



Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Analysis of vitamin D receptor gene polymorphisms in women with and without endometriosis

Fábia Lima Vilarino, Bianca Bianco*, Tatiana Goberstein Lerner, Juliana Souto Teles, Fernanda Abani Mafra, Denise Maria Christofolini, Caio Parente Barbosa

Division of Pathological Gynecology and Human Reproduction, Department of Gynecology and Obstetrics, Faculdade de Medicina do ABC, Santo André/São Paulo, Brazil

ARTICLE INFO

Article history:

Received 2 November 2010

Accepted 13 January 2011

Available online 25 January 2011

Keywords:

Autoimmunity

Endometriosis

Infertility

Vitamin D receptor gene

Polymorphism

ABSTRACT

An aberrant immunologic mechanism has been suggested to be involved in the pathogenesis of endometriosis. Genetic alterations in the vitamin D receptor gene (*VDR*) may lead to important defects in gene activation that principally affect immune function. We have hypothesized a possible relationship between endometriosis and/or infertility and the *VDR* polymorphisms (*Apal*, *TaqI*, *FokI*, and *BmsI*). The study was a case–control study including 132 women with endometriosis-related infertility, 62 women with idiopathic infertility, and 133 controls. *VDR* polymorphisms were studied by restriction fragment length polymorphism. We found relatively similar *VDR* polymorphism genotype frequencies in cases and controls. When patients with minimal/mild and moderate/severe endometriosis were studied separately, no difference was found. When we compared infertile groups with and without endometriosis there was no statistically significant difference. The data suggest that *VDR* polymorphisms did not play an important role in the pathogenesis of endometriosis and/or infertility in the Brazilian women studied.

© 2011 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc.

Open access under the [Elsevier OA license](http://www.elsevier.com/locate/elsevier/oa-elsevier).

1. Introduction

Endometriosis is a common estrogen-dependent gynecologic 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]. Susceptibility to endometriosis depends on a complex interaction of immunologic, genetic, and hormonal factors [2,3].

Numerous hypotheses have been put forward to explain the presence of ectopic endometrial tissue and stroma. Levander [4] attempted to link 2 previous theories: metaplasia proposed by Meyer [5] and retrograde tubal endometrial reflux proposed by Sampson [6]. The presence of this abnormal menstrual reflux would irritate the peritoneum. In defending itself, the peritoneum would secrete activating and growth factors, which facilitate implantation and growth and could thus induce metaplasia [4]. This unifying theory is supported by modern immunologic concepts. The immune system participates in the homeostasis of the peritoneal cavity. Modifications in the peritoneal cavity functioning have been advanced to explain endometriosis and its consequences [7,8].

Some authors have suggested that endometriosis may have an autoimmune component because it is often associated with the presence of antinuclear, antiphospholipid, and antiendometrial autoanti-

bodies as well as an abrogated cell-mediated immunity reaction manifested, for example, by decreased activity of natural killer cells and cytotoxic T lymphocytes [7–9]. Genetic factors play a role in the pathogenesis of endometriosis [3,10] and autoimmunity genes are therefore reasonable candidate genes for endometriosis [11].

Vitamin D is a hormone that has essential roles in endocrine functions, regulating cell replication, and other metabolic pathways, such as those involved in immune response. Vitamin D suppresses lymphocyte proliferation and immunoglobulin synthesis and inhibits the action of proinflammatory transcription factors and the production of different cytokines, such as interleukin 2 and interleukin 12, among others [12]. Most of the biologic activities of vitamin D are mediated by a high-affinity receptor that acts as a transcription factor activated by the ligand receptor gene of vitamin D [*VDR*; OMIM 601769]. *VDR*, located on chromosome 12 (12q13.11), is a member of the family of steroid receptors that mediates the effects of vitamin D in regulating the transcription of multiple genes [13]. The *VDR* gene is expressed in most cells of the immune system, including CD4⁺ and CD8⁺, as well as antigen-presenting cells, macrophages, and dendritic cells; these cells also possess the first hydroxylase, which catalyzes the synthesis of active vitamin D [14].

Genetic alterations in the *VDR* gene may lead to important defects in gene activation principally affecting calcium metabolism, cell proliferation, and immune function. The function of the *VDR* gene is influenced by several genetic polymorphisms associated with susceptibility to a range of diseases, such as osteoar-

* Corresponding author.

E-mail address: bianca.bianco@hotmail.com (B. Bianco).

thritis, diabetes, cancer, rheumatoid arthritis, and Graves disease [15].

In addition, vitamin D has been reported to increase responsiveness to estradiol and is used in osteoporosis treatment of postmenopausal women [16]. Thus, because endometriosis is an estrogen-dependent disease, serum levels of vitamin D associated with polymorphisms in its receptor gene could increase disease susceptibility.

Based on this observation, we have hypothesized the existence of a relationship between endometriosis and/or infertility and polymorphisms (*Apal*, *TaqI*, *FokI*, and *BmsI*) of the vitamin D receptor gene.

2. Subjects and methods

2.1. Patients

Three hundred seventy-four infertile women with endometriosis were selected to participate in the study. From these patients, 132 infertile women with endometriosis (mean age, 35.1 ± 3.9 years) from the Endometriosis Outpatient Clinic of the Human Reproduction Service of the Faculdade de Medicina do ABC (FMABC) were studied once they met the criteria selection. The studied women were diagnosed with endometriosis by laparoscopy and classified according to the American Society for Reproductive Medicine [17] with histologic confirmation of disease. Women with acute or chronic medical conditions, especially autoimmune diseases, were excluded. In the endometriosis group, disease stage was minimal/mild (stage I and II) in 72 cases (54.5%) and moderate/severe (stage III and IV) in 60 cases (45.5%). Sixty-two women with idiopathic infertility (mean age, 35.7 ± 5.0 years) were screened at the Human Reproduction Service of the FMABC. For the control group, 133 fertile women (mean age, 39.7 ± 3.2 years) without autoimmune diseases were selected from the Family Planning Outpatient Clinic of the FMABC among a group submitted for tubal ligation and with confirmed absence of endometriosis.

The cause of infertility was investigated according to the minimum propedeutic procedure for infertile couples: hormonal and biochemistry profile, testing for sexually transmitted diseases, imaging examinations, investigation of genetic and/or immunologic abnormalities, semen analysis, hysterosalpingography, hysteroscopy, and laparoscopy (laparoscopy was performed in all women up to 36 years old as well as in patients over 36 years old whenever there were symptoms or abnormalities on imaging examinations). In the absence of abnormalities in any of these exams, infertility was considered idiopathic. Women with endometriosis who did not achieve pregnancy after at least 6 natural or induced cycles following laparoscopy were considered infertile. Women with partners having any male factors associated with infertility were excluded from the study.

Clinical data and peripheral blood samples were collected only after the objectives of the study were explained and signed informed consent was obtained, as approved by the Research Ethics Committee of the Faculdade de Medicina do ABC.

2.2. Molecular analysis

Peripheral blood was collected from each patient and control in an EDTA-containing tube. Genomic DNA was extracted from peripheral blood lymphocytes according to Lahiri and Numberger [18].

The *VDR* gene polymorphisms were studied by restriction fragment length polymorphism PCR, according to the protocol of Györfy et al. [19], with modifications. In general, the PCR procedure was carried out in a total volume of 25- μ L reaction mixture containing $10\times$ 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 primer (sense and antisense). The cycling profile consisted of denaturation at 95°C for 30 seconds; annealing temperature varied according to polymorphism (Table 1), and extension was at 72°C for 30 seconds, except for the first cycle, when denaturation was extended to 5 minutes. The PCR product was digested with 5 U (*BmsI* and *FokI*) or 10 U (*Apal* and *TaqI*) of the restriction enzyme (New England Biolabs, Ipswich, MA), and the reaction mixture was incubated at 65°C for 15 minutes. The digestion product was subjected to electrophoresis on a gel containing 2% agarose stained with ethidium bromide and visualized under ultraviolet light.

A random subset (~20% of samples) was also evaluated by quantitative PCR to confirm the results of commercially available *BmsI* (rs15444410) and *TaqI* (rs731236) polymorphism. Taqman primers and probes for *BmsI* and *TaqI* polymorphisms were used (C_8716062_10 and C_2404008_10, respectively; Applied Biosystems, Foster City, CA). Assays were performed with Taqman Universal Master Mix (Applied Biosystems, Foster City, CA) with 50 ng of DNA per reaction. PCR conditions were as recommended by the manufacturer: initial denaturation at 95°C (15 minutes), followed by 40 denaturation cycles at 95°C (15 seconds) and a final annealing/extension cycle at 60°C (1 minute).

2.3. Statistical analysis

Statistical analyses were carried out using SPSS for Windows 11.0 (SPSS, Inc., Chicago, IL). The χ^2 test was used to compare allele and genotype frequencies between groups, to estimate Hardy-Weinberg equilibrium, and to calculate the power of the test. The odds ratio (OR) and range with 95% confidence interval (95% CI) were calculated for the presence of the reference genotype using a logistic regression model. The association between the combined genotypes of *VDR* gene polymorphisms and risk of infertility-related endometriosis was also evaluated by the study of haplotypes using Haploview software version 4.1 (<http://www.hapmap.org>). All *p* values were two-tailed, and 95% CIs were calculated. A *p* value < 0.05 was considered statistically significant.

3. Results

The genotype and allele distributions of *Apal*, *TaqI*, *FokI*, and *BmsI* polymorphisms of *VDR* gene in infertile women with endometriosis, women with idiopathic infertility, and controls are summarized in Table 2.

Table 1
VDR polymorphisms and respective primers, restriction enzymes, and annealing temperatures

Polymorphism	rs	Location	Primer	Restriction enzyme	Annealing temperature (°C)
<i>Apal</i> G1025-49T	11168271	Exon 2	F: CAG AGC ATG GAC AGG GAG CAA R: GCA ACT CCT CAT GGC TGA GGT CTC	<i>Apal</i>	68
<i>TaqI</i> T1056C	731236	Exon 9	F: CAG AGC ATG GAC AGG GAG CAA R: CAC TTC GAG CAC AAG GGG CGT TAG C	<i>TaqI</i>	68
<i>FokI</i> T2C	10735810	Exon 9	F: AGC TGG CCC TGG CAC TGA CTC TGC TCT R: ATG GAA ACA CCT TGC TTC TTC TCC CTC	<i>FokI</i>	60
<i>BmsI</i> G1024+283A	1544410	Intron 8	F: AACCAAGACTACAAGTACCGCTCAGTGA R: AACCAGCGGGAAGAGGTCAAGGG	<i>BmsI</i>	62

Table 2Genotype and allele frequencies of *VDR* polymorphisms *Apal*, *TaqI*, *BsmI*, and *FokI* in women with endometriosis, women with idiopathic infertility, and controls

VDR polymorphism	n	Genotypes			Alleles		p ^a	OR (95% CI)	p ^b
		n (%)	n (%)	n (%)	n (%)	n (%)			
<i>Apal</i>		GG	GA	AA	G	A			
G1025-48T									
Endometriosis-associated infertility patients	132	44 (33.4)	72 (54.5)	16 (12.1)	160 (60.6)	104 (39.4)	0.806	1.06 (0.75–1.51)	0.823
Minimal/mild endometriosis	72	21 (29.1)	41 (57.0)	10 (13.9)	83 (57.7)	61 (42.3)	0.446	1.20 (0.79–1.81)	0.920
Moderate/severe endometriosis	60	23 (38.3)	31 (51.7)	6 (10.0)	77 (64.2)	43 (35.8)	0.777	0.91 (0.58–1.43)	0.471
Idiopathic infertile patients	62	33 (35.5)	29 (46.8)	11 (17.7)	73 (58.9)	51 (41.1)	0.631	1.14 (0.74–1.76)	
Controls	133	49 (36.8)	67 (50.4)	17 (12.8)	165 (62.0)	101 (38.0)			
<i>TaqI</i>		TT	TC	CC	T	C			
T1056C									
Endometriosis-associated infertility patients	132	55 (41.7)	62 (47.0)	15 (11.3)	172 (65.1)	92 (34.9)	0.841	0.96 (0.67–1.37)	0.124
Minimal/mild endometriosis	72	31 (43.1)	34 (47.2)	7 (9.7)	96 (66.7)	48 (33.4)	0.654	0.90 (0.59–1.38)	0.112
Moderate/severe endometriosis	60	24 (40.0)	28 (46.7)	8 (13.3)	76 (63.4)	44 (36.6)	1.0	1.04 (0.67–1.63)	0.335
Idiopathic infertile patients	62	20 (32.2)	30 (48.4)	12 (19.3)	70 (56.4)	54 (43.5)	0.195	1.39 (0.90–2.14)	
Controls	133	50 (37.6)	71 (53.4)	12 (9.0)	171 (64.3)	95 (35.7)			
<i>FokI</i>		TT	TC	CC	T	C			
T2C									
Endometriosis-associated infertility patients	132	60 (45.5)	61 (46.2)	11 (8.3)	181 (68.5)	83 (31.5)	1.0	0.99 (0.69–1.43)	0.493
Minimal/mild endometriosis	72	30 (41.7)	33 (45.8)	9 (12.5)	93 (64.6)	51 (35.4)	0.497	1.19 (0.77–1.82)	0.203
Moderate/severe endometriosis	60	30 (50.0)	28 (46.7)	2 (3.3)	88 (73.3)	32 (26.7)	0.393	0.79 (0.49–1.27)	1.0
Idiopathic infertile patients	62	31 (50.0)	28 (45.2)	3 (4.8)	90 (72.6)	34 (27.4)	0.475	0.82 (0.51–1.31)	
Controls	133	59 (44.4)	64 (48.1)	10 (7.5)	182 (68.4)	84 (31.6)			
<i>BsmI</i>		GG	GA	AA	G	A			
G1024+283A									
Endometriosis-associated infertility patients	132	53 (40.1)	69 (52.3)	10 (7.6)	175 (66.3)	89 (33.7)	0.537	1.14 (0.79–1.64)	0.920
Minimal/mild endometriosis	72	30 (41.6)	37 (51.4)	5 (7.0)	97 (67.4)	47 (32.6)	0.791	1.09 (0.70–1.68)	0.920
Moderate/severe endometriosis	60	23 (38.3)	32 (53.3)	5 (8.4)	78 (65.0)	42 (35.0)	0.488	1.21 (0.77–1.91)	1.0
Idiopathic infertile patients	62	24 (38.7)	34 (54.8)	4 (6.5)	82 (66.1)	42 (33.9)	0.631	1.15 (0.73–1.81)	
Controls	133	59 (44.4)	66 (49.6)	8 (6.0)	184 (69.2)	82 (30.8)			

OR = odds ratio; CI = confidence interval.

^aVersus control group.^bVersus idiopathic infertile group.

We found relatively similar *VDR* polymorphism genotype frequencies in cases and controls and we did not observe any association between polymorphisms in *Apal*, *TaqI*, *FokI*, and *BsmI* and endometriosis risk in endometriosis-related infertility or idiopathic infertile groups. When patients with minimal/mild endometriosis and moderate/severe endometriosis were studied separately, no difference was found for any *VDR* polymorphism. When we compared infertile groups with and without endometriosis there was no statistically significant difference related to the studied polymorphism frequency.

Statistical analyses showed that the genotype distribution in endometriosis-related infertility, idiopathic infertility, and control groups for all polymorphisms studied were in Hardy–Weinberg equilibrium. Haplotype analysis showed that none of the *VDR* haplotypes was associated with the endometriosis-related infertility sample (Fig. 1). The power of the test calculated was <0.50 ($\alpha = 0.05$) to the endometriosis-related infertility group.

4. Discussion

We did not find a significant difference in the frequencies of the *VDR* polymorphisms either between infertile women with endometriosis and controls or between idiopathic infertile women and controls. To our knowledge, this is the first study in the literature to investigate the association between *VDR* polymorphisms (*Apal*, *TaqI*, *FokI*, and *BsmI*) and endometriosis and/or infertility.

Single nucleotide polymorphisms are common in the human genome and often provide correlative evidence for the involvement of specific genes in human disease. A polymorphism is a genetic variant that appears in at least 1% of the population. Changes in the regulatory parts of the gene could affect the degree of expression of the gene and thus the levels of the protein. For instance, changes in the 5' promoter of the *VDR* gene can affect mRNA expression patterns and levels, whereas 3' untranslated region sequence variations can affect mRNA stability and protein translation efficiency

[15]. There is not much information regarding how the polymorphisms affect vitamin D receptor transcription. The *VDR FokI* polymorphism in exon 2 leads to an alternative transcription initiation site, resulting in a *VDR* protein with the addition of 3 amino acids. The *VDR BsmI*, *TaqI*, and *Apal* polymorphisms have no established functional role yet [20].

In animal and cell culture studies, tolerogenic dendritic cells are induced by active vitamin D treatment and promote the induction of Tregs, regulatory T cells that are critical for maintaining immune tolerance, which are suggested to prevent autoimmune diseases

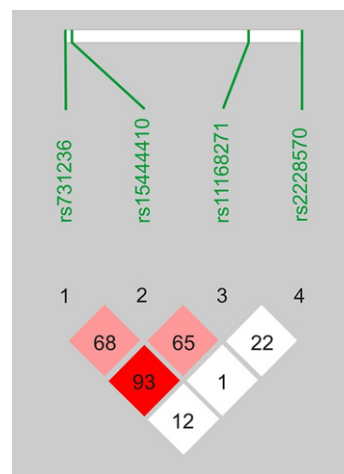


Fig. 1. Graphical representation of the linkage disequilibrium structure of the *VDR* haplotype block, obtained with Haploview v. 4.1 software. Squares represent the pairwise calculation of r^2 (top) and D' (bottom) in the female control cohort (values within the squares, $100\times$) for each combination of single nucleotide polymorphisms. The red scale represents proximity to 1 (lighter red, r^2 close to 0; darker red, r^2 close to 1).

because of their immunosuppressive activity [21,22]. Prietl et al. [23] demonstrated that vitamin D intake significantly increased the percentage of Tregs in the peripheral circulation.

The possible link between endometriosis and the vitamin D system has been poorly investigated in the past. The first observation was reported by Hartwell et al. [24], who observed higher serum levels of 1,25-dihydroxyvitamin D3 and similar levels of 25-hydroxyvitamin D3 in a small group of women with endometriosis compared with controls. Recently, Vigano et al. [25] demonstrated that human endometrium can be included among those sites capable of extrarenal synthesis of active vitamin D. The enzyme that catalyzes the synthesis of 1,25-dihydroxyvitamin D3, 1 α -hydroxylase, is expressed in both eutopic and ectopic endometrium and its expression is enhanced in the eutopic endometrium of women with endometriosis. Measurement of 1,25-dihydroxyvitamin D3 levels in the supernatant of endometrial cells treated with 25-hydroxyvitamin D3 confirmed that endometrium represents a site of local conversion from the precursor to the active form.

Somigliana et al. [26] studied serum levels of 25-hydroxyvitamin D3, 1,25-dihydroxyvitamin D3, and Ca²⁺ by radioimmunoassay in 87 women with endometriosis and 53 controls. The authors observed that the levels of 25-hydroxyvitamin D3 were significantly increased in the serum of women with endometriosis. A biologic gradient indicating more striking differences in patients with advanced stages was also noted, resulting in the conclusion that endometriosis is associated with higher serum levels of vitamin D.

In a recent study, Faserl et al. [27] identified differences in protein expression in serum that might shed light on the pathophysiology of endometriosis. The authors found 25 protein spots with a significant difference in abundance between women with endometriosis and controls, including acute-phase proteins and complement components. The abundance of vitamin D-binding protein was higher in all endometriosis pools compared with the control pool ($p < 0.02$). Faserl et al. concluded that the inability to sufficiently activate phagocytic function of macrophages in women with endometriosis may allow endometriotic tissues to implant in the peritoneal cavity.

Many epidemiologic studies have linked vitamin D and an increased prevalence of autoimmune disease [28–30]. Although endometriosis has been considered an autoimmune disease, in the present study we did not find any association between VDR polymorphisms and endometriosis-related infertility or idiopathic infertility. When we studied patients with minimal/mild endometriosis and moderate/severe endometriosis separately, no difference was found. The finding suggests that the VDR (*Apal*, *TaqI*, *FokI*, and *BmsI*) polymorphisms are not related to endometriosis pathogenesis in the Brazilian population. When we compared infertile groups with and without endometriosis to determine whether the polymorphism was linked to endometriosis or infertility, there was no statistically significant difference related to the frequencies of the studied polymorphisms, which suggests that VDR polymorphisms are not related to infertility risk in the Brazilian population.

A major limitation of our study is the relatively low number of patients, which reduced the statistical power to detect associations between the studied polymorphisms and unexplained female infertility and/or endometriosis. However, the small number of studied patients is the result of selection criteria once all patients included in this study were operated on using laparoscopy and classified according to endometriosis stage with histologic confirmation of the disease. None of the patients had a clinical history of autoimmune disease.

In conclusion, the results suggest that VDR (*Apal*, *TaqI*, *FokI*, and *BmsI*) polymorphisms do not play a role in the pathogenesis of idiopathic infertility and endometriosis-related infertility in Brazilian women. However, the results do exclude a role of

vitamin D in endometriosis. Perhaps other polymorphisms and mutations can act in the vitamin D influences the disease. It would be of great interest to characterize the actual relation between these mutations and endometriosis and/or infertility in a large number of cases.

Acknowledgments

The authors thank FAPESP for granting students Tatiana Goberstein Lerner (2010/01104-6) and Juliana Souto Teles (2009/01960-2) a Scientific Initiation scholarship and for research grants 2010/00459-5.

References

- [1] Barbosa CP, de Souza AM, Bianco B, Christofolini DM, Mafrá FA, de Lima GR. OC-125 immunostaining in endometriotic lesion samples. Arch Gynecol Obstet 2009. [Epub ahead of print, 12 April 2009].
- [2] Bianco B, Christofolini DM, Mafrá FA, Brandes A, Zulli K, Barbosa CP. +1730 G/A polymorphism of the estrogen receptor beta gene (ERbeta) may be an important genetic factor predisposing to endometriosis. Acta Obstet Gynecol Scand 2009;88:1397–401.
- [3] Gomes FM, Bianco B, Teles JS, Christofolini DM, de Souza AM, Guedes AD, et al. PTPN22 C1858T polymorphism in women with endometriosis. Am J Reprod Immunol 2010;63:227–32.
- [4] Levander G. Über die pathogenese bei Endometriose. Arch Klin Chirg 1941; 202:497–515.
- [5] Meyer R. Ueber den Stand der Frage der adenomyositis und adenomyome in algemeinen und insbesondere über adenomyositis serosoepithelialis und adenomyometritis sarcomatosa. Zentralbl Gynäkol 1919;43:745–50.
- [6] Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927;14: 422–8.
- [7] Matarese G, De Placido G, Nikas Y, Alviggi C. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends Mol Med 2003; 9:223–8.
- [8] Vinatier D, Dufour P, Oosterlynck D. Immunological aspects of endometriosis. Hum Reprod Update 1996;2:371–84.
- [9] Nothnick WB. Treating endometriosis as an autoimmune disease. Fertil Steril 2001;76:223–31.
- [10] Giudice LC, Kao LC. Endometriosis. Lancet 2004;364:1789–99.
- [11] Vigano P, Lattuada D, Somigliana E, Abbiati A, Candiani M, Di Blasio AM. Variants of the CTLA4 gene that segregate with autoimmune diseases are not associated with endometriosis. Mol Hum Reprod 2005;11:745–9.
- [12] Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, Cantorna MT. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Mol Endocrinol 2003;17:2386–92.
- [13] Whitfield GK, Hsieh JC, Jurutka PW, Selznick SH, Haussler CA, MacDonald PN, et al. Genomic actions of 1,25-dihydroxyvitamin D3. J Nutr 1995;125(6 suppl): 1690S–4S.
- [14] Lehman B. The vitamin D3 pathway in human skin and its role for regulation of biological processes. Photochem Photobiol 2005;81:1246–51.
- [15] Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. Clin Chim Acta 2006;371:1–12.
- [16] Kurabayashi T, Tomita M, Matsushita H. Association of vitamin D and estrogen receptor gene polymorphism with the effect of hormone replacement therapy on bone mineral density in Japanese women. Am J Obstet Gynecol 1999;180: 1115–20.
- [17] Revised American Society for Reproductive Medicine classification of endometriosis. Fertil Steril 1997;67:817–21.
- [18] Lahiri DK, Numberger JL. A rapid non-enzymatic method for preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444.
- [19] Györfy B, Vászrhelyi B, Krikovszky D, Madácsy L, Tordai A, Tulassay T, et al. Gender-specific association of vitamin D receptor polymorphism combinations with type 1 diabetes mellitus. Eur J Endocrinol 2002;147:803–8.
- [20] Mory DB, Rocco ER, Miranda WL, Kasamatsu T, Crispim F, Dib SA. Prevalence of vitamin D receptor gene polymorphisms FokI and BsmI in Brazilian individuals with type 1 diabetes and their relation to beta-cell autoimmunity and to remaining beta-cell function. Hum Immunol 2009;70:447–51.
- [21] Gorman S, Kuritzky LA, Judge MA, Dixon KM, McGlade JP, Mason RS, et al. Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4⁺CD25⁺ cells in the draining lymph nodes. J Immunol 2007;179:6273–83.
- [22] Adorini L, Penna G. Dendritic cell tolerogenicity: a key mechanism in immunomodulation by vitamin D receptor agonists. Hum Immunol 2009;70:345–52.
- [23] Prietl B, Pilz S, Wolf M, Tomaschitz A, Obermayer-Pietsch B, Graninger W, et al. Vitamin D supplementation and regulatory T cells in apparently healthy subjects: vitamin D treatment for autoimmune diseases? Isr Med Assoc J 2010;12: 136–9.
- [24] Hartwell D, Rødbro P, Jensen SB, Thomsen K, Christiansen C. Vitamin D metabolites—relation to age, menopause and endometriosis. Scand J Clin Lab Invest 1990;50:115–21.

- [25] Vigano P, Lattuada D, Mangioni S, Ermellino L, Vignali M, Caporizzo E, et al. Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system. *J Mol Endocrinol* 2006;36:415–24.
- [26] Somigliana E, Panina-Bordignon P, Murone S, Di Lucia P, Vercellini P, Vigano P. Vitamin D reserve is higher in women with endometriosis. *Hum Reprod* 2007;22:2273–8.
- [27] Faserl K, Golderer G, Kremser L, Lindner H, Sarg B, Wildt L, et al. Polymorphism in vitamin D-binding protein as a genetic risk factor in the pathogenesis of endometriosis. *J Clin Endocrinol Metab* 2010;96:E233–41.
- [28] Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001;358:1500–3.
- [29] Ponsonby AL, McMichael A, van der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology* 2002;181-182:71–8.
- [30] Cantorna MT, Mahon BD. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. *Exp Biol Med* Maywood 2004;229:1136–42.