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Original Article

Estrogen receptor and laminin genetic polymorphism among women with pelvic organ prolapse

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ABSTRACT

Objective: Laminin is a connective tissue component. The LAMC1 gene encodes for gamma-1 chain of laminin, which is associated with familial clustering of POP. The ERα gene which encodes for cellular estrogen receptor has also been associated with POP. The aim of this study was to evaluate a possible correlation between polymorphism in these genes and the risk for developing POP.
Materials and methods: Blood samples were drawn from 33 women with advanced POP (study group) and 33 women without POP (control group). DNA was extracted, and the presence of the rs10911193 C/T mutation in LAMC1 and of the rs2228480 G/A mutation in ERα was detected using the PCR technique.
Results: 26 samples were available for each group regarding ERα. 33 samples were available for each group, regarding LAMC1. The prevalence of homozygotes for the ERα rs2228480 G/A mutation was 19.2% and 0% among women with and without POP, respectively (OR 39.77, 95% CI 1.93–817.0, P = 0.00046). The prevalence of heterozygotes for this mutation was 83.3% and 11.5%, respectively (OR 19.2, 95% CI 4.15–88.6, P < 0.0001). The prevalence of homozygotes for the LAMC1 gene rs10911193 C/T mutation was 3.6% and 6.1% among women with and without POP (NS), while the respective for heterozygotes for this mutation was 21.4% and 33.3% (NS).
Conclusions: Polymorphism in the ERα gene is associated with an increased risk for advanced POP. However, polymorphism in the LAMC1 gene does not seem to be associated with such risk.

Introduction

Pelvic organ prolapse (POP) is a common condition, with a lifetime prevalence of 15–20% [1]. Its incidence increases with age reaching up to 40% in postmenopausal women, with a peak incidence at the 7th and 8th decades of life [2]. Implications of this disorder are beyond the individual suffering of each affected subject, and influence economical and social issues as well. Pathophysiology of POP is considered to be multifactorial. Known risk factors include age, obesity, multiparty, vaginal birth, increased infant birth weight, instrumental deliveries, extended second stage of labor, increased intra-abdominal pressure (such as chronic constipation or chronic obstructive lung disease) and smoking [3–5]. Other than these environmental risk factors, genetic predisposition is considered to play an important role in the pathophysiology of this disorder.

The pathophysiology of POP suggests vulnerability of connective tissue, making the investigation of its components a fertile basis for further studies. Apart from its cellular components, connective tissue is formed from extra-cellular matrix (ECM). Laminin, an important component of the ECM is composed of three chains: Alpha, beta and gamma, creating 15 different isoforms. The laminin gamma-1 chain, is a heterogeneous group of extra-cellular matrix glycoproteins, making the bulk of the noncollagenous excerpt of the basement membrane.

First-degree familial clustering has been identified as a possible risk factor for POP by Chiaffarino et al. [6]. Thereafter, Rinne et al. [7]

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set the familial incidence of genital prolapse at 30%. An autosomal dominant mode of inheritance was established in a three generation family affected by early-onset POP and hernias [8]. This study was able to identify a high risk family with a high prevalence of LAMC1 rs10911193 minor T allele, making the first report of this polymorphism. In 2012, Wu et al. evaluated LAMC1 genetic variants in the manifestation of advanced POP among non-hispanic white women, and could not associate between LAMC1 polymorphism and nonfamilial POP [9]. They argued that further investigation would be of use, especially in view of the limited sample size of their study. More than one polymorphism was investigated in a study by Chen et al. among nonrelated Caucasian and African American females, however, no association was found to be statistically significant [10].

The integrity and normal function of the connective tissue is modifiable by ECM proteases. These proteases which degrade ECM are up-regulated by estrogen [11]. Estrogen receptor (ER) mediates the function of estrogen. Being a nuclear protein, it binds to DNA and performs as a transcription factor. Low levels of estrogen receptor were found in premenopausal women diagnosed with POP. This was established by Lang et al. [12] when investigating the histologic characteristics of supporting ligaments in the pelvic floor presumed to be the cause of POP. Another research by Ewies et al. [13] used an immunohistochemical study to evaluate the differential expression of gonadal steroid receptors in human cardinal ligaments of women with prolapsed uteri. Individuals affected by POP expressed 1.5–2.5 times more ER alpha (ERα) positive cells. However, twice as high expression of ERβ was found in premenopausal women without POP.

The association between estrogen and POP was investigated by Moon et al. [14] by examining microarray gene expression profiles using functional clustering and quantitative polymerase chain reaction. Fifty-nine genes were identified to be associated with signal transduction and transcription, of which 4 genes were associated with estrogen. ER-related receptor-α was down-regulated, while death-associated protein kinase 2, signal transducing adapter protein-2 and interleukin 15 were up-regulated in patients with POP.

In view of the non-conclusive data presented above, the aim of this study was to evaluate possible correlation between POP and polymorphism in LAMC1 and ERα genes, based on a group of patients with advanced POP and a matched control group of patients with no significant prolapse.

Materials and methods

This was a case-control study, with prospective patient evaluation and genetic analysis. The study protocol was approved by the Institutional Review Board Committee for Human Subjects in Carmel Medical Center, Haifa, Israel, and all participants gave their written informed consent upon enrollment. Subjects were women of Ashkenazi-Jewish origin who visited the gynecology outpatient clinic at Carmel and Lin Medical Centers in Haifa, Israel. Women with known connective tissue disorders, such as Marfan and Ehler-Danlos syndromes, ongoing pregnancy, cancer involving reproductive or pelvic organs or stress urinary incontinence (including occult stress urinary incontinence) were excluded from the study. The study group consisted of women with advanced (stages 3 or 4) POP, and the control group consisted of women with no or mild (stages 0 or 1) prolapse, who were matched to the study group with respect to age, body mass index (BMI) and parity. Diagnosis was established by a physical examination of the external genitalia and vaginal canal, according to the POP quantification system (POPQ) as advocated by the International Continence Society, the American Urogynecologic Society, and the Society of Gynecologic Surgeons [15]. Stress Urinary Incontinence was ruled out based on medical history, cough stress test and two validated symptom-impact questionnaires – the Urogenital Distress Inventory (UDI) and the Incontinence Impact Questionnaire (IIQ) [16].

Blood samples were drawn from all subjects in tubes containing ethylenediaminetetraacetic acid. Genomic DNA was extracted from whole-blood leukocytes using commercially available kit (High pure PCR template preparation kit, Roche, Mannheim, Germany). DNA was stored at −20 °C until used. In order to determine the presence or absence of polymorphisms in the ERα and LAMC1 genes, relevant segments from both genes were amplified by two-step polymerase chain reaction (PCR) using 500–1000 ng DNA as template. For amplification, Taq DNA polymerase (Sigma, St. Louis, MO, USA) was used together with the following ordered primers: For ERα: forward: 5′-GCTCTACTTCATCGCATTCC-3'; reverse: 5′-CCACTAAGAACTGAGCAAGC-3'. The primer set for the LAMC1 gene was: forward- 5′-CACTGGCTGGTTACACTTTACCTCT-3′ and reverse: 5′-CCTTTTGAGTCCTAATGTCCAAGAC-3'. The PCR conditions were as follows: preceding denaturation at 94 °C for 3 min followed by 30 cycles of 1 min denaturation at 94 °C, annealing of 1 min at 56 °C for the ERα gene and at 60 °C for the LAMC1, and extension at 72 °C for 1 min. Final extension of 10 min was performed. Amplified DNA segments supposed to harbor potential polymorphic regions were analyzed on 3% agarose gel electrophoresis following digestion with suitable restriction enzymes. For the ERα rs2228480 G/A mutation BtgI restriction enzyme was used. Upon digestion with BtgI enzyme, the 'G' allele is represented by two fragments of 174 bp and 64 bp, and the 'A' allele by one fragments of 238 bp (Fig. 1). The rs10911193 C/T LAMC1 mutation was diagnosed using the MaeII restriction enzyme. The 'C' allele presents two fragments of 141 bp and 59 bp and the 'T' allele one fragment of 200 bp (Fig. 2).

Statistical analysis was performed using SPSS version 21 package for Windows. Power calculations were performed prior to recruitment, based on previous reports on genetic polymorphism

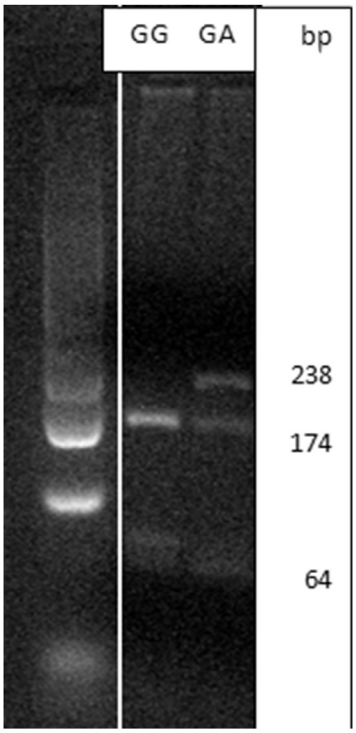

Fig. 1. Agarose gel DNA electrophoresis of the ERα rs2228480 G/A segment after restriction by BtgI enzyme.

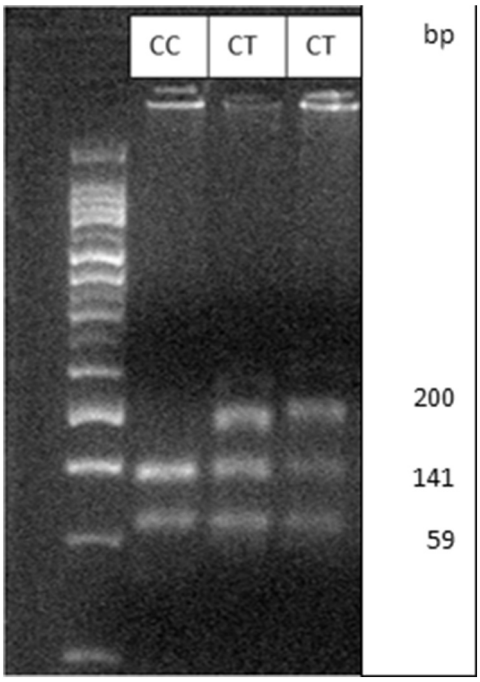

Fig. 2. DNA separation of the LAMC1 rs10911193 C/T segment gene following MaeII restriction on 3% agarose gel.

and POP [8,17]. Assuming similar prevalence of genetic polymorphism, a sample size of 30 women in each group would be required in order to detect an absolute difference of 30% or higher in the prevalence of the mutant genotype, with power of 80% and a P value < 0.05. Two-side student *t* test, Chi square (χ^2) test, Mann–Whitney *U* test and Hardy–Weinberg Equilibrium test were used to assess differences between the study and control group as well as correlation between LAMC1 and ERα gene mutations and the risk for developing POP. P < 0.05 was considered statistically significant for all comparisons.

Results

A total of 33 female patients with advanced POP and 33 controls with no significant POP were included in the study. The two groups matched with respect to age, body mass index (BMI) and parity (Table 1). All patients in the study group had POPQ stage 3 or above (most prolapsed component at more than +1 cm), and all patients in the control group had no more than stage 1 prolapse (most prolapsed component −1 cm or less). Spontaneous DNA degradation occurred in several blood samples. Therefore, only 26 samples were available for genetic analysis of ERα gene in both the study and control groups. Regarding LAMC1 gene, all 33 blood samples were available for genetic analysis in the control group, while only 28 were available in the study group.

Genetic analysis of the entire studied population revealed the following distribution of genotypes for the ERα gene: The native genotype GG: 55.8%; the mutant heterozygote genotype GA: 34.6%; and the mutant homozygote genotype AA: 9.6%. There was a higher incidence of the heterozygote (57.7% vs. 11.5%, OR = 19.2, 95% CI 4.1–88.6, P < 0.0001) and homozygote (19.2% vs. 0%, OR = 39.77, 95% CI 1.93–817.0, P = 0.00046) mutant genotypes among patients with POP as compared to patients without POP (Table 2). Genetic analysis of the LAMC1 gene revealed the following distribution: the native genotype CC: 67.2%; the mutant heterozygote genotype CT: 27.9%; and the mutant homozygote genotype TT: 4.9%.

The prevalence of the mutant homozygote genotype (TT) was 3.6% and 6.1% among the study and control groups, respectively (OR = 0.48, 95% CI 0.04–5.67, NS) (Table 3). The prevalence of the mutant heterozygote genotype (CT) was 21.4% and 33.3% among the study and control groups, respectively (OR = 0.52, 95% CI 0.16–1.67, NS).

Discussion

These results indicate that while ERα genetic polymorphism seems to be associated with an increased risk for developing POP, LAMC1 polymorphism showed no correlation with this disorder. Several published studies have suggested a genetic background for POP, presenting evidence of genetic predisposition. Weber and Richter [18] claimed that the pathophysiology of POP was multifactorial and proposed a multiple-hit process model. According to this model, genetically susceptible women may be exposed to life events such as multiple vaginal deliveries, that would ultimately result in the development of clinically significant POP. McLennan et al. [19] suggested a family history of POP to be a risk factor for the disease. A cohort of 458 women were classified into either having or not having a family history of POP. The study was adjusted for other commonly known environmental risk factors such as vaginal deliveries, urinary incontinence and hysterectomy, and found a 1.4 times higher risk among women with a family history of POP (P < 0.001). Buchsbaum et al. [20] assessed prevalence of POP among postmenopausal nulliparous women and their sisters, and found a high concordance in pelvic support disorders between the two groups.

The stability and strength of the pelvic floor is largely dependent on the molecular composition of the ECM. Laminin, an important component of the ECM, which belongs to a family of glycoproteins, modulating cell proliferation, migration and differentiation [21]. It has been shown to have a vital effect on the way other ECM molecules interact, possibly explaining the reduced collagen content in pelvic tissue of patients with POP [22,23]. Moreover, the age-dependent changing hormonal environment has been shown to

Table 1
Demographic and clinical characteristics of the study and control groups.

Characteristic	Study group (n = 33)	Control group (n = 33)	P
Age	61.8 ± 10.1	58.2 ± 8.7	0.11
BMI* (kg/m^2)	26.3 ± 3.0	26.5 ± 5.0	0.84
Parity	2 (1–3)	2 (1–3)	0.6
Instrumental deliveries	7 (8.5)	7 (8.0)	0.91
Macrosomic babies	5 (6.1)	7 (8.0)	0.62
Menopause	30 (83.3)	30 (83.3)	1.0
Smoking	6 (16.7)	11 (30.6)	0.17
Chronic constipation rate	4 (11.1)	6 (16.7)	0.50

* Body Mass Index.
Values are presented as mean ± SD, median (range) or *no.* (%).

Table 2
Estrogen receptor alpha genetic polymorphism – Distribution of genotypes among groups.

Genotype	Study group (n = 26)	Control group (n = 26)	P value	Odds ratio (95% CI)
GG	6 (23.1)[a]	23 (88.5)	<0.0001	Ref
GA	15 (57.7)	3 (11.5)	<0.0001	19.2 (4.15–88.6)
AA	5 (19.2)	0 (0)	0.00046	39.7 (1.93–817.0)[b]

GG: native genotype; GA: mutant heterozygote genotype; AA: mutant homozygote genotype.
[a] Values are presented as no. (%).
[b] Statistical analysis was calculated using the Hardy–Weinberg Equilibrium.

Table 3
LAMC1 genetic polymorphism- Distribution of genotypes among groups.

Genotype	Study group (n = 28)	Control group (n = 33)	P value	Odds ratio (95% CI)
CC	21 (75)[a]	20 (60.6)	0.233	Ref
CT	6 (21.4)	11 (33.3)	0.301	0.52 (0.16–1.67)
TT	1 (3.6)	2 (6.1)	>0.99	0.48 (0.04–5.67)

CC: The native genotype; CT: The mutant heterozygote genotype; TT: The mutant homozygote genotype.
[a] Values are presented as no. (%).

be associated with laminin [22,24]. Chen et al. [17] suggested that both the absence or up-regulation of laminin, can alter the normal composition of the basement membrane and cause tissue integrity disruption. Studies of high risk families with multigenerational early onset POP identified the LAMC1 SNP rs10911193 minor T allele to be more prevalent among family members with POP [8]. In this particular family, the penetrance of the condition appeared to be high. Thereafter, Chen et al. reported no significant clustering of the LAMC1 gene among 102 Caucasian and 63 African–American females with POP [10]. In the current study, we investigated 33 Ashkenazi Jewish women with advanced POP, and 33 matched controls with no prolapse. Twenty-six samples were available for genetic analysis of ERα gene (both in the study and the control groups). Regarding the LAMC1 gene, all 33 blood samples were available for genetic analysis in the control group, while only 28 were available in the study group. We have found no statistically significant clustering of the LAMC1 alleles among patients with POP.

Estrogen receptors are thought to be of special importance in the pathophysiology of POP. They are identified on the nuclei of connective tissue and smooth muscle cells of the bladder trigone, urethra, vaginal mucosa, levator ani muscles and uterosacral ligaments [25]. These are ligand-activated transcription factors, which include two main subtypes: ERα, the dominant receptor in the adult uterus, and ERβ which is dominant in other estrogen-target tissues. These subtypes seem to play a role in maintaining the integrity of the pelvic floor by either increasing the synthesis or decreasing breakdown of collagen and other ECM proteins. McIntush et al. [26] and Brincat et al. [27] reported that estrogen controls the expression of matrix metalloproteinases (MMP), which in turn degrade the collagen contents in skin, endometrium and ovary. Moali et al. [28] reported that the expression of MMP is inhibited by estrogen in vaginal epithelium and fibroblasts of uterine supportive tissue. These data indicate that decreased levels of estrogen or defective estrogen receptor may contribute to abnormal function of the pelvic floor ECM and supportive mechanisms. This notion is further supported by animal studies where production of ERα gene transcripts was found to be decreased in sheep with vaginal prolapse [29]. Bai et al. [30] found significantly decreased estrogen and progesterone receptors in uterosacral ligaments of prolapsed uteri. Chen et al. [17] investigated ERα genetic polymorphism in 83 women with POP vs. 153 controls. Five genotypes were evaluated in the study, however, only one (ESR1 rs2228480) was significantly associated with POP. Our study investigated the ERα rs2228480 genotype in a cohort of 26 POP patients and 26 control subjects. In concordance with Chen's results, we found a statistically significant correlation between ERα polymorphism and advanced POP. Both the heterozygote and homozygote forms of this genotype were associated with a higher risk for developing POP.

The main limitation of this study is the low number of participants, partially attributed to spontaneous DNA degradation which occurred in some of the blood samples. Despite this limitation, we have found that polymorphism in the ERα gene is associated with an increased risk for advanced POP, while polymorphism in the LAMC1 gene does not. Furthermore, statistical significance and tangible results were reached for the ERα polymorphism, supporting its role in the pathophysiology of POP. Clinical implications of this study include the potential ability to identify women who are at a higher risk for developing POP, and to consult them antenatally regarding the preferable mode of delivery. Avoiding fetal macrosomia, prolonged second stage of labor and instrumental deliveries may be indicated in this high risk group. Elective cesarean delivery may also be considered in some of these women in order to prevent future POP. Since estrogen deficiency seems to be a major risk factor for POP, future research should also concentrate on the possible role of vaginal and systemic estrogen replacement therapy in post-menopausal patients carrying the high-risk ERα mutant genotype.

Conflicts of interest

The authors declare that they have no conflict of interest.

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