

Elastin genetic point mutation and the risk of pelvic organ prolapse

N. Haya¹, I. Feferkorn¹, F. Fares², N. Azzam², R. Auslender¹, Y. Abramov¹

¹Division of Urogynecology and Reconstructive Pelvic Surgery, Department of Obstetrics and Gynecology, Carmel Medical Center, The Ruth and Bruce Rappaport Faculty of Medicine, Technion University, Haifa

²Faculty of Natural Sciences, University of Haifa (Israel)

Summary

Aim: A missense mutation in the elastin gene (g28197A > G) is associated with an increased risk for inguinal hernias. Due to the shared epidemiological and pathophysiological features between pelvic organ prolapse (POP) and inguinal hernias, the authors hypothesized that a similar association exists between elastin gene polymorphism and POP. **Materials and Methods:** Patients of Ashkenazi Jewish origin with advanced (stage III-IV) POP (as assessed by POP-Q) and healthy controls were compared for the presence of the elastin gene g28197A > G missense mutation. **Results:** The missense mutation in the elastin gene was not found in neither the study or the control group. **Conclusion:** The elastin gene g28197A > G missense mutation was not found to be associated with an increased risk for POP.

Key words: Elastin; Genetic polymorphism; Pelvic organ prolapse; Point mutation.

Introduction

Pelvic organ prolapse (POP) is the abnormal descent or herniation of the pelvic organs to or beyond the vaginal walls. Women with POP often experience symptoms which impact on their daily activities, body image, sexuality, and quality of life. The prevalence of this disorder is estimated to be approximately 40% of all postmenopausal women [1], although most women are diagnosed with stage 1 or 2 disease according to the POP quantification system (POP-Q) [2], and about 11% would require a reconstructive surgical procedure [3]. With the gradual increase in life expectancy in western communities, the impact of POP on healthcare is likely to expand [4].

Disruption or dysfunction of the levator ani muscle complex and the endopelvic fascia can lead to loss of support and eventually to POP. The causes for this dysfunction are likely to be multifactorial. Among the established risk factors are obesity, parity, obstetric risk factors, such as vaginal birth, and specifically, instrumental vaginal deliveries, increasing age, chronic constipation, and connective tissue disorders [5]. Data from trials showing a higher prevalence of POP among siblings and among women of certain ethnic backgrounds imply that there may also be a genetic factor involved in the pathophysiology of this disorder. Recent meta analyses suggest that polymorphism in the collagen type 3 alpha 1 chain (COL3A1) and collagen type 1 alpha 1 chain (COL1A1) genes may be associated with a higher risk for POP [6,7].

POP and hernia share several pathophysiological and epidemiological features. It has been demonstrated that patients with advanced POP have a higher prevalence of hiatal

and inguinal hernias [8]. This association may be explained by similar pathophysiological mechanisms and, possibly, a shared genetic susceptibility. Disorders of elastin, a major component of connective tissue, are thought to be associated with a higher prevalence of inguinal hernia and genetic conditions affecting the elastin protein, such as Cutis Laxa, are associated with a higher prevalence of inguinal hernia [9]. To date, the only mutation in the elastin gene (not part of a genetic syndrome) that was significantly associated with inguinal hernia is a point mutation found by Rodriguez *et al.* (a g28197A > G missense mutation) leading to an S422G amino acid substitution in the elastin hydrophobic domain [10]. The present authors assumed that a higher prevalence of this polymorphism would also be present in women diagnosed with advanced POP. The aim of this study was to compare the prevalence of the g28197A > G missense mutation polymorphism in the elastin gene between women with and without POP.

Materials and Methods

All women of Ashkenazi-Jewish origin who visited the present gynecologic outpatient clinic between December 2006 and March 2007, were asked to participate in this study. Women with stress urinary incontinence (SUI), connective tissue disorders (such as Marfan and Ehlers-Danlos syndromes), ongoing pregnancy or cancer involving reproductive or pelvic organs were excluded from this study. Women with advanced (stage III and above) symptomatic POP were allocated to the study group, while women with no or mild (stage 0 or 1) POP were allocated to the control group. The study and control groups were matched with regards to age and parity. Patients in both groups underwent a pelvic examination by a fellowship-trained urogynecologist in order to di-

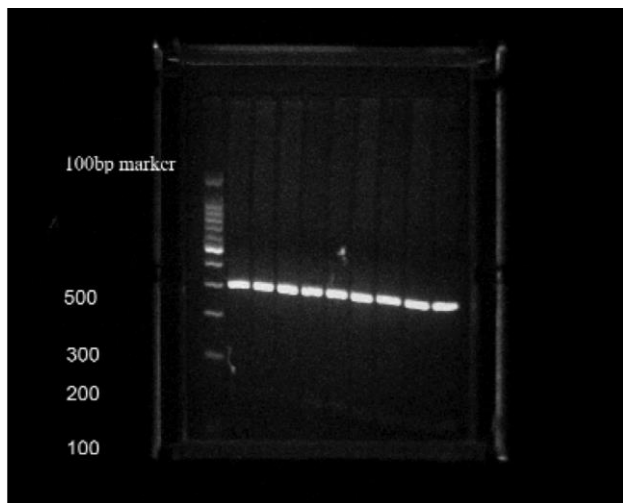


Figure 1. — MaeI cut of DNA from healthy (control) women. The presence of only one band signifies that no mutation is present.

agnose or rule out advanced POP. POP staging was determined according to the POP-Q system as advocated by the International Continence Society, the American Urogynecologist Society, and the Society of Gynecologic Surgeons [11].

Blood samples were obtained from all subjects in tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted from whole-blood leukocytes using a commercially available kit (high pure PCR template preparation kit). DNA was stored at -20°C until used. Amplification of exon 20 of the elastin gene, resulting in a 297 bp band, was carried out by a polymerase chain reaction (PCR), using a thermocycler and Taq DNA polymerase, with the following primers: Forward: 5'-CTC TTT CCC AAT CCA TCA GCA TC -3' and Reverse: 5'-CCC ATC CCT TCT CAA CCC ATG TC -3'. 500 ng of DNA served as a template for each reaction. The conditions of the PCR were as follows: preceding denaturation at 94°C for three minutes followed by 30 repeated amplification cycles of denaturation at 94°C for one minute, annealing at 60°C for one minute, and elongation at 72°C for one minute. A final primer extension was carried out at 72°C for ten minutes. PCR samples were stored at minus 20°C until analysis. For analysis PCR products were digested with the restriction enzyme MaeI, which cuts the DNA at a restriction site found in the mutant DNA (CTAG), to produce two bands of 199 and 96bp, but not at the wild type DNA (harboring the CTGG sequence). The products were then separated with electrophoresis on 2% agarose gel, using a 100 v charge and, while comparing to a 100 bp marker. Images of gels were taken under ultraviolet light using an imaging system.

Power calculations were performed prior to recruitment, based on the report by Rodriguez *et al.* [10], which found an increased prevalence of the g28197A > G missense mutation in patients with inguinal hernias as compared to healthy controls (95.9% vs. 32% respectively). Assuming a common etiology for POP and inguinal hernias, a sample size of 11 women in each group would be required in order to detect an absolute difference of 63.9% or higher in the prevalence of the mutant genotype with power of 80% and a p value < 0.05. Statistical analysis was performed using statistics (SPSS) version 22. Chi square test was used for comparing categorical variables and independent t -test or Mann Whitney U test as appropriate, for comparing continuous variables. A p value < 0.05 was considered statistically significant for all com-

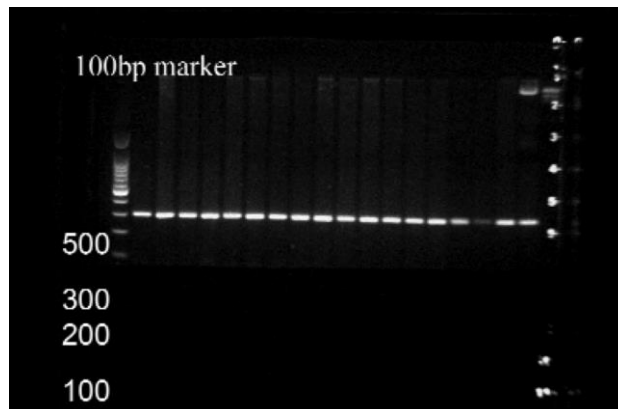


Figure 2. — MaeI cut of DNA from women with POP. The presence of only one band signifies no mutation is present.

parisons. The study protocol was approved by the Institutional Review Board Committee for Human Subjects, and the Israeli Ministry of Health (Ref. 021-21780) and all participants gave their written informed consent upon enrollment.

Results

Seventy-two women enrolled in the study, 36 in each group. The two groups did not significantly differ with respect to known risk factors for POP such as age, BMI, smoking habits, menopausal status, constipation, parity, vaginal births, instrumental deliveries, as well as delivery of macrosomic infants (Table 1). The g28197A > G missense mutation in the elastin gene was not found in any of the patients in either the control or the study group (Table 2, Figures 1 and 2). Due to the fact that neither POP DNA samples nor control samples were restricted by Mae I, the authors carried out a positive control cut with Mae I of a commercial vector named pEXP in order to be confident that enzyme used was active. The vector contains six sites for Mae I, and the resulting DNA bands confirmed the activity of the enzyme.

Discussion

Recently, there has been growing evidence that hereditary factors play a role in the occurrence of POP. The high prevalence of POP in women with diagnosed connective tissue disorders or in women whose family members suffer from POP, implies that there is a genetic predisposition for POP and that some genetic variation, alone or in combination with other known risk factors, may be involved in the pathophysiology of this disorder. Most studies searching for such candidate genes have focused on those involved in the formation or metabolism of connective tissue, as the latter provides mechanical support for the pelvic organs. Recent meta-analyses suggested an association between variations in the subtypes of collagen, the main structural

Table 1. — Demographic data of the study and control groups.

Characteristic	Study group (n=36)	Control group (n=36)	p value
Mean age \pm SD	61.8 \pm 10.1	58.2 \pm 8.7	0.11
Mean BMI (kg/m ²) \pm SD	26.3 \pm 10	26.5 \pm 5.0	0.84
Mean overall parity \pm SD	2.4 \pm 0.8	2.5 \pm 0.8	0.66
Mean vaginal parity \pm SD	2.3 \pm 0.8	2.4 \pm 0.8	0.49
Total instrumental deliveries (%)	7 (8.5)	7 (8.0)	0.91
Total macrosomic infants (%)	5 (6.1)	7 (8.0)	0.62
Menopause rate, n (%)	30 (83.3)	30 (83.3)	1
Smoking rate n, (%)	6 (16.7)	11 (30.6)	0.17
Chronic constipation rate, n (%)	4 (11.1)	6 (16.7)	0.5

Values are presented as mean \pm SD or no. (%).

Table 2. — POP-Q stage of the study and control groups.

Control group (n=36)	Study group (n=36)	POP-Q stage
4 (11.1)	0	0
32 (89.9)	0	1
0	0	2
0	33 (91.7)	3
0	3 (8.3)	4

Values are presented as no. (%)

protein in connective tissue, and POP [6, 7].

Elastin is another major component of connective tissue which provides elasticity and resilience to many tissues including ligaments, tendons, and skin. Several studies have shown an association between reduced elastin expression and POP. One study assessed the extracellular matrix proteins in postmenopausal women with and without POP and found a higher expression of collagen type III and lower quantities of elastin in the cardinal ligaments of women with uterine prolapse [12]. Yamamoto *et al.* assessed the expression of the elastin gene in cardinal ligaments and showed reduced expression of this gene in the cardinal ligaments of patients with prolapsed uteri, as compared to age matched controls [13]. Similarly, reduced elastin content in the endopelvic fascia was found to be associated with uterine prolapse [14]. A study assessing elastin expression and elastic fiber width in the anterior vaginal wall also showed reduced elastin expression and fiber width in the vaginal wall of patients with large cystoceles as compared to age-matched controls [15]. Alterations in elastin metabolism (increased elastolytic protease activity) were also shown to be present in postmenopausal women with POP [16]. Catabolism of the extracellular matrix was shown to be of importance in both animal models and humans and increased matrix metalloproteinase 9 (MMP-9) activity was associated with POP in both animal models and humans [17, 18].

A previous study by Rodriguez *et al.* [10] found a higher prevalence of the elastin gene g28197A > G missense mutation in subjects with inguinal hernia (24 out of 49 subjects were heterozygous for the mutation and 23 were

homozygous for the mutation), as compared to controls (17 out of 75 subjects were heterozygous for the mutation and seven out of 75 were homozygous for the mutation). The present authors have previously found a substantial overlap in the occurrence of POP and hernias (hiatal or inguinal) possibly due to common pathophysiologic mechanisms such as connective tissue biomechanical weakness [8]. The present authors therefore hypothesized that a higher prevalence of the g28197A > G missense mutation in the elastin gene would also be present in women with advanced POP, similar to the higher prevalence of this mutation in patients with inguinal hernia. The results of the current study do not confirm this hypothesis, as this mutation was not found in any of the patients in either the study or the control group. The discrepancy between this study and those cited above may be attributed to the heterogeneity between Brazilian and Ashkenazi-Jewish populations. Another study by Ferrel *et al.* did not find an association between a single nucleotide polymorphism in the promotor of the lysyl oxidase homolog 1 (LOXL1) gene (which is essential for elastin synthesis) and the risk for advanced POP [19]. A recent, genome wide association study, identified four susceptibility loci underlying inguinal hernia formation. All four genes found in that study were expressed in connective tissue and deserve further research to their relation with POP [20]. While discrepancies between studies examining different populations and using various analytical methodologies remain to be resolved, the present authors believe that further research, possibly using whole exome sequencing, can refine our understanding of the genetic background for POP and help guide clinicians regarding preventive measures and tailored treatment for populations which may be genetically predisposed.

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Corresponding Author:

I. FEFERKORN, M.D.

Department of Obstetrics and Gynecology

Carmel Medical Center

7 Michal Street

Haifa, 34632, (Israel)

e-mail: idofeferkorn@gmail.com

idofe@clalit.org.il