RESEARCH ARTICLE

Association of FSHR gene polymorphisms with endometriosis in women visiting tertiary-care hospitals of Lahore, Pakistan

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Abstract

Objectives: The study aimed to explore the association of endometriosis risk factors with single nucleotide polymorphisms rs6166 and rs6165 (Asn680Ser and Ala307Thr) of follicle stimulating hormone receptor (FSHR) gene in Pakistani women.

Methods: This study was conducted from 2013 to 2016. The sampling and extraction of DNA was done in Department of Zoology GC University, Lahore, while the sequencing was performed at Yale University, USA. This case control study consisted of 364 subjects including 156 women diagnosed with endometriosis and 208 conveniently recruited controls. Subjects diagnosed at stage II-IV endometriosis with infertility were pooled for study. The women with adenomyosis, ovarian cancer and leiomyoma were excluded. The whole blood leukocytes were used for DNA extraction. Two important polymorphisms of exon 10 of FSHR gene were analyzed by direct DNA sequencing both in endometriosis and controls.

Results: Genetic variant SNP rs6166 in the affected endometriosis subjects exhibited high incidence of allele "A" (Asn/Asn) 68.3% as compared to controls 33.7% (OR= 4.240; P =0.001). Similarly, the allele "A" of SNP rs6165 (Thr/Thr) was more frequent in endometriosis 67.3% than in control subjects 37.5% (OR =3.430, P =0.001). The occurrence of haplotype AA (Asn/Thr) was 45.5% in endometriosis and 11 % in control subjects (P= 0.001). Remarkably, the incidence of haplotype GG (Ser/Ala) was contrary to previous observations, since only 9.9% occurred in endometriosis as opposed to 45.2% in controls (P= 0.001).

Conclusion: Investigation of FSHR gene polymorphisms rs6165and rs6166 (Ala307Thr and Asn680Ser) in the current study showed that haplotype AA (680Asn/307Thr) was associated with endometriosis in Pakistani women.

Keywords: Endometriosis, Receptors, FSH, Polymorphism, Single Nucleotide, Haplotypes, Linkage Disequilibrium.
(JPMA 71: 1118; 2021) **DOI: https://doi.org/10.47391/JPMA.836**

Introduction

Infertility is among the prevailing health issues plaguing young women worldwide. Endometriosis is one of the important factors to be considered in female back pain, pelvic pain and infertility. The global burden of endometriosis is almost 7-10% which affects women of all reproductive ages,[1,2] is increasing day by day,[3] resulting in high healthcare expenditures and loss of reproductivity.[4]

Endometriosis is a "sex hormone dependent" disease that reverts menopause and subsequently affects fertility of women.[5] In this disorder, endometrial tissue grafts also develop out side the uterus and consequently affect ovarian function, fallopian tubes and uterus.[6] Despite enormous research on endometriosis, consistent mechanism of its development is not fully understood. Similarly, pathogenesis leading to infertility also varies in different studies, although approximately 20-50% women with endometriosis showed variations in their fertility status.[7] Many hypotheses were proposed to elucidate the exact cause of disease development; these include pelvic factors, alteration in peritoneal function, adhesions in pelvic anatomy, ovarian factors that alter oocytes and uterine factors.[8] In previous decade, several studies involving various ethnicities evaluated association of SNPs of various genes with endometriosis.[9,10] The investigations involving various genes and SNPs have reported different results.

The follicle stimulating hormone (FSH) plays a significant role in the development and maturation of follicles, growth of granulosa cells, steroid synthesis regulation and synthesis of aromatase enzyme that induces androgen conversion.[11,12] FSH hormone acts by binding with a trans-membrane glycoprotein receptor encoded by FSHR gene at 2p16.3.[13] However, the role of FSHR gene in signal transduction pathway is still not clear.[14] The amino acids of this region are found in extracellular domain of FSH binding protein. This important region is responsible for signal transduction mechanism which

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affect hormone binding capacity of the gene.[15,16] Many genetic variants (SNPs) are present in FSHR gene that have shown to affect the pathway of signal transduction.[17]

Very little work is available on the FSHR gene polymorphism in relation to endometriosis. However, in FSHR gene, more work is available on polymorphism rs6166 the rs6165 (Asn680Ser and Ala307Thr).[18] The homozygous polymorphism GG (Ser/Ser) in SNP rs6166 of FSHR gene; boosts aromatase action at 680, which later induces estrogens synthesis motivating the propagation of endometriosis.[19] Furthermore, in Taiwanese population endometriosis was studied with non-synonymous homozygous GG (Ser/Ser) SNP as well as heterozygous GA (Ser/Asn) at 680 of FSHR gene. To date, only four studies with conflicting results have described association of FSHR gene polymorphism with endometriosis.[19-23] In observation of significant role of FSHR gene in endometriosis studies, we examined genomic variant associated with risk of endometriosis at FSHR gene in infertile Pakistani women.

Patients and Methods

This study was permitted by "Institutional Bioethics Committee" of GC University Lahore, Pakistan. This was a case control study. The sampling technique was convenient.

This study consisted of 156 infertile women (clinically and laproscopically) diagnosed with endometriosis and 208 fertile women without any history of endometriosis. Endometriosis patients were screened from the outpatient's clinics of tertiary care government hospitals (Lady Willington hospital and Sir Ganga Ram hospital Lahore, Pakistan). All women (both with and without endometriosis) had normal levels of prolactin, thyroid and follicle stimulating hormone as well as both functional ovaries with normal morphology and ovulatory cycle of 25-35 days. The average age of women with endometriosis was 30.25 ± 5.46 years, while Body Mass Index (BMI) was ≤ 25. All women of control group were fertile with average age of 30.59 ± 4.95 years and matched BMI of ≤ 25. The detailed inclusion and exclusion criteria were according to published lliterature.[24] Sample size calculated by formula ($n = z^2_{\alpha/2}\ \pi\ (1-\pi)/e^2$) was 156.[25]

The protocol for DNA extraction, DNA sequencing, genotyping panel, primer design and PCR (Polymerase Chain Reaction Analysis) conditions were followed as described previously by Liaqat at el.[24,26] PCR was performed in a reaction volume of 25 µl. PCR product was confirmed with 2% agarose gel electrophoresis. The PCR product was cleaned using Qiaquick PCR kit (Qiagen, Hilden Germany) and sent for direct sequencing with forward primer.

Hardy-Weinberg equilibrium was tested in both endometriosis and controls by chi-square test. The confidence interval and odd ratios were calculated by Sigma Plot 14.0. Data was tested for normality and Student t-test was performed to compare means of anthropometric parameters. The calculation of haplotype frequency, standard errors and linkage disequilibrium was performed by HAPLO.[27] The significance level for statistical analysis was set at $p \leq 0.05$.

Results

The genotype, alleles and haplotype incidences of both polymorphisms i.e. rs6166 and rs6165 were in accordance to "Hardy-Weinberg equilibrium" in all subjects. The genotype and allele occurrence with odd ratios, confidence

Table-1: Genotype and Allele frequency distribution of SNPs of FSHR gene in endometriosis and controls.

SNPs	Amino acid residue	Genotype and allele frequencies	Endometriosis (n= 156) n (%)	Controls (n= 208) n (%)	OR (95% CI)	p
rs6166	Asn/Asn	AA	72(46.2)	24(11.5)	6.57	
N680S	Ser/Asn	AG	69(44.2)	92(44.2)	(2.39-18.01)	0.001
	Ser/Ser	GG	15(9.6)	92(44.2)		
		Allele frequency				
		A Allele	213(68.3)	140(33.7)	4.24	
		G Allele	99(31.7)	276(66.3)	(2.37- 7.57)	0.001
rs6165	Thr/Thr	AA	72(46.2)	28(13.5)	5.511	
A307T	Ala/Thr	GA	66(42.3)	100(48.1)	(2.09-14.46)	0.001
	Ala/Ala	GG	18(11.5)	80(38.5)		
		Allele frequency				
		A Allele	210(67.3)	156(37.5)	3.43	
		G Allele	102(32.7)	260(62.5)	(1.94-6.07)	0.001

SNPs: single nucleotide polymorphism; P: Significance; OR: Odd ratios; CI: Confidence interval.

Table-2: Haplotype %age of Ala307Thr/G>A and Asn680Ser/A>G SNPs of the FSHR gene in endometriosis group and control.

Haplotypes	Groups Endometriosis (%)	Control (%)	p
307Thr680Asn/AA	45.5	16.3	0.001
307Ala680Asn/GA	21.8	21.2	0.94
307Thr680Ser/AG	22.8	17.3	0.195
307Ala680Ser/GG	9.9	45.2	0.001

p: Significance.

Table-3: Summary of association studies between FSHR (Ala307Thr and Asn680Ser) gene Polymorphisms and endometriosis.

Sr. No	Population	FSHR Polymorphism	Allele Frequency				Population Characteristics	Conclusions	Reference
			Endometriosis		Control				
			A allele n (%)	G allele n (%)	A allele n (%)	G allele n (%)			
1	Taiwanese Chinese	Asn680Ser	417(69.5)	183(30.5)	425(70.83)	249(41.5)	300 women with endometriosis; 337 controls	Endometriosis not associated with polymorphisms	Wang et al. (2011)[19]
2	Taiwanese Chinese	Ala307Thr	402(67)	198(33)	447(66.32)	227(33.67)	300 women with endometriosis; 337 control	Endometriosis not associated with polymorphisms	Wang et al. (2012)[20]
3	Brazilian	Asn680Ser	Data not shown				67 women with endometriosis; 65 control	Endometriosis not associated with polymorphisms	Schmitz et al. (2015)[21]
4	Turkish	Ala307Thr	125 62.5)	75 (37.5)	142 (71.0)	58 (29.0)	100 women with endometriosis; 100 control	Polymorphisms not associated with Endometriosis development	Kerimoglu et al. (2015)[22]
5	Brazilian	Asn680Ser	98 (49.0)	102 (51.0)	107 (53.5)	93 (46.5)	352 women with endometriosis; 510 control	Polymorphisms associated with Endometriosis	Andre´ et al. (2018)[23]
6	Present study	Ala307Thr and	140(67.3)	68(32.7)	156(37.5)	260(62.5)	156 women with endometriosis; 204 control	Polymorphisms associated with Endometriosis	

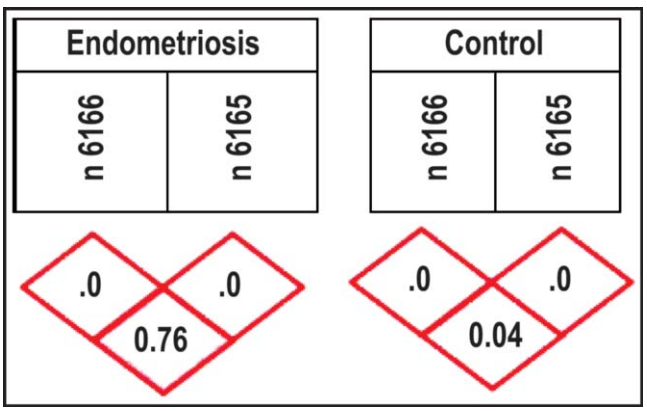

Figure: Linkage disequilibrium (LD) between polymorphisms (rs6166 and rs6165) of FSHR gene in endometriosis and control. The LD value (0.76) showed strong linkage between two sites of polymorphisms in FSHR gene.

interval and level of significance are presented in Table-1. In rs6166 SNP (A>G), the genotype frequency of AA (Asn/Asn), GG (Ser/Ser) homozygous and AG (Ser/Asn) heterozygous were 46.2%, 9.6 % and 44.2% respectively in subjects with endometriosis and 11.5%, 44.2% and 44.2% respectively in control subjects. Whereas, in rs6166 SNP, a higher frequency of "allele A" (OR= 4.240, 95% CI. 2.37- 7.57, P =0.001) was observed in endometriosis subjects (68.3%) in contrast to control subjects (33.7%). In rs6165 SNP (A>G), frequencies of Asn/Asn, Ser/Asn and Ser/Ser were 46.2%, 42.3% and 11.5% in endometriosis cases, whereas 13.5%, 48.1% and 38.5% in controls, as illustrated in Table-1. Both the haplotypes AA (Asn/Thr; 45.5% in endometriosis vs 16.3% in control) and GG (Ser/Ala; 9.9% in endometriosis vs 45.2% in control) exhibited distinct changes in both studied groups (Table-2). The pairwise linkage disequilibrium was 0.76 for observed sites in endometriosis as is presented in Figure. This LD value proposes that the two SNPs were in adjacent linkage disequilibrium.

Discussion

The present study investigated polymorphism of FSHR gene as a risk of endometriosis development in selected

Pakistani women. The genotype frequency of AA (Asn/Asn) of rs6166 SNP was observed more frequently in endometriosis (46.2%) as compared to control subjects (11.5%) (P =0.001). In addition, the combined allele haplotypes of Asn 680 and Thr 307 (AA) were more frequent in endometriosis as compared to controls. Similarly, haplotype GG was frequently found in controls as compared to endometriosis. The results of previous findings were conflicting from strong association to no link between FSHR polymorphism and endometriosis (Table-3). The findings of Wang et al.[19,20] showed no significant association between FSHR polymorphisms and endometriosis in Taiwanese population. His studies revealed that genotype AA (307Ala/Ala) and AG (307Ala/Thr) polymorphism, as well as, both GG genotype (680Ser/Ser) and GA genotype (680Ser/Asn) were linked with a significantly lesser risk of endometriosis. Similarly, FSHR polymorphism was studied in Turkish women with endometriosis and controls. The genotype frequencies showed no significant difference in Turkish women;[22] results are contrary to the present study where strong association was observed at both (Ala307Thr and Asn680Ser) polymorphisms. A recent study on Brazilian infertile endometriosis women exhibited no relationship between FSHR gene polymorphism and onset of disease.[21] Finally, a more recent (2018) study on Brazilian women exxibited no significant difference for both (Ala307Thr and Asn680Ser) polymorphisms between endometriosis and control group. However, when women were divided according to fertility status and stage of severity, a positive association was observed in fertile endometriosis women and FSHR gene polymorphism.[23] This result seems similar with the current study where significant (P=0.001) association was observed between endometriosis and FSHR gene variants.

Conclusion

In the light of above discussion it is concluded that the Pakistani population showed diversity in their genetic makeup on the basis of significant association of FSHR gene with endometriosis.

Disclaimer: Article is part of PHD thesis research work.

Conflict of Interest: None

Funding Disclosure: None

References

1. Baldi A, Campioni M, Signorile PG. Endometriosis: pathogenesis, diagnosis, therapy and association with cancer. Oncol Rep. 2008; 19:843-6.
2. Eisenberg VH, Weil C, Chodick G, Shalev V. Epidemiology of endometriosis: a large population-based database study from a healthcare provider with 2 million members. BJOG: Int J Obstet Gynaecol. 2018; 125:55-62.
3. Tomassetti C, D'Hooghe T. Endometriosis and infertility: Insights into the causal link and management strategies. Best Pract Res Clin Obstet Gynaecol. 2018 Aug 1;51:25-33.
4. Simoens S, Hummelshoj L, D'Hooghe T. Endometriosis: cost estimates and methodological perspective. Hum Reprod Update. 2007; 13:395-404.
5. Kitawaki J, Kado N, Ishihara H, Koshiba H, Kitaoka Y, Honjo H. Endometriosis: the pathophysiology as an estrogen-dependent disease. J Steroid Biochem Mol Biol. 2002; 83:149-55.
6. Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. Ann NY Acad Sci. 2008; 1127:106.
7. Abrao MS, Muzii L, Marana R. Anatomical causes of female infertility and their management. Int J Gynecol Obstet. 2013; 123:S18-24.
8. Šalamun V, Verdenik I, Laganà AS, Vrtačnik-Bokal E. Should we consider integrated approach for endometriosis-associated infertility as gold standard management? Rationale and results from a large cohort analysis. Arch Gynecol Obstet. 2018; 297:613-21.
9. Tempfer CB, Simoni M, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part II—endometriosis. Hum Reprod Update. 2008; 15:97-118.
10. Isidor B, Latypova X, Ploteau S. Familial deep endometriosis: A rare monogenic disease?. Eur J Obstet Gynecol Reprod Biol. 2018; 221:190-3.
11. Themmen AP, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocrine Rev. 2000; 21:551-83.
12. Gu BH, Park JM, Baek KH. Genetic variations of follicle stimulating hormone receptor are associated with polycystic ovary syndrome. Int J Mol Med. 2010; 26:107-12.
13. Lussiana C, Guani B, Mari C, Restagno G, Massobrio M, Revelli A. Mutations and polymorphisms of the FSH receptor (FSHR) gene: clinical implications in female fecundity and molecular biology of FSHR protein and gene. Obstet Gynecol Surv. 2008; 63:785-95.
14. Borgbo T, Klučková H, Macek Sr M, Chrudimska J, Kristensen SG, Hansen LL, et al. The common Follicle-stimulating hormone receptor (Fshr) Promoter Polymorphism Fshr– 29g> a affects androgen Production in normal human small antral Follicles. Front Endocrinol. 2017; 8:122.
15. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. Hum Reprod Update. 2002; 8:413-21.
16. Kene PS, Dighe RR, Mahale SD. Delineation of regions in the extracellular domain of follicle-stimulating hormone receptor involved in hormone binding and signal transduction. Am J Reprod Immunol. 2005; 54:38-48.
17. Simoni M, Tempfer CB, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: Part I: Polycystic ovary syndrome and ovarian response. Hum Reprod Update. 2008; 14:459-84.
18. Mohiyiddeen L, Nardo LG. Single-nucleotide polymorphisms in the FSH receptor gene and ovarian performance: future role in IVF. Hum Fertil. 2010; 13:72-8.
19. Wang HS, Cheng BH, Wu HM, Yen CF, Liu CT, Chao A, Wang TH. A mutant single nucleotide polymorphism of follicle-stimulating hormone receptor is associated with a lower risk of endometriosis. Fertil Steril. 2011; 95:455-7.
20. Wang HS, Wu HM, Cheng BH, Yen CF, Chang PY, Chao A, et al. Functional analyses of endometriosis-related polymorphisms in the estrogen synthesis and metabolism-related genes. PLoS One. 2012; 7:e47374.
21. Schmitz CR, de Souza CA, Genro VK, Matte U, de Conto E, Cunha-

Filho JS. LH (Trp8Arg/Ile15Thr), LHR (insLQ) and FSHR (Asn680Ser) polymorphisms genotypic prevalence in women with endometriosis and infertility. J Assist Reprod Genet. 2015; 32:991-7.
22. Kerimoglu OS, Yılmaz SA, Pekin A, Nergiz S, Incesu F, Dogan NU, et al. Follicle-stimulating hormone receptor gene polymorphisms in women with endometriosis. Arch Gynecol Obstet. 2015; 291:1411-6.
23. André GM, Trevisan CM, Pedruzzi IN, Fernandes RF, Oliveira R, Christofolini DM, et al. The impact of FSHR gene polymorphisms Ala307Thr and Asn680Ser in the endometriosis development. DNA Cell Biol. 2018; 37:584-91.
24. Liaqat I, Jahan N, Lone KP, Pakstis A, Taylor HS. Genetic polymorphisms associated with endometriosis in Pakistani women. J Endo Pelvic Pain Disord. 2013; 5:134-43.
25. Lwanga SK, Lemeshow S. Sample size determination in health studies: a practical manual. World Health Organization, 1991.
26. Liaqat I, Jahan N, Krikun G, Taylor HS. Genetic polymorphisms in Pakistani women with polycystic ovary syndrome. Reprod Sci. 2015; 22:347-57.
27. Hawley ME, Kidd KK. HAPLO: a program using the EM algorithm to estimate the frequencies of multi-site haplotypes. J Hered. 1995; 86:409-11.