PTPN22 C1858T Polymorphism in Women with Endometriosis

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Problem
Endometriosis has been suggested to be an autoimmune disease and recently, an allelic variation of the *PTPN22* (C1858T) gene was revealed to be associated with the development of autoimmunity. The aim of the study was to determine the frequency of the *PTPN22* (C1858T) polymorphism in Brazilian women with endometriosis as compared with controls.

Method of study
Case–control study included 140 women with endometriosis and a control group consisting of 180 healthy fertile women without a history of endometriosis and/or autoimmune diseases from the ABC School of Medicine. The *PTPN22* (C1858T) polymorphism was studied by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR).

Results
Genotypes CC, CT and TT of *PTPN22* polymorphism presented frequencies of 67.9, 30.0 and 2.1% in the women with endometriosis ($P = 0.008$); 76.2, 19.0 and 4.8% in women with minimal/mild endometriosis ($P = 0.173$); 61.0, 39.0 and 0.0% in women with moderate/severe endometriosis ($P \leq 0.001$) and 82.8, 16.1 and 1.1% in control group. Allele C and T were present in 82.9 and 17.1%; 85.7 and 14.3%; 80.5 and 19.5%; and 90.8 and 9.2% respectively, in women with endometriosis ($P = 0.004$), women with minimal/mild endometriosis ($P = 0.148$), women with moderate/severe endometriosis ($P = 0.002$) and control group.

Conclusion
The data suggest that in Brazilian women polymorphism *PTPN22* (C1858T) may be an important genetic predisposing factor for endometriosis, especially, in advanced disease.

Introduction

Endometriosis is a common disease, defined as the growth of endometrial tissue outside the uterine cavity that often results in a vast array of gynecologic problems including dyspareunia, dysmenorrhea, pelvic pain, and infertility.[1] Numerous hypotheses have been put forward to explain the presence of ectopic

endometrial tissue and stroma. The theory of pelvic implantation by retrograde menstruation of endometrial tissues proposed by Sampson[2] is the most widely accepted theory on the pathogenesis of endometriosis. However, retrograde menstruation occurs in up to 80% of women during their reproductive life, and the discrepancy between the incidence of this phenomenon and the occurrence of endometriosis might be explained by the presence of further permissive factors that promote the implantation and growth of endometrial cells.[3] Recently, other familial predispositions, as well as immunologic, genetic, cell adhesion-related, angiogenic and hormonal factors have been proposed.[4]

Some authors have suggested that endometriosis may be an autoimmune disease, because it is often associated with the presence of antinuclear, antiphospholipid and anti-endometrial autoantibodies as well as an abrogated cell-mediated immunity reaction manifested, for example, by decreased activity of natural killer cells and cytotoxic T-lymphocytes.[3,5,6] Genetic factors play a role in the pathogenesis of endometriosis,[1] and autoimmunity genes are therefore reasonable candidate genes for endometriosis.[7]

Protein tyrosine phosphatase non-receptor 22 (*PTPN22*) is located on chromosome 1 (1p13.3–13.1) and encodes a lymphoid-specific phosphatase known as Lyp that is an important downregulator of T-cell activation.[8] Recently, an allelic variation of the *PTPN22* gene – C1858T – was revealed to be associated with the development of autoimmunity.[9] So far, the *PTPN22* C1858CT polymorphism (also called LYP-W620) has been reported to be associated with type 1 diabetes,[10] systemic lupus erythematosus,[11] rheumatoid arthritis,[12] Grave's disease[13] and some other autoimmune disorders.[14]

Thus, the aim of this study was to determine the frequency of the *PTPN22* C1858T polymorphism in Brazilian women with endometriosis and controls.

Materials and methods

Patients

One hundred and forty women affected by endometriosis (mean age: 34.3 ± 4.3 years) were recruited from the Endometriosis Outpatient clinic of the ABC School of Medicine. Endometriosis had been confirmed and classified in these patients, both by laparoscopic and histopathologic examinations, according to the revised American Society for Reproductive Medicine classification.[15] The study group consisted of 63 patients (45.0%) with minimal/mild (stage I/II) and 77 patients (55.0%) with moderate/severe (stage III/IV) disease. All patients had normal blood counts on admittance and, according to basic laboratory tests and clinical interviews, did not suffer from any serious chronic disease, including autoimmune disorder(s). The control group was composed of 180 fertile women, recruited from the Family Planning Outpatient clinic, without a history of autoimmune diseases (mean age: 39.8 ± 4.5 years) who underwent tubal ligation, which allows the confirmation of the absence of endometriosis.

Clinical data and peripheral blood samples were collected after explaining the objectives of the study and obtaining signed informed consent from all patients and controls, approved by the local ethics committee.

Genotyping of *PTPN22* C1858T Polymorphisms

Peripheral blood was collected from each patient and control in an ethylene diamine tetra-acetic acid (EDTA)-containing tube. Genomic DNA was extracted from peripheral blood lymphocytes using the Illustra blood genomicPrep Mini Spin Kit, according to the manufacturer's instructions (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK).

The PCR procedure was carried out according to Ammendola et al.[6] with modifications, in a total volume of 25 μL reaction mixture containing 10× reaction buffer (500 mM KCl, 100 mM Tris-Cl; pH 8.3), 2.5 mM $MgCl_2$, 0.8 mM dNTP, 2.0 U Taq polymerase, and 50 nM of each *PTPN22* (5′-TCACCAGCTT CCTCAACCACA-3′) sense and (5′-GATATTGTTGCTT CAACGGAA TTT-3′) antisense primer. PCR generated a fragment of 220 base pairs (bp). The cycling profile consisted of denaturation at 95°C for 30 s, annealing at 60°C for 45 s, and extension at 72°C for 30 s, except for the first cycle when denaturation was extended to 5 min. The PCR product was digested with 2 U of *Xcm*I restriction enzyme, and the reaction mixture was incubated at 37°C for 2 hr. The digestion product was subjected to electrophoresis on a gel containing 3% agarose and stained with ethidium bromide.

Statistical Analysis

The Pearson's chi-square was used to compare allele and genotype frequencies between groups and to

estimate the Hardy–Weinberg equilibrium. Statistical tests of significance and chi-square analysis were carried out using SPSS for Windows 8.0 (SPSS, Inc., Chicago, IL, USA). All *P*-values were two-tailed, and 95% confidence intervals (CIs) were calculated. A *P*-value of <0.05 was considered statistically significant.

Results

The CC, CT and TT genotype frequencies of the *PTPN22* C1858T polymorphism in the patients with endometriosis were 67.9% (95/140), 30.0% (42/140) and 2.1% (3/140) respectively ($P = 0.008$). Among the women with minimal/mild endometriosis (stage I/II) 76.2% (48/63) presented the normal homozygous genotype GG, 19.0% (12/63) the heterozygous genotype GA, and 4.8% (3/63) the mutated homozygous genotype AA ($P = 0.173$). In the patients with moderate/severe endometriosis (stage III/IV) the frequencies of genotypes GG, GA and AA were 61.0% (47/77), 39.0% (30/77) and 0.0%, respectively, $P \leq 0.001$. In the control group, 82.8% (149/180) presented the normal homozygous genotype CC, 16.1% (29/180) the heterozygous genotype CT, and 1.1% (2/180) the homozygous mutated genotype TT (Table I).

Considering the alleles, allele C was present in 82.9% of the patients with endometriosis and in 90.8% of the control group, whereas allele T was present in 17.1 and 9.2% respectively, in the patients with endometriosis and control group ($P = 0.004$). Of the women with minimal/mild endometriosis, 85.7% presented allele C and 14.3% presented allele T ($P = 0.148$). The frequencies of the alleles C and T were 80.5 and 19.5% respectively, in patients with moderate/severe endometriosis ($P = 0.002$) (Table I).

Statistical analysis showed that the genotype distribution in endometriosis and control groups was in Hardy–Weinberg equilibrium.

Discussion

Single nucleotide polymorphisms (SNPs) are common in the human genome and often provide correlative evidence for the involvement of specific genes in human disease.[16] Single nucleotide polymorphisms that affect the function of crucial components of the T-cell antigen receptor (TCR) signaling pathways could have profound effects on the function of the immune system and thus on the development of autoimmune diseases.[17] Protein tyrosine phosphatases (PTPs) are particularly good candidates for carrying disease-related SNPs because they are involved in preventing spontaneous T-cell activation and they restrict the response to antigen by dephosphorylating and inactivating TCR-associated kinases and their substrates.[18]

Immunologic theories have suggested that in women with endometriosis alterations in T-cell-mediated immunity may facilitate implantation of endometrial fragments or cells in ectopic locations, either directly interfering with T-cell cytotoxicity or indirectly altering their cooperation with other cell types of the immune network [macrophages, natural killer (NK) cells, B cells].[19,20]

The missense single-nucleotide polymorphism at nucleotide 1858 causes a substitution of arginine at codon 620 (CGG) for tryptophan (TGG). The autoimmune-associated *PTPN22* C1858T variant does not bind kinases well and appears to encode a gain-

Table I Genotype and Allele Frequencies of Polymorphism *PTPN22* C1858T in Endometriosis Patients and Controls

		PTPN22 C1858T genotype					Alleles			
Subjects	*n*	CC (%)	CT (%)	TT (%)	*P*	OR (95% CI)[a]	C (%)	T (%)	*P*	OR (95% CI)
Endometriosis	140	95 (67.9)	42 (30.0)	3 (2.1)	0.008	2.28 (1.35–3.85)	232 (82.9)	48 (17.1)	0.004	2.05 (1.28–3.29)
Minimal/mild endometriosis	63	48 (76.2)	12 (19.0)	3 (4.8)	0.173	1.50 (0.75–3.02)	108 (85.7)	18 (14.3)	0.148	1.65 (0.89–3.05)
Moderate/severe endometriosis	77	47 (61.0)	30 (39.0)	0 (0.0)	<0.001	3.07 (1.68–5.59)	124 (80.5)	30 (19.5)	0.002	2.40 (1.40–4.10)
Controls	180	149 (82.8)	29 (16.1)	2 (1.1)			327 (90.8)	33 (9.2)		

OR, odds ratio; CI, confidence interval.
[a]CT + TT versus CC genotype.

of-function enzyme.[14,21] The mechanism of action of *PTPN22* in autoimmunity still needs to be clarified. However, increased inhibition of T-cell-receptor signaling caused by the *PTPN22* C1858T polymorphism could predispose to autoimmunity, either by affecting thymic deletion of autoreactive T cells or by affecting the development or function of peripheral regulatory T cells.[22]

Ammendola et al.[6] studied 132 Italian women hospitalized for endometriosis and 232 healthy controls (163 men and 69 women) by PCR and found an association between the *PTPN22* C1858T polymorphism and endometriosis ($P = 0.008$). Similarly, Płoski et al.[23] studied 171 Polish women with endometriosis and 310 anonymous and unrelated adults as controls, however, the results disclosed no association between the *PTPN22* C1858T polymorphism and endometriosis. In this study, the genotype frequencies of the *PTPN22* C1858T polymorphism were statistically different in women with endometriosis ($P = 0.008$) when compared with controls. When we studied the patients with minimal/mild endometriosis and moderate/severe endometriosis separately, the difference was more evident in the women with advanced disease, $P = 0.173$ and $P \leq 0.001$ respectively. The finding suggests that the *PTPN22* C1858T polymorphism is actually related to endometriosis development in Brazilian population, especially in moderate/severe endometriosis.

The inconsistency among studies could have resulted from a difference in the ethnic makeup. The genetic background is different between ethnic groups and may have produced a variation in genetic factors involved in the pathogenesis of endometriosis in these populations (Table II). Another possible reason for the difference observed between the studies could be resulting from the selection of the control group. Most of the published studies on endometriosis have used heterogeneous control groups, such as healthy men and women,[6,23] however, the absence of symptomatology in women does not rule out endometriosis, taking into account that about 16% of the patients with this disease are fertile and asymptomatic.[24,25] Our control group was carefully selected among fertile, non-menopausal women and without history of autoimmune diseases that had been submitted to tubal ligation for family planning reasons and had no sign of endometriosis in their clinical history.

Besides, *PTPN22* C1858T polymorphism was significantly associated with only stage III/IV endometriosis, not with stage I/II, in Brazilian women. Superficial endometriosis has been described as a cyclical and normal phenomenon in the life of a woman, but in some women the development and progression of this disease occurs as a result of immunologic and genetic alterations. Some authors have considered superficial endometriosis as a physiological and intermittent condition in women during their reproductive years, whereas its progression, characterized as deep infiltrative endometriosis and endometrial ovarian cysts, is considered to be the true disease.[26–28] Divergences persist regarding the natural history of endometriosis, its symptoms, extent, location, and staging.[26]

The peritoneal fluid of women with endometriosis shows a marked increase in the number of macrophages.[25] This increase causes higher local secretion of various products. Among these are growth factors and cytokines, which may be involved in the mechanism for the implantation and subsequent development and proliferation of endometriotic implants. Moreover, some authors have suggested that there is a correlation between serum levels of CA-125 and the proliferative activity of the epithelial cells in endometriotic lesions,[29] because endometriosis is an inflammatory process associated with altered function of immune-related cells in the peritoneum and

Table II Frequency of Polymorphism *PTPN22* C1858T in Endometriosis Patients and Controls, in Different Population Studies

Study	Population studied	Conclusion of the study
Ammendola et al.[6]	132 Italian women with endometriosis and 232 healthy blood donors (163 males and 69 females)	Association of polymorphism *PTPN22* C1858T with endometriosis
Płoski et al.[23]	171 Polish endometriosis patients and 310 anonymous, unrelated adults	No association of endometriosis with polymorphism *PTPN22* C1858T
Present study	140 Brazilian women with endometriosis and 180 fertile women without history of endometriosis and/or autoimmune disease	Association of polymorphism *PTPN22* C1858T with endometriosis, especially advanced disease

may be viewed as a local disease with systemic and subclinical inflammation.[30] These alterations contribute to the mechanism of infertility, resulting from an intraperitoneal exudate of unknown cause, even in the presence of normal ovulatory function.[20]

Autoimmune diseases, which affect about 5% of the population and disproportionately affect women, comprise a heterogeneous group of disorders.[29] The recent recognition that these diseases need to be considered, at a genetic and mechanistic level, as a related group of diseases has helped the field forward. Recent genetic analyses have supported the existence of common pathways to autoimmunity and have identified susceptibility loci shared by several autoimmune diseases.[30] These observations suggest that there are common pathways of intracellular signaling or cellular interactions that are dysregulated in more than one autoimmune disease. Moreover, they provide an explanation for the evidence that these diseases cluster in families with different individuals having different diseases.

The immune system probably plays a role in the onset and development of endometriosis, and the *PTPN22* genotype could perhaps be used as one component of a set of predictor genes for endometriosis. In conclusion, our results suggest that in the Brazilian population studied endometriosis is associated with the *PTPN22* C1858T polymorphism, especially in women with advanced disease, which is a marker for a variety of autoimmune disorders. However, further studies with much larger samples are needed to evaluate whether or not the association is real.

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Conflict of interest

None of the authors has any conflict of interest to disclose.

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