the INSR gene in studied groups

| SNPs | Cases F(%F) | Controls F(%F) |
|------------------------|-------------|----------------|
| NsiI based RFLP | | |
| GG | 45(90) | 41(87.2) |
| AG | 5(10) | 6(12.8) |
| AA | 0(0) | 0(0) |
| G | 95(95) | 88(93.6) |
| A | 5(5) | 6(6.38) |
| PmlI based RFLP | | |
| CC | 17(34) | 13(27.66) |
| CT | 29(58) | 29(61.7) |
| TT | 4(8) | 5(10.64) |
| C | 63(63) | 55(58.51) |
| T | 37(37) | 39(41.49) |

SNPs (rs1799817) resulted in three genotypes of CC, CT, and TT with C and T alleles. The frequencies of PmlI (rs1799817) SNP in the INSR gene were 37(37%) and 63(63%) in cases, also 39(41.49%) and 55(58.51%) in controls regarding T and C alleles. The frequencies of PmlI (rs1799817) SNP in the INSR gene were 4(8%), 29(58%), and 17(34%) in cases, also 5(10.64%), 29(61.7%), and 13(27.66%) in controls regarding TT, TC, and CC genotypes, respectively.

Discussion

PCOS has a variety of etiologies including gene-gene and gene-environment interactions (18). Numerous data support the idea that PCOS has been associated with insulin resistance (19), type 2 diabetes mellitus (20, 21), hyperinsulinemia (22), hyperandrogenism (23, 24), inflammation (25), cardiovascular disease (26, 27), and hypertension (28). PCOS women are mainly diagnosed by hyperandrogenism, menstrual irregularity and infertility (29), and are usually overweight or obese (30). In this case-control study, an attempt was made to investigate whether NsiI/PmlI based SNPs in the INSR gene were associated with PCOS risk. Our findings in the present study showed no statistically significant differences in the allele and genotype frequencies regarding INSR/NsiI (rs2059806) and INSR/PmlI (rs1799817) SNPs between PCOS women and controls. The results of several studies are consistent with our findings (2, 7, 8, 31-33). Investigation of several genes are necessary to understand the pathophysiology of PCOS. Our research had some limitations such as lack of precise data from tested genes, low number of cases and poor quality of registry information. Further studies with big sample size and extra information from other genetic variations and haplotypes are required for supplementary analysis.

Conclusion

This report as the first analysis in its own kind in Iranian Azeri Turkish women showed no statistically significant differences between women with PCOS and controls regarding NsiI (rs2059806) and PmlI (rs1799817) SNPs in the INSR gene.

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Conflict of Interest

We declare that there is no conflict of interest with any commercial or other associations in connection with the submitted article.

References

1. Ranjzad F, Mahmoudi T, Irani Shemirani A, Mahban A, Nikzamir A, Vahedi M, et al. A common variant in the adiponectin gene and polycystic ovary syndrome risk. *Mol Biol Rep.* 2012;39(3):2313-9.
2. Mukherjee S, Shaikh N, Khavale S, Shinde G, Meherji P, Shah N, et al. Genetic variation in exon 17 of INSR is associated with insulin resistance and hyperandrogenemia among lean Indian women with polycystic ovary syndrome. *Eur J Endocrinol.* 2009; 160(5):855-62.
3. Talbot JA, Bicknell EJ, Rajkhowa M, Krook A, O'Rahilly S, Clayton RN. Molecular scanning of the insulin receptor gene in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab.* 1996; 81(5):1979-83.
4. Chen ZJ, Shi YH, Zhao YR, Li Y, Tang R, Zhao LX, et al. [Correlation between single nucleotide polymorphism of insulin receptor gene with polycystic ovary syndrome]. *Zhonghua Fu Chan Ke Za Zhi.* 2004;39(9):582-5. Chinese.
5. Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet.* 2011; 43(1):55-9.
6. Lee EJ, Yoo KJ, Kim SJ, Lee SH, Cha KY, Baek KH. Single nucleotide polymorphism in exon 17 of the insulin receptor gene is not associated with polycystic ovary syndrome in a Korean population. *Fertil Steril.* 2006;86(2):380-4.
7. Jin L, Zhu XM, Luo Q, Qian Y, Jin F, Huang HF. A novel SNP at exon 17 of INSR is associated with decreased insulin sensitivity in Chinese women with PCOS. *Mol Hum Reprod.* 2006;12(3):151-5.
8. Siegel S, Futterweit W, Davies TF, Concepcion ES, Greenberg DA, Villanueva R, et al. A C/T single nucleotide polymorphism at the tyrosine kinase domain of the insulin receptor gene is associated with polycystic ovary syndrome. *Fertil Steril.* 2002; 78(6):1240-3.
9. Xu X, Zhao H, Shi Y, You L, Bian Y, Zhao Y, et al. Family association study between INSR gene polymorphisms and PCOS in Han Chinese. *Reprod Biol Endocrinol.* 2011;9:76.
10. Ranjzad F, Mahban A, Shemirani AI, Mahmoudi T, Vahedi M, Nikzamir A, et al. Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic

- ovary syndrome. *J Assist Reprod Genet.* 2011;28(3):225-32.
11. Taylor SI, Kadowaki T, Kadowaki H, Accili D, Cama A, McKeon C. Mutations in insulin-receptor gene in insulin-resistant patients. *Diabetes Care.* 1990;13(3):257-79.
12. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R. The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1996;81(9):3299-306.
13. Ott J, Wattar L, Kurz C, Seemann R, Huber JC, Mayerhofer K, et al. Parameters for calcium metabolism in women with polycystic ovary syndrome who undergo clomiphene citrate stimulation: a prospective cohort study. *Eur J Endocrinol.* 2012;166(5):897-902.
14. Farmakiotis D, Katsikis I, Panidis D. Calcium homeostasis and anovulatory infertility. *Hum Reprod.* 2007;22(12):3264.
15. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril.* 2004;81(1):19-25.
16. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. Boston: Blackwell Scientific Publications; 1992. 377 p.
17. Miller SA, Dykes DD, Polesky HF. A simple salt-ing out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16(3):1215.
18. Sagodi L, Kiss-Toth E, Barkai L. [Role of genetic and environmental factors in the development of polycystic ovary syndrome]. *Orv Hetil.* 2013;154(17):650-7. Hungarian.
19. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev.* 1997;18(6):774-800.
20. Stepto NK, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, et al. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulinaemic clamp. *Hum Reprod.* 2013;28(3):777-84.
21. Barber TM, Franks S. The link between polycystic ovary syndrome and both Type 1 and Type 2 diabetes mellitus: what do we know today? *Womens Health (Lond Engl).* 2012;8(2):147-54.
22. Nestler JE. Role of hyperinsulinemia in the pathogenesis of the polycystic ovary syndrome, and its clinical implications. *Semin Reprod Endocrinol.* 1997;15(2):111-22.
23. Carmina E. Ovarian and adrenal hyperandrogenism. *Ann N Y Acad Sci.* 2006;1092:130-7.
24. Markopoulos MC, Rizos D, Valsamakis G, Deligeorgoglou E, Grigoriou O, Chrousos GP, et al. Hyperandrogenism in women with polycystic ovary syndrome persists after menopause. *J Clin Endocrinol Metab.* 2011;96(3):623-31.
25. Sirmans SM, Weidman-Evans E, Everton V, Thompson D. Polycystic ovary syndrome and chronic inflammation: pharmacotherapeutic implications. *Ann Pharmacother.* 2012;46(3):403-18.
26. Schmidt J, Landin-Wilhelmsen K, Brannstrom M, Dahlgren E. Cardiovascular disease and risk factors in PCOS women of postmenopausal age: a 21-year controlled follow-up study. *J Clin Endocrinol Metab.* 2011;96(12):3794-803.
27. Wild S, Pierpoint T, McKeigue P, Jacobs H. Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. *Clin Endocrinol (Oxf).* 2000;52(5):595-600.
28. Bentley-Lewis R, Seely E, Dunaif A. Ovarian hypertension: polycystic ovary syndrome. *Endocrinol Metab Clin North Am.* 2011;40(2):433-49.
29. Usadi RS, Legro RS. Reproductive impact of polycystic ovary syndrome. *Curr Opin Endocrinol Diabetes Obes.* 2012;19(6):505-11.
30. Bhattacharya SM. Insulin resistance and overweight-obese women with polycystic ovary syndrome. *Gynecol Endocrinol.* 2010;26(5):344-7.
31. Ramezani Tehrani F, Daneshpour M, Hashemi S, Zarkesh M, Azizi F. Relationship between polymorphism of insulin receptor gene, and adiponectin gene with PCOS. *Iran J Reprod Med.* 2013;11(3):185-94.
32. Unsal T, Konac E, Yesilkaya E, Yilmaz A, Bideci A, Ilke Onen H, et al. Genetic polymorphisms of FSHR, CYP17, CYP11A1, CAPN10, INSR, SERPINE1 genes in adolescent girls with polycystic ovary syndrome. *J Assist Reprod Genet.* 2009;26(4):205-16.
33. Urbanek M, Sam S, Legro RS, Dunaif A. Identification of a polycystic ovary syndrome susceptibility variant in fibrillin-3 and association with a metabolic phenotype. *J Clin Endocrinol Metab.* 2007;92(11):4191-8.