Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 750–754

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

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

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ARTICLE INFO

Article history:
Accepted 1 June 2017

Keywords:
Estrogen receptor alpha gene (ERα)
Genetic polymorphism
LAMC1 gene
Laminin gamma-1 chain
Pelvic organ prolapse

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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https://doi.org/10.1016/j.tjog.2017.10.008

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

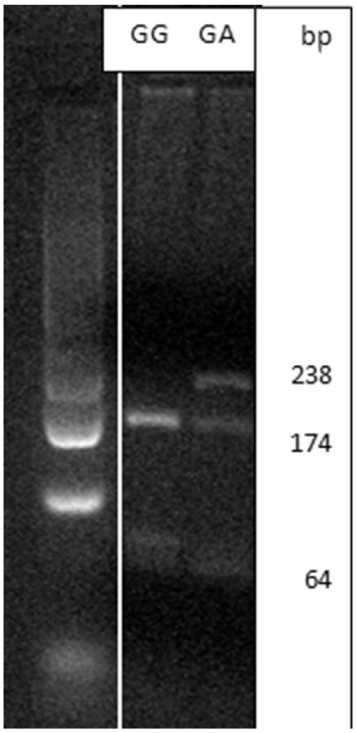

Fig. 1. Agarose gel DNA electrophoresis of the ERα rs2228480 G/A segment after restriction by BtgI enzyme.