The role of *IL-16* gene polymorphisms in endometriosis

MICHAIL MATALLIOTAKIS[1,2*], MARIA I. ZERVOU[3*], ELIAS ELIOPOULOS[4], CHAROULA MATALLIOTAKI[1], NILUFER RAHMIOGLU[5,6], IOANNIS KALOGIANNIDIS[2], KRINA ZONDERVAN[5,6], DEMETRIOS A. SPANDIDOS[7], IOANNIS MATALLIOTAKIS[1] and GEORGE N. GOULIELMOS[3]

[1]Department of Obstetrics and Gynecology, Venizeleio General Hospital, 71409 Heraklion, Crete; [2]3rd Department of Obstetrics and Gynaecology, Aristotle University of Thessaloniki, 54124 Thessaloniki; [3]Section of Molecular Pathology and Human Genetics, Department of Internal Medicine, School of Medicine, University of Crete, 71003 Heraklion, Crete; [4]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, 11855 Athens, Greece; [5]Wellcome Trust Centre for Human Genetics, and [6]Endometriosis CaRe Centre, Nuffield Department of Obstetrics and Gynaecology, University of Oxford, OX3 7BN Oxford, UK; [7]Laboratory of Clinical Virology, Medical School, University of Crete, 71003 Heraklion, Crete, Greece

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Abstract. Endometriosis is one of the most common gynecological diseases affecting up to 10% of the female population of childbearing age and a major cause of pain and infertility. It is influenced by multiple genetic, epigenetic and environmental factors. Interleukin-16 (IL-16) is a proinflammatory cytokine playing a pivotal role in many inflammatory and autoimmune diseases as well as in the pathogenesis of endometriosis. The aim of the present study was to evaluate the association of two *IL-16* gene single nucleotide polymorphisms (SNPs), rs4072111 and rs11556218, with the risk of endometriosis in women from Greece as well as to gain insight about the structural consequences of these two exonic SNPs regarding development of the disease. A total of 159 women with endometriosis (stages I-IV) hospitalized for endometriosis, diagnosed by laparoscopic intervention and histologically confirmed, and 146 normal controls were recruited and genotyped. Subjects were genotyped using a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) strategy. A significant association was detected regarding the GG and GT genotype as well as 'G' allele of rs11556218 in patients with endometriosis. The rs4072111 SNP of the *IL-16* gene was not found to be associated with an increased susceptibility to endometriosis either for all patients (stages I-IV) or for stage III and IV of the disease only. Our results demonstrated that rs11556218 is associated with endometriosis in Greek women, probably by resulting in the aberrant expression of *IL-16*, as suggested by the bioinformatics analysis conducted on the SNP-derived protein sequences, which indicated a possible association between mutation and functional modification of Pro-IL-16.

Correspondence to: Dr George N. Goulielmos, Section of Molecular Pathology and Human Genetics, Department of Internal Medicine, School of Medicine, University of Crete, Voutes Area, 71003 Heraklion, Crete, Greece
E-mail: goulielmos@med.uoc.gr

*Contributed equally

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Introduction

Endometriosis is an estrogen-driven inflammatory condition, defined by a misplacement of endometrium outside of the uterine cavity, most commonly in the pelvic cavity and is one of the common causes of infertility (1,2). It affects 6 to 10% of women of reproductive age (3), but with varying symptoms including severe dysmenorrhea (4,5), chronic pelvic pain, dysfunctional uterine bleeding (5), as well as urinary tract and gastrointestinal symptoms (6). Notably, endometriosis possesses many features of a benign neoplastic process with the potential for malignant transformation (7). Endometriosis is a major problem of women's health, which affects dramatically the quality of life. It has been accepted that multiple factors contribute to the development of this condition, including genetic and environmental ones. However, the exact molecular and pathophysiological pathways leading to endometriosis are still unclear, as at present only various hypotheses have been suggested (8-10). Thus, it may be assumed that all cases of endometriosis are not able to be explained by one theory only. Genetic factors contribute to the heritability of endometriosis (11-14) and the overall heritability has been estimated at approximately 50%, as shown from twin studies (15,16). Notably, the impact of epigenetics in endometriosis has been under investigation in recent years and significant progress regarding DNA methylation and histone post-translational modifications has been achieved (17,18). The epigenetic disruption of gene expression plays an important role in the

development of endometriosis through interaction with environmental changes.

Candidate gene association studies, genome-wide association studies and various meta-analyses have led to the identification of many endometriosis-risk loci that may be initially involved in the pathogenesis of endometriosis (19-24). These gene loci have been categorized according to the function of their gene products, i.e., growth factors, matrix remodeling, cell cycle regulation and signaling, oncogenes, hormone receptors and metabolism, adhesion molecules, transcription regulation, cytokines, inflammation, immune and oxidative stress (reviewed in ref. 25). However, a portion of these data has been rather disappointing due to the absence of replication in independent populations (26). At present, 19 single nucleotide polymorphisms (SNPs) associated with endometriosis have been identified, which can explain approximately 5.19% of the disease variance (27). The list of novel endometriosis-associated loci is enriching considering that recent studies are focused mainly on the severe stages of the disease (stage III/IV endometriosis), thus suggesting the greater genetic burden of moderate to severe endometriosis cases compared to that of minimal or mild disease (stage I/II) (22,28).

Previous findings have shown that various cytokines may be used as potential biomarkers for the diagnosis of endometriosis, given that they were detectable in the serum and peritoneal fluid (29). In this framework, IL-16 has been found in amniotic fluid with its levels declining over gestation, while IL-16 transcripts were detected in whole tissue extracts of fetal gut, skin and placenta (30,31). Interleukin-16 (IL-16), also known as a lymphocyte chemoattractant factor, is a polypeptide proinflammatory cytokine that plays a pivotal (decisive) role in most immune and inflammatory responses as well as in the pathogenesis of endometriosis (32). It is produced by a variety of cell types previously found in association with complex disorders and it is now clear that this cytokine plays a critical role in the regulation of cellular functions. The precise mechanism by which IL-16 functions as an inflammatory mediator is still under investigation and not fully clarified.

Accumulated data suggest that IL-16 activates T lymphocytes, thus resulting in the secretion of several proinflammatory cytokines (33). It is produced mainly by CD8 lymphocytes as a 67-kDa precursor protein (34). Human IL-16 is normally produced as a 631-amino acid precursor protein, Pro-IL-16, which is then cleaved at the subsequent step by the enzyme caspase-3 to release the biologically active C-terminal domain, consisting of 121 amino acids (35-37). Two functional polymorphisms in this gene (rs4072111 C/T and rs11556218 T/G) have been reported to be associated with various cardiovascular (38-40), neurodegenerative (41), infectious (42), inflammatory or autoimmune diseases (43-46), as well as with various types of cancer (47-50). Of the two, rs11556218 is a missense exon-SNP, located in the exon 6 region, leading to an amino acid change (Asn446Lys) on position 446 of the shorter isomorph 2 (631 aminoacids) of Pro-IL-16 (Fig. 1), which may alter protein structure and function. The rs4072111 is another missense SNP (Pro434Ser) appearing on position 434 of the second PDZ domain of the longer isomorph 1, i.e., the neuronal nPro-IL-16 (1,331 amino acids) (Fig. 1). PDZ domains were originally identified as repeated sequences conserved in two proteins, postsynaptic density protein PSD95, *Drosophila* discs large tumor suppressor protein DLG (51). PDZ domains are now known to be present in many proteins (52). Additionally, they function as motifs for protein-protein interaction (PPI) (53,54). Proteins with PDZ motifs have been associated with neoplasia and alterations in cell proliferation as originally described (51). While the majority of PDZ-containing proteins appear to participate in PPIs in the cytoplasm at the sites of cell-cell contact, a number of PDZ domain-containing proteins have been identified to localize in the nucleus (52-54).

Encouraged by the *IL-16* association with endometriosis detected by Azimzadeh *et al* (55) recently, we conducted the current study to investigate whether rs4072111 and rs11556218 SNPs of the *IL-16* gene were associated with the risk for endometriosis and/or with progression to the severe stages (III-IV) of this condition in the Greek population. Furthermore, we attempted to detect any ethnic-specific differences regarding the genetic association of these SNPs with endometriosis, considering that population differences for endometriosis have been reported previously in terms of genetic susceptibility and disease manifestations (56-58).

Patients and methods

Patient population and study design. In this case control association study, 305 women were enrolled (159 endometriosis patients and 146 controls) followed in the Department of Obstetrics and Gynecology of Venizeleion General Hospital of Heraklion (Heraklion, Crete). All the women had undergone surgery in the aforementioned tertiary care centre. The average age of the Greek endometriosis and control cohorts was 32.25±7.1 and 29.49±6.7 years, respectively. The women with endometriosis were diagnosed surgically (laparotomy or laparoscopy), and the disease was confirmed histologically from biopsies. Staging of the disease was performed according to the revised American Fertility Society classification (59). All the members of the control group had given birth to 2-5 (2.3±0.6) children and had no previous medical record of chronic pelvic pain, dysmenorrhea, or dyspareunia. According to the revised American Fertility Society Classification (1985), 81 (50.94%) stage I-II patients and 78 (49.06%) patients had moderate to severe endometriosis (stage III-IV). All the subjects were of self-reported Greek origin. Written informed consent was obtained from both patients and controls. The study was performed in the Section of Molecular Pathology and Human Genetics of the Medical School of Crete, after obtaining the approval of the Research Committee of the Venizeleion General Hospital of Heraklion and was carried out in compliance with the declaration of Helsinki.

Genotyping. Whole blood was collected preoperatively in ethylenediaminetetraacetic acid (EDTA)-containing tubes. Genomic DNA was isolated from peripheral blood leukocytes by using the commercial kit Invitrogen (PureLink® Genomic DNA Mini kit; Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted DNA was stored at -20˚C until analyzed. Genotyping of two common SNPs in the *IL-16* gene, rs4072111 (Pro434Ser) and rs11556218 (Asn446Lys) was performed by following the restriction fragment length polymorphism (RFLP) approach, by using *Bsm*AI and *Nde*I, respectively,

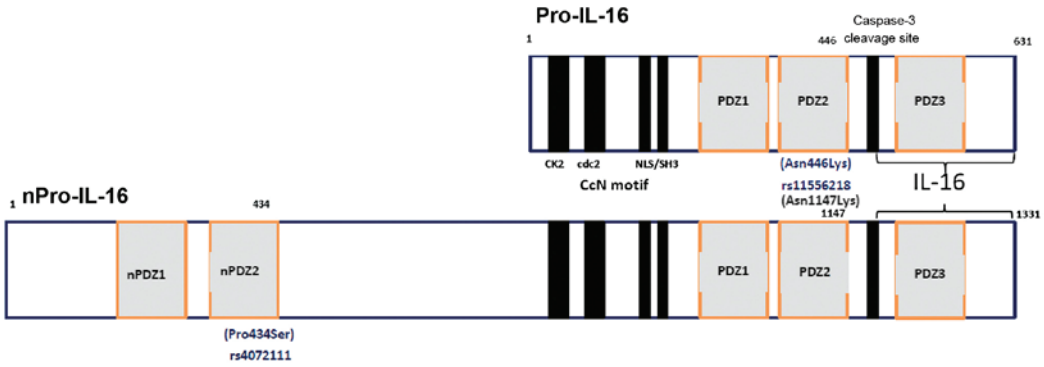

Figure 1. Schematic representation of domain structure of Pro-IL-16 and nPro-IL-16. N-terminal Pro-IL-16 contains a CcN motif and two PDZ domains. N-terminal neuronal Pro-IL-16 contains two nPDZ domains, a CcN motif and a further two PDZ domains. C-terminal mature IL-16 is processed by caspase-3, and contains a PDZ domain. rs11556218 SNP leads to an amino acid change of both Pro-IL-16 and nPro-IL-16 on their PDZ2 domain (Asn446Lys or Asn1147Lys, respectively). The rs4072111 is another missense SNP that leads to an amino acid change of nPro-IL-16 only on its nPDZ2 domain (Pro434Ser). The figure was adapted from the study by Bannert *et al* (75). IL-16, interleukin-16; SNP, single nucleotide polymorphism.

as described elsewhere (47,55). Amplification of the genomic fragments harboring the polymorphic sites was carried out using the GoTaq polymerase provided by Promega Corporation (Madison, WI, USA). Briefly, the upstream primers 5'-CAC TGT GAT CCC GGT CCA GTC-3' and 5'-GCT CAG GTT CAC AGA GTG TTT CCA TA-3' as well as the downstream primers 5'-TTC AGG TAC AAA CCC AGC CAG C-3' and 5'-TGT GAC AAT CAC AGC TTG CCT G-3', based on the human *IL-16* gene sequence (accession no. NC_000015.10) were used to generate the regions of the rs4072111 (164 bp) (C>T) and rs11556218 (171 bp) (T>G) SNPs of the *IL-16* gene, respectively.

Polymerase chain reactions (PCR) were carried out in the final volume of 25 μl containing: 10X PCR buffer and 2 mM $MgCl_2$ (both from Roche Diagnostics GmbH, Mannheim, Germany), 0.4 mM of each dNTP (Fermentas, St. Leon-Rot, Germany), 5 pmol of each primer, 50 ng template DNA, 1 unit Taq DNA polymerase (Roche Diagnostics GmbH) and sterile distilled water up to 25 μl. A hot start was used with initial heating at 94˚C for 5 min and then 30 cycles of denaturing (at 94˚C for 30 sec), annealing for 30 sec (60˚C for rs11556218 and 60˚C for rs4072111) and chain extension (at 72˚C for 30 sec), followed with a final extension step at 72˚C for 5 min. Both undigested and digested PCR products were visualized in 2.5% agarose gel stained with ethidium bromide in reference to a molecular weight marker (100 bp DNA ladder; Invitrogen Life Technologies). The genotyping of the samples for rs4072111 was performed by digesting the 164-bp PCR product with *Bsm*AI restriction enzyme (New England BioLabs, Inc., Beverly, MA, USA), which digested the DNA that was amplified from the 'T' allele, thus generating two fragments of 140 and 24-bp. The presence of allele 'C' resulted in the absence of the *Bsm*AI restriction site (47). In addition, the genotyping of the samples for rs11556218 was performed by digesting the 171-bp PCR product with *Nde*I restriction enzyme (New England BioLabs, Inc.), which digested the DNA that was amplified from the 'G' allele, thus generating two fragments of 147 and 24-bp. The presence of allele 'T' resulted in the absence of the *Nde*I restriction site (47). Genotypes were scored blindly, and analysis of all the ambiguous samples was repeated. Furthermore, to ensure accuracy of the results, 10% of the samples were amplified twice.

Construction of IL-16 domain three-dimensional (3D) model. SNPs bioinformatics analysis was performed using NCBI dbSNP (for nucleotide sequence analysis), UNIPROT (for protein sequence analysis) and PDB (for protein structure analysis) databases. PyMOL (DeLano Scientific, San Carlos, CA, USA) was used for 3D structural positioning, mutation analysis and visualization. The crystal structures of IL-16 PDZ and PDZ12 domains (PDB codes, 1X6D2QT5 and 2KA9) were used as the initial models. The 3D structure modeling was performed using SWISS-MODEL (60).

Statistical analysis. All cases and controls used in the analysis were unrelated. The GraphPad Prism statistical program (GraphPad Software, San Diego, CA, USA) was used in the framework of the analyses performed. A two-tailed P-value <0.05 was defined as statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. The χ^2 test, with one or two degrees of freedom or Fisher's exact test was used to examine differences of genotype and allele frequencies between patients and controls, where all SNPs had a call rate of >98%. The possible deviation from Hardy-Weinberg equilibrium (HWE) was performed by using the program 'Calculate' (copyright TRG, SR, INMD, 2008). The distribution of genotypes in case group for both SNPs examined was found to be under HWE (P>0.01).

Results

rs11556218 IL-16 T/G SNP. In the case of the rs11556218 SNP, a statistical significant difference was found in the frequencies of GG and GT genotypes in patients and controls (P<0.0001, OR=7.01; 95% CI, 3.65-13.46; and P<0.0001, OR=4.17; 95% CI, 2.30-7.56, respectively) (Table I). Additionally, we observed a significant difference in the distribution of the 'G' allele between the endometriosis and control groups (P<0.0001, OR=3.02; 95% CI, 2.17-4.20), being associated with an increased susceptibility for endometriosis.

Of note, when patients were analyzed according to the severity of the disease, a significant association was detected regarding the GG and GT genotypes of this SNP in patients with stage III/IV of the disease and controls (P=0.0004, OR=3.92; 95% CI, 1.87-8.22; and P=0.0178, OR=2.37; 95% CI,

Table I. Genotypes and alleles frequency of the *IL-16* rs11556218 SNP analyzed in 159 women with endometriosis and 146 healthy controls.

rs11556218	Patients	Controls	P-value	OR (95% CI)
Genotypes	n=159	n=146		
G/G	64 (40.25%)	27 (18.49%)	**<0.0001**	7.01 (3.65-13.46)
G/T	72 (45.28%)	51 (34.93%)	**<0.0001**	4.17 (2.30-7.56)
T/T	23 (14.46%)	68 (46.58%)		1.00 (Reference)
Alleles	n=318	n=292		
G	200 (62.89%)	105 (35.96%)	**<0.0001**	3.02 (2.17-4.20)
T	118 (37.11%)	187 (64.04%)		1.00 (Reference)

Bold, statistically significant difference.

Table II. Genotypes and alleles frequency of the *IL-16* rs11556218 SNP analyzed in 78 women with endometriosis (stage III and IV) and 146 healthy controls.

rs11556218	Patients	Controls	P-value	OR (95% CI)
Genotypes	n=78	n=146		
G/G	28 (35.88%)	27 (18.49%)	**0.0004**	3.92 (1.87-8.22)
G/T	32 (41.04%)	51 (34.93%)	**0.0178**	2.37 (1.20-4.69)
T/T	18 (23.08%)	68 (46.58%)		1.00 (Reference)
Alleles	n=156	n=292		
G	88 (56.41%)	105 (35.96%)	**<0.0001**	2.30 (1.55-3.43)
T	68 (43.59%)	187 (64.04%)		1.00 (Reference)

Bold, statistically significant difference.

Table III. Genotypes and alleles frequency of the *IL-16* rs4072111 SNP analyzed in 159 women with endometriosis and 146 healthy controls.

rs4072111	Patients	Controls	P-value	OR (95% CI)
Genotypes	n=159	n=146		
C/C	137 (86.16%)	117 (80.14%)	0.46	3.51 (0.14-87.07)
C/T	22 (13.84%)	28 (19.18%)	1	2.37 (0.09-61.01)
T/T	0 (0%)	1 (0.68%)		
Alleles	n=318	n=292		
C	296 (93.08%)	262 (89.72%)	0.15	1.54 (0.87-2.74)
T	22 (6.92%)	30 (10.28%)		

1.20-4.69, respectively) (Table II). The 'G' allele was also associated with endometriosis in this analysis (P<0.0001, OR=2.30; 95% CI, 1.55-3.43).

The rs4072111 IL-16 SNP. We further evaluated the effect of the rs4072111 SNP in the development of endometriosis. Based on the genotyping as well as the allelic data obtained, no association with endometriosis was detected either for CC genotype or for allele 'C' (Table III) (P=0.46, OR=3.51; 95% CI, 0.14-87.07; and P=0.15, OR=1.54; 95% CI, 0.87-2.74, respectively). Furthermore, when patients were analyzed according to the severity of the disease (stage III/IV), no association was detected either (data not shown). The genotyping success for all the SNPs analyzed was >98%.

Developing 3D models of IL-16 protein. We located the rs4072111 and rs11556218 SNPs on the aminoacid sequences of the isomorphs of Pro-IL-16 (Fig. 1). We then attempted to approach the functional role of both SNPs under investigation, by constructing 3D models of the respective PDZ domains of nPro-IL-16 isomorph 1 (Fig. 2) and Pro-IL-16 isomorph 2 (Fig. 3) proteins. The first model consists of the N-terminal

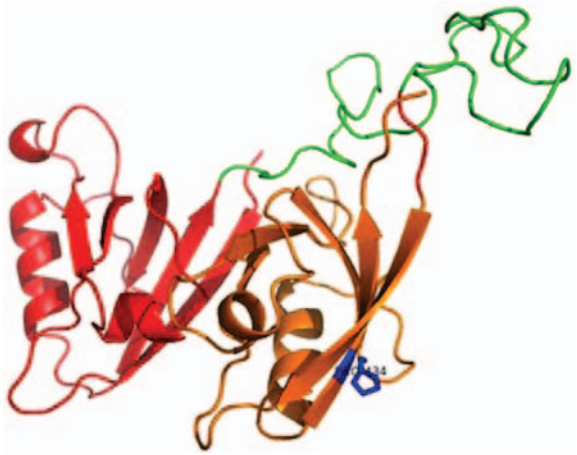

Figure 2. Schematic ribbon view of the modeled nPDZ1,2 domains of neuronal Pro-IL-16 isomorph 1. The rs4072111 SNP-derived mutant Pro434Ser (in blue) is located on the β3 strand of the nPDZ2 domain. Elements of secondary structure of the protein domain are shown as ribbons. The figure was created using the PYMOL v1.8 program for the nPro-IL-16 isomorph 1 structure. IL-16, interleukin-16; SNP, single nucleotide polymorphism.

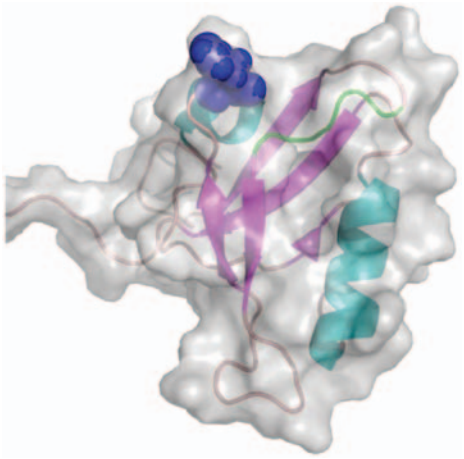

Figure 3. Surface view of the PDZ2 domain of Pro-IL-16 isomorph 2. The rs11556218 SNP-derived mutant Asn446Lys is located on the β3/α1 loop at the entrance of the recognition cavity of the PDZ domain running from top to bottom (see purple arrows) and in close proximity to the GLGF motif (in green), known as the 'carboxylate binding loop'. Elements of secondary structure of the protein domain are shown as ribbons (cyan for the α-helix and purple for the β strands). The figure was created using the PYMOL v1.8 program for the IL-16 structure (PDB code, 1X6D) (76). IL-16, interleukin-16; SNP, single nucleotide polymorphism.

two PDZ domains (residues 211-443) of nPro-IL-16. The location of the mutant residue is predicted on the last β-strand of the second PDZ domain (Fig. 2). The second model is based on the NMR structure of the second PDZ domain (PDB code, 1X6D) (61) of the N-terminal Pro-IL-16 isomorph 2. The rs11556218SNP-derived mutant Asn446Lys is located on the β3/α1 loop, at the entrance of the recognition cavity of the PDZ domain and in close proximity to the GLGF motif, known as the 'carboxylate binding loop' (Fig. 3).

Discussion

Recent advances in genetics and relevant technology during the current post-genomic era resulted in the identification and a better understanding of genetic risk factors associated with endometriosis. In the present study, we investigated the role of two SNPs of the *IL-16* gene with regard to risk of endometriosis susceptibility in Greek women. To the best of our knowledge, this is the first study to screen for *IL-16* gene polymorphisms in patients with endometriosis in a European population. The *IL-16* gene is located on chromosome 15 (15q26.1) (62).

Although GWAS have detected numerous endometriosis susceptibility genes, it is clear that there are many differences in genetic associations with endometriosis across different world populations and, therefore, it is important to study the genetic basis of the disease in multiple populations (63,64). This would be particularly important considering that some major endometriosis risk factors such as *WNT4*, *VEZT* and *FSHB* were shown by a recent study of our group, focused on the same (Greek) population analyzed in the present study, to have a specific geographic distribution (58). In this framework, genetic association studies involving rs4072111 and rs11556218 SNPs of the *IL-16* gene in the Greek population showed that rs11556218 only is strongly associated with an increased susceptibility for the development of endometriosis at both the genotypic and allelic level.

The results of a recent study conducted in Iran showed that genotype and allelic distribution in the two *IL-16* exonic polymorphisms rs4072111 and rs11556218 was significantly different between endometriosis patients and healthy individuals (65). Of note, allele 'G' of rs11556218 was found to be protective for endometriosis and did not increase the risk for the disease, as found in the Greek population in the present study. No significant differences were detected in the genotype and allele frequencies of the rs11556218 SNP between patients with endometriosis and controls either in a Chinese (66) or a Korean population (67). Of note, the allele frequencies that we obtained for the Greek control population for rs11556218 SNP vary significantly in comparison with Iranian (55) and Chinese (18,40,47,66), as published in the literature. These observations underline the importance of assessing genetic variants in different ethnic and/or racial populations in any attempt to approach the genetic basis of endometriosis and the specific effects of various alleles in different populations.

Genetic variation in the DNA sequence of the *IL-16* gene may lead to altered cytokine production and/or activity, and this variation may modulate an individual's susceptibility to endometriosis. Notably, in patients with colorectal cancer or gastric cancer, IL-16 serum levels were significantly higher than those in the healthy controls, although no significant association between *IL-16* polymorphisms and serum levels of IL-16 was observed (47). Several cytokines have been shown to appear differences in women with endometriosis in comparison with controls. Thus, among members of the interleukin family evaluated, IL-16 exhibited elevated levels (68). Nevertheless, few studies have directly examined the mechanisms by which IL-16 is involved in the development and progression of endometriosis. IL-6 was found to be elevated in the peritoneal fluid and serum of women with endometriosis (69,70). However, in another study, it was found that although the concentrations of IL-16 in peritoneal fluid and sera were both lower in women with endometriosis, the observed differences did not reach a

level of statistical significance (71). Apparently, these findings should be validated in larger studies, in order to clarify the role of this molecule in endometriosis.

Although the molecular mechanisms by which *IL-16* gene polymorphisms are associated with endometriosis remain unclear, additional functional studies, in combination with genetic studies involving subjects from various ethnicities, may provide valuable information concerning this issue. GWAS have detected many regions harboring interesting disease-candidate genes but the risk alleles may not always act in obvious ways. As a consequence, it is necessary to accumulate evidence of their functional significance by performing gene expression studies, epigenetic analyses or further functional studies. In our attempt to approach the functional role of the SNPs studied, we constructed 3D models of the respective PDZ domains of nPro-IL-16 isomorph 1 and Pro-IL-16 isomorph 2 proteins. It is apparent that the rs11556218 SNP polymorphism leading to the Asn446Lys mutation on the PDZ2 domain of Pro-IL-16 affected the function of the protein. The introduction of the positive charge on the side chain of the lysine no. 446 amino acid residue, located on the rim of the GLGF carboxylate binding loop, may deregulate protein-protein recognition.

A recent study by Xiao *et al* (72) presented a disease network of endometriosis that integrated human PPIs and known disease-causing genes. Considering that most human diseases reflect the phenotype of the co-operative function of many causative gene alleles (73), gene networks confer information for the underlying disease mechanisms. The construction of this network was based on endometriosis-causing genes that were identified from disease-gene databases and subsequent calculations and approaches using bioinformatics. However, *IL-16* was not included in this network.

The pathogenesis of endometriosis is highly complex given that it involves genetic, epigenetic and environmental factors, with all of them interacting with each other in order to yield the disease phenotype. Thus, conflicting studies appear frequently and the interpretation of the data collected remains a challenge. A major advantage of our study was the homogeneous patient cohort and control group selected, thus minimizing the possibility that our results are biased by sampling. The major weakness of the present study was the small sample size, leading to a low statistical power, probably non-efficient to detect a weak genetic effect. Furthermore, it should be mentioned that laparoscopy is an expensive and invasive procedure, and women with no symptoms of endometriosis have had low adherence to accepting this diagnostic procedure. The failure to confirm previous findings from another study for rs4072111 is largely attributed to population differences or, probably, to interactions with genetic and/or non-genetic factors (74). Together, the results from the present study demonstrate the difficulty in identifying common, generalizable risk alleles in a complex disease, such as endometriosis.

In conclusion, the present study has shown that the *IL-16* polymorphisms analyzed may be involved in the development of endometriosis but additional studies in different populations are needed in an attempt to validate the present results. Moreover, further investigations to clarify the possible role of the gene studied in the clinical course of endometriosis are required to provide functional insight into the role of *IL-16* in endometriosis and elucidate the mechanism by which the *IL-16* gene polymorphisms affect the risk for this disease.

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Competing interests

K.Z. has scientific collaborations in the area of endometriosis with Bayer AG (Leverkusen, Germany), Roche Diagnostics (Basel, Switzerland) and MDNA. Demetrios A. Spandidos is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article.

References

1. Giudice LC and Kao LC: Endometriosis. Lancet 364: 1789-1799, 2004.
2. Halis G and Arici A: Endometriosis and inflammation in infertility. Ann N Y Acad Sci 1034: 300-315, 2004.
3. Burney RO and Giudice LC: Pathogenesis and pathophysiology of endometriosis. Fertil Steril 98: 511-519, 2012.
4. Bazot M, Lafont C, Rouzier R, Roseau G, Thomassin-Naggara I and Daraï E: Diagnostic accuracy of physical examination, transvaginal sonography, rectal endoscopic sonography, and magnetic resonance imaging to diagnose deep infiltrating endometriosis. Fertil Steril 92: 1825-1833, 2009.
5. Koninckx PR, Ussia A, Adamyan L, Wattiez A and Donnez J: Deep endometriosis: Definition, diagnosis, and treatment. Fertil Steril 98: 564-571, 2012.
6. Maroun P, Cooper MJ, Reid GD and Keirse MJ: Relevance of gastrointestinal symptoms in endometriosis. Aust N Z J Obstet Gynaecol 49: 411-414, 2009.
7. Varma R, Rollason T, Gupta JK and Maher ER: Endometriosis and the neoplastic process. Reproduction 127: 293-304, 2004.
8. Sampson J: Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 14: 422-469, 1927.
9. Batt RE, Smith RA, Buck GM, Severino MF and Naples JD: Müllerianosis. Prog Clin Biol Res 323: 413-426, 1990.
10. Vercellini P, Viganò P, Somigliana E and Fedele L: Endometriosis: Pathogenesis and treatment. Nat Rev Endocrinol 10: 261-275, 2014.
11. Simpson JL, Elias S, Malinak LR and Buttram VC Jr: Heritable aspects of endometriosis. I. Genetic studies. Am J Obstet Gynecol 137: 327-331, 1980.
12. Lamb K, Hoffmann RG and Nichols TR: Family trait analysis: A case-control study of 43 women with endometriosis and their best friends. Am J Obstet Gynecol 154: 596-601, 1986.
13. Coxhead D and Thomas EJ: Familial inheritance of endometriosis in a British population. A case control study. J Obstet Gynaecol 13: 42-44, 1993.
14. Stefansson H, Geirsson RT, Steinthorsdottir V, Jonsson H, Manolescu A, Kong A, Ingadottir G, Gulcher J and Stefansson K: Genetic factors contribute to the risk of developing endometriosis. Hum Reprod 17: 555-559, 2002.
15. Treloar SA, O'Connor DT, O'Connor VM and Martin NG: Genetic influences on endometriosis in an Australian twin sample. sueT@qimr.edu.au. Fertil Steril 71: 701-710, 1999.
16. Saha R, Pettersson HJ, Svedberg P, Olovsson M, Bergqvist A, Marions L, Tornvall P and Kuja-Halkola R: Heritability of endometriosis. Fertil Steril 104: 947-952, 2015.
17. Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ and Zhao K: Combinatorial patterns of histone acetylations and methylations in the human genome. Nat Genet 40: 897-903, 2008.

18. Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, Rousseaux S, Rajagopal N, *et al*: Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell 146: 1016-1028, 2011.
19. Falconer H, D'Hooghe T and Fried G: Endometriosis and genetic polymorphisms. Obstet Gynecol Surv 62: 616-628, 2007.
20. Nyholt DR, Low SK, Anderson CA, Painter JN, Uno S, Morris AP, MacGregor S, Gordon SD, Henders AK, Martin NG, *et al*: Genome-wide association meta-analysis identifies new endometriosis risk loci. Nat Genet 44: 1355-1359, 2012.
21. Albertsen HM, Chettier R, Farrington P and Ward K: Genome-wide association study link novel loci to endometriosis. PLoS One 8: e58257, 2013.
22. Rahmioglu N, Nyholt DR, Morris AP, Missmer SA, Montgomery GW and Zondervan KT: Genetic variants underlying risk of endometriosis: Insights from meta-analysis of eight genome-wide association and replication datasets. Hum Reprod Update 20: 702-716, 2014.
23. Zondervan KT, Rahmioglu N, Morris AP, Nyholt DR, Montgomery GW, Becker CM and Missmer SA: Beyond endometriosis genome-wide association study: From genomics to phenomics to the patient. Semin Reprod Med 34: 242-254, 2016.
24. Uimari O, Rahmioglu N, Nyholt DR, Vincent K, Missmer SA, Becker C, Morris AP, Montgomery GW and Zondervan KT: Genome-wide genetic analyses highlight mitogen-activated protein kinase (MAPK) signaling in the pathogenesis of endometriosis. Hum Reprod 32: 780-793, 2017.
25. Kobayashi H, Imanaka S, Nakamura H and Tsuji A: Understanding the role of epigenomic, genomic and genetic alterations in the development of endometriosis (Review). Mol Med Rep 9: 1483-1505, 2014.
26. Rahmioglu N, Montgomery GW and Zondervan KT: Genetics of endometriosis. Wom Health Lond 11: 577-586, 2015.
27. Sapkota Y, Steinthorsdottir V, Morris AP, Fassbender A, Rahmioglu N, De Vivo I, Buring JE, Zhang F, Edwards TL, Jones S, *et al*; iPSYCH-SSI-Broad Group: Meta-analysis identifies five novel loci associated with endometriosis highlighting key genes involved in hormone metabolism. Nat Commun 8: 15539, 2017.
28. Sapkota Y, Attia J, Gordon SD, Henders AK, Holliday EG, Rahmioglu N, MacGregor S, Martin NG, McEvoy M, Morris AP, *et al*: Genetic burden associated with varying degrees of disease severity in endometriosis. Mol Hum Reprod 21: 594-602, 2015.
29. Drosdzol-Cop A and Skrzypulec-Plinta V: Selected cytokines and glycodelin A levels in serum and peritoneal fluid in girls with endometriosis. J Obstet Gynaecol Res 38: 1245-1253, 2012.
30. Athayde N, Romero R, Maymon E, Gomez R, Pacora P, Araneda H and Yoon BH: A role for the novel cytokine RANTES in pregnancy and parturition. Am J Obstet Gynecol 181: 989-994, 1999.
31. Thornton CA, Holloway JA, Shute JK, Holloway JW, Diaper ND and Warner JO: Human mid-gestation amniotic fluid contains interleukin-16 bioactivity. Immunology 126: 543-551, 2009.
32. Mathy NL, Scheuer W, Lanzendörfer M, Honold K, Ambrosius D, Norley S and Kurth R: Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. Immunology 100: 63-69, 2000.
33. Brait VH, Arumugam TV, Drummond GR and Sobey CG: Importance of T lymphocytes in brain injury, immunodeficiency, and recovery after cerebral ischemia. J Cereb Blood Flow Metab 32: 598-611, 2012.
34. Baier M, Bannert N, Werner A, Lang K and Kurth R: Molecular cloning, sequence, expression, and processing of the interleukin 16 precursor. Proc Natl Acad Sci USA 94: 5273-5277, 1997.
35. Center DM, Kornfeld H and Cruikshank WW: Interleukin-16. Int J Biochem Cell Biol 29: 1231-1234, 1997.
36. Chupp GL, Wright EA, Wu D, Vallen-Mashikian M, Cruikshank WW, Center DM, Kornfeld H and Berman JS: Tissue and T cell distribution of precursor and mature IL-16. J Immunol 161: 3114-3119, 1998.
37. Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW and Kornfeld H: Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem 273: 1144-1149, 1998.
38. Bowler RP, Bahr TM, Hughes G, Lutz S, Kim YI, Coldren CD, Reisdorph N and Kechris KJ: Integrative omics approach identifies interleukin-16 as a biomarker of emphysema. OMICS 17: 619-626, 2013.
39. Tong Z, Li Q, Zhang J, Wei Y, Miao G and Yang X: Association between interleukin 6 and interleukin 16 gene polymorphisms and coronary heart disease risk in a Chinese population. J Int Med Res 41: 1049-1056, 2013.
40. Liu XL, Du JZ, Zhou YM, Shu QF and Li YG: Interleukin-16 polymorphism is associated with an increased risk of ischemic stroke. Mediators Inflamm 2013: 564750, 2013.
41. Khoshbakht T, Soosanabadi M, Neishaboury M, Kamali K, Karimlou M, Bazazzadegan N and Khorram Khorshid HR: An association study on IL16 gene polymorphisms with the risk of sporadic Alzheimer's disease. Avicenna J Med Biotechnol 7: 128-132, 2015.
42. Romani S, Hosseini SM, Mohebbi SR, Kazemian S, Derakhshani S, Khanyaghma M, Azimzadeh P, Sharifian A and Zali MR: Interleukin-16 gene polymorphisms are considerable host genetic factors for patients' susceptibility to chronic hepatitis B infection. Hepat Res Treat 2014: 790753, 2014.
43. Glas J, Török H-P, Unterhuber H, Radlmayr M and Folwaczny C: The -295T-to-C promoter polymorphism of the IL-16 gene is associated with Crohn's disease. Clin Immunol 106: 197-200, 2003.
44. Gu XJ, Cui B, Zhao ZF, Chen HY, Li XY, Wang S, Ning G and Zhao YJ: Association of the interleukin (IL)-16 gene polymorphisms with Graves' disease. Clin Immunol 127: 298-302, 2008.
45. Xue H, Gao L, Wu Y, Fang W, Wang L, Li C, Li Y, Liang W and Zhang L: The IL-16 gene polymorphisms and the risk of the systemic lupus erythematosus. Clin Chim Acta 403: 223-225, 2009.
46. Luo SX, Li S, Zhang XH, Zhang JJ, Long GH, Dong GF, Su W, Deng Y, Liu Y, Zhao JM and Qin X: Genetic polymorphisms of interleukin-16 and risk of knee osteoarthritis. PLoS One 10: e0123442, 2015.
47. Gao LB, Rao L, Wang YY, Liang WB, Li C, Xue H, Zhou B, Sun H, Li Y, Lv ML, *et al*: The association of interleukin-16 polymorphisms with IL-16 serum levels and risk of colorectal and gastric cancer. Carcinogenesis 30: 295-299, 2009.
48. Li S, Deng Y, Chen ZP, Huang S, Liao XC, Lin LW, Li H, Peng T, Qin X and Zhao JM: Genetic polymorphism of interleukin-16 influences susceptibility to HBV-related hepatocellular carcinoma in a Chinese population. Infect Genet Evol 11: 2083-2088, 2011.
49. Batai K, Shah E, Murphy AB, Newsome J, Ruden M, Ahaghotu C and Kittles RA: Fine-mapping of IL16 gene and prostate cancer risk in African Americans. Cancer Epidemiol Biomarkers Prev 21: 2059-2068, 2012.
50. Mo CJ, Peng QL, He Y, Wang J, Xie L, Li TJ, Li S and Qin X: Positive association between IL-16 rs11556218 T/G polymorphism and cancer risk: A meta-analysis. Asian Pac J Cancer Prev 15: 4697-4703, 2014.
51. Poulat F, de Santa Barbara P, Desclozeaux M, Soullier S, Moniot B, Bonneaud N, Boizet B and Berta P: The human testis determining factor SRY binds a nuclear factor containing PDZ protein interaction domains. J Biol Chem 272: 7167-7172, 1997.
52. Fanning AS and Anderson JM: Protein-protein interactions: PDZ domain networks. Curr Biol 6: 1385-1388, 1996.
53. Sherman DL and Brophy PJ: A tripartite nuclear localization signal in the PDZ-domain protein L-periaxin. J Biol Chem 275: 4537-4540, 2000.
54. Hsueh YP, Wang TF, Yang FC and Sheng M: Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2. Nature 404: 298-302, 2000.
55. Azimzadeh P, Khorram Khorshid HR, Akhondi MM and Shirazi A: Association of interleukin-16 polymorphisms with disease progression and susceptibility in endometriosis. Int J Immunogenet 43: 297-302, 2016.
56. Hsieh YY, Bau DT, Chang CC, Tsai CH, Chen CP and Tsai FJ: XRCC4 codon 247*A and XRCC4 promoter -1394*T related genotypes but not XRCC4 intron 3 gene polymorphism are associated with higher susceptibility for endometriosis. Mol Reprod Dev 75: 946-951, 2008.
57. Altinkaya SO, Ugur M, Ceylaner G, Ozat M, Gungor T and Ceylaner S: Vascular endothelial growth factor +405 C/G polymorphism is highly associated with an increased risk of endometriosis in Turkish women. Arch Gynecol Obstet 283: 267-272, 2011.
58. Matalliotakis M, Zervou MI, Matalliotaki C, Rahmioglu N, Koumantakis G, Kalogiannidis I, Prapas I, Zondervan K, Spandidos DA, Matalliotakis I and Goulielmos GN: The role of gene polymorphisms in endometriosis. Mol Med Rep 16: 5881-5886, 2017.

59. The American Fertility Society: Revised American Fertility Society classification of endometriosis: 1985. Fertil Steril 43: 351-352, 1985.
60. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, Kiefer F, Gallo Cassarino T, Bertoni M, Bordoli L and Schwede T: SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Res 42: W252-258, 2014.
61. Sato M, Koshiba S, Inoue M, Kigawa T and Yokoyama S: RIKEN Structural Genomics/Proteomics Initiative, deposition 2005-05-23.
62. Kim HS: Assignment of human interleukin 16 (IL16) to chromosome 15q26.3 by radiation hybrid mapping. Cytogenet Cell Genet 84: 93, 1999.
63. Mori M, Yamada R, Kobayashi K, Kawaida R and Yamamoto K: Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. J Hum Genet 50: 264-266, 2005.
64. Gregersen PK and Olsson LM: Recent advances in the genetics of autoimmune disease. Annu Rev Immunol 27: 363-391, 2009.
65. Azimzadeh P, Romani S, Mohebbi SR, Kazemian S, Vahedi M, Almasi S, Fatemi SR and Zali MR: Interleukin-16 (IL-16) gene polymorphisms in Iranian patients with colorectal cancer. J Gastrointestin Liver Dis 20: 371-376, 2011.
66. Gan XL, Lin YH, Zhang Y, Yu TH and Hu LN: Association of an interleukin-16 gene polymorphism with the risk and pain phenotype of endometriosis. DNA Cell Biol 29: 663-667, 2010.
67. Kim JG, Kim H, Ku S-Y, Kim SH, Choi YM and Kim JH: The association between single nucleotide polymorphisms of interleukin (IL)-10, IL-10 receptor antagtonist and IL-16 genes and endometriosis. Fertil Steril 100 (Suppl): S364, 2013.
68. Koga K, Osuga Y, Yoshino O, Hirota Y, Yano T, Tsutsumi O and Taketani Y: Elevated interleukin-16 levels in the peritoneal fluid of women with endometriosis may be a mechanism for inflammatory reactions associated with endometriosis. Fertil Steril 83: 878-882, 2005.
69. Cheong YC, Shelton JB, Laird SM, Richmond M, Kudesia G, Li TC and Ledger WL: IL-1, IL-6 and TNF-alpha concentrations in the peritoneal fluid of women with pelvic adhesions. Hum Reprod 17: 69-75, 2002.
70. Somigliana E, Viganò P, Tirelli AS, Felicetta I, Torresani E, Vignali M and Di Blasio AM: Use of the concomitant serum dosage of CA 125, CA 19-9 and interleukin-6 to detect the presence of endometriosis. Results from a series of reproductive age women undergoing laparoscopic surgery for benign gynaecological conditions. Hum Reprod 19: 1871-1876, 2004.
71. Zhang X, Lin J, Deng L, Chen Z and Chen L: Peritoneal fluid and serum concentration of interleukin-16 in women with endometriosis. Acta Obstet Gynecol Scand 84: 297-298, 2005.
72. Xiao H, Yang L, Liu J, Jiao Y, Lu L and Zhao H: Protein-protein interaction analysis to identify biomarker networks for endometriosis. Exp Ther Med 14: 4647-4654, 2017.
73. Schadt EE: Molecular networks as sensors and drivers of common human diseases. Nature 461: 218-223, 2009.
74. Ma Y, Tian J, Cen H, Li J, Xu WD, Wang DG, Pan HF and Ye DQ: Association of c-Jun gene polymorphism with susceptibility to systemic lupus erythematosus in a Chinese population. DNA Cell Biol. 31: 1274-1278, 2012.
75. Bannert N, Vollhardt K, Asomuddinov B, Haag M, König H, Norley S and Kurth R: PDZ Domain-mediated interaction of interleukin-16 precursor proteins with myosin phosphatase targeting subunits. J Biol Chem 278: 42190-42199, 2003.
76. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN and Bourne PE: The Protein Data Bank. Nucleic Acids Res 28: 235-242, 2000.