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# Association of rs1800790 in FGB and Endometrium in Iranian Women

**P. Zohre Omidi[1], Mehrdad Hashemi[2] and Ahmad Ebrahimi[3*]**

[1]Islamic Azad University, Tehran Medical Sciences Branch,Tehran, Iran;hoda_pink@yahoo.com
[2]Molecular Genetics, Islamic Azad University, Tehran Medical Sciences Branch,Tehran, Iran; drmehashemi@gmail.com
[3]Molecular Genetics,The Cellular and Molecular Endocrine, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran; ae35m@yahoo.com

## Abstract

**Background/Objectives:** Endometriosis is a disease with the growth of endometrium-like tissue in aberrant locations outside of the uterine.Variants of a number of genes have been associated to endometriosis; however, the contributions of these genetic variants in different ethnic groups are not similar. **Methods/Statistical Analysis:** Our study isAssociation of rs1800790 in FGB and endometrium in Iranian women. The case–control study included 100 affected patients and 100 controls. The genetic variants on FGB gene was genotyped using Tetra Amplification Refractory Mutation System-Polymerase Chain Reaction (Tetra-ARMS-PCR).**Findings:** Association of risk allele with endometriosis was examined using SPSS software. Results showed that FGB gene polymorphism genotype frequencies were compared in the patients and control. Frequency of AA, AG and GG genotypes of the gene polymorphism fibrinogen beta polypeptide chain were28, 48and 24% in patients respectively, and66, 31and 3% in control group respectively. Results showed that relation between patients and control group is significant (P=4.619e-11).**Applications/Improvements:** This study showed that there was meaningful relationship between FGB gene polymorphism and increased risk of endometriosis in women studied.

**Keywords:** Endometriosis, FGB Gene, rs1800790, Women

## 1. Introduction

Endometriosis is characterized by the growth of endometrium-like tissue in aberrant locations outside of the uterine. However, the exact prevalence of the disease is not clear, about 5–10% of women of reproductive age are estimated to have endometriosis. Recent studies showed that both environmental and genetic factors have been implicated in the disease. Although the exact etiology and pathogenesis is still unclear, the role of genetic factors has been supported by familial and twin studies[1–27]. Recent genetic studies have revealed an association between the development of endometriosis and the polymorphisms of several genes[2,3], including the genes related to estrogen metabolism[4–6]in different ethnic groups, the contributions of these genetic change are not similar. Among all endometriosis susceptibility genes studied before, the strong association has been found with variants in Fibrinogen Beta Polypeptide Chain (FGB) gene and pathogenesis of endometriosis. The protein encoded by FGB (Fibrinogen Beta Polypeptide Chain) gene is the beta component of fibrinogen, a blood borne glycoprotein comprised of three pairs of no identical polypeptide chains. Mutations in this gene lead to several disorders, including a fibrinogenemia, hypodys fibrinogenemia and thrombotic tendency[10–13].

Since, mutations in FGB gene are the most frequent known genetic causes of thrombotic tendency; the prior probability that variants at this locus are associated with endometriosis is likely to be above the null. Therefore, several studies have investigated the association between polymorphisms in FGB gene and endometriosis across different ethnic populations; however, the results have been inconsistent. Accordingly, this study was carried out

* *Author for correspondence*

to extend earlier studies to examine whether FGB variation (rs1800790) per se is associated with endometriosis incidence in Iranian population[16,17,19].

## 2. Material and Methods

This Study was conducted on 100 patients with clinically confirmed as endometriosis cases and 100 healthy controls. The DNA was extracted from the peripheral blood using the standard phenol-chloroform method. The concentration and quality of extracted DNA was evaluated using Nano drop (2000, Termofisher Co.) and subsequently rs1800790 was genotyped using Tetra Amplification Refractory Mutation System-Polymerase Chain Reaction (Tetra -ARMS-PCR) using designed primers (Table 1).

**Table 1.** Designed primers (reverse and forward)

| Primers | Tm (°C) | Sequence (5' → 3') | CG% | (Size) bp |
|---|---|---|---|---|
| Reverse Normal | 53.2 | ATTCTATTTCAAAAG-GCGCC | 40 | 20 |
| Reverse Common | 54.5 | GACTTCAGAAATGGT-TACC | 47.4 | 19 |
| Forward Common | 53.2 | CATTTAGTCTGTGAG-CATAC | 40 | 20 |
| Forward Mutant | 53.5 | CATTACTATT-GATTTTACTA | 30.4 | 20 |

The reaction mixture was prepared in total volume of 20µL using ready to use Ampliqon2X-PCR master mix. The amplification were done using PCR program with initial denaturation for 5 min at 95˚C, followed by 35 cycles of including, 40 sec at 94˚C, 40 sec at 52˚C, 38 sec at 72˚C with a final elongation for 5 min at 72˚C. The PCR products were loaded on 2% agarose gel supplemented with Gel Red for DNA product visualization under UV light[28,29].

Allelic and genotypic associations were evaluated by the Chi−square and Fisher's exact tests using the SPSS statistical software package, version 20.0 after age adjustment (SPSS Inc, Chicago, IL, USA).

## 3. Results and Discussion

Analyses of affected and controls showed that heterozygote genotype "AG" has the highest frequency (48.0%) in case group (Table 2). However, in control group the highest genotype frequency belonged to GG (66.0%). The results also indicated significant differences between case and control groups in terms of genotype frequency (p =7.754e-06).

**Table 2.** The frequency of different genotypes in case and control groups

| | | | Genotype | | | Total |
|---|---|---|---|---|---|---|
| | | | GG | AG | AA | |
| Group | Control | Count | 66 | 31 | 3 | 100 |
| | | % within Group | 66% | 31% | 3% | 100.0% |
| | Case | Count | 24 | 48 | 28 | 100 |
| | | % within Group | 24% | 48% | 28% | 100.0% |
| | | Count | 90 | 79 | 31 | 200 |
| **Total** | | **% within Group** | **45%** | **39.5%** | **15.5%** | **100.0%** |

In this study, allele "G" had the highest frequency (76.5%) in control, meanwhile; allele "A" had the highest frequency (76.5%) in case (Figure 1). The results also showed that the allele frequency had significant differences between case and control groups p =2.348e-12).

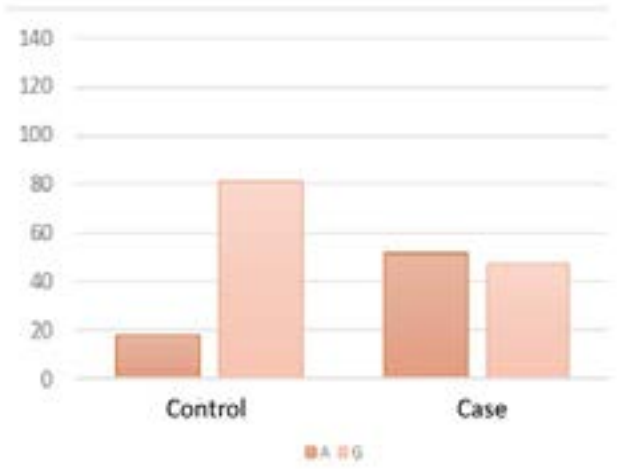


**Figure 1.** Genotype frequency in control and case.

The results obtained in this study indicated that the presence of mutant allele "A" significantly increase susceptibility to endometriosis in Iranian population compares to individuals carrying wide allele (odds ratio: 4.77, 95% CI: 3.03-7.50, p = 2.348e-12) (Table 3).

**Table 3.** Risk estimate based on allele frequency between case and control group

| Allele | Case | Control | Total | P | OR | C.I |
|---|---|---|---|---|---|---|
| G | 48 | 81.5 | 129.5 | Reference | 4.77 | 3.03-7.50 |
| A | 52 | 18.5 | 70.5 | 2.34e-12 | | |
| **Total** | **100** | **100** | **200** | | | |

In addition, the results revealed that risk of endometriosis is about four times higher in individuals with AG genotype compared to normal individuals having GG genotype (odds ratio: 4.25, 95% CI: 2.22-8.15, p = 7.75e-06) (Table4).

**Table 4.** Risk estimate based on genotype frequency between case and control group

| Allele | Case | Control | Total | P | OR | C.I |
|---|---|---|---|---|---|---|
| GG | 66 | 24 | 90 | Reference | 4.25 | 2.22-8.15 |
| AG | 31 | 48 | 79 | 7.75e-06 | 25.66 | 7.14-92.22 |
| AA | 3 | 28 | 31 | 6.63e-10 | | |
| **Total** | **100** | **100** | **200** | | | |

Over the past 10 years, in some studies, genetic polymorphisms have checked out as one contributing factor to the development of endometriosis[4]. A number of candidate genes have been detected as being associated with endometriosis, using a variety of techniques for the analysis of genetic polymorphisms.The clinical relevance of the way Single Nucleotide Polymorphisms (SNPs) can modify physiology can be illustrated by the occurrence of Follicle Stimulating Hormone (FSH) receptor polymorphisms[18,21–25].

Although its exact etiology and pathogenesis are unclear, two main theories for the pathogenesis have been proposed: metastatic implantation after the reflux of endometrial cells through the fallopian tubes[1], and met aplastic development such as coelomic metaplasia[12,13].Have been studied a several candidate genes (for example: FGB gene) a possible factor contributing to the development of endometriosis. FGB gene is encodes fibrinogen protein. Fibrinogen as a key regulator of inflammation in disease and clotting pathways and an established risk factor for Cardiovascular Disease (CVD)[6]. In response to cytokines released during inflammation, infection, neoplasia or tissue damage, fibrinogen production is up regulated[9].

In[7] author indicated that support potential interaction between two fibrinogen Single Nucleotide Polymorphisms (SNPs), FGB1437 (rs1800790) and FGA6534 (rs6050) in determining fibrinogen levels. These single nucleotide polymorphisms appeared in one model which included their main effects only and a second model including only their interaction term.Have been marginally significant interactions between IL-6 level and fibrinogen single nucleotide polymorphisms located in the promoter regions of FGA and FGG so that in individuals having the homozygous rare FGG902 (rs1800792) genotype or lacking the rare FGA251 (rs2070006) allele, the slope describing the association between fibrinogen level and ln(IL-6) level was not as steep as with the other genotypes. These results are consistent with a recessive effect for FGG902 whereas the similar slopes for individuals with either 1 or 2 minor alleles are consistent with a dominant gene effect for FGA251, though the overlap of the confidence intervals should be noted[7].

Author in[7]reported that in single SNP analyses without interaction terms, each FGG902 allele was significantly associated with higher fibrinogen and each FGA251 minor allele was associated with significantly lower fibrinogen levels[28,29].

## 4. Conclusion

This study revealed the association between the presence of a allele and endometriosis among Iranian women which is in agreement with previous results in other ethnicities. However, further extensive researches with a large number of samples from different populations and ethnicities are required to validate the results obtained in this study[7].

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