The relationship between *COL3A1* exon 31 polymorphism and pelvic organ prolapse

Jeon Myung Jae

Department of Medicine

The Graduate School, Yonsei University

The relationship between *COL3A1* exon 31 polymorphism and pelvic organ prolapse

Directed by Professor Bai Sang Wook

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medicine

Jeon Myung Jae

June 2008

This certifies that the Master's Thesis of

Jeon Myung Jae is approved.

Thesis Supervisor: Bai Sang Wook

Thesis Committee Member#1: Kim Sei Kwang

Thesis Committee Member#2: Park Soon Jung

The Graduate School
Yonsei University

June 2008

ACKNOWLEDGEMENTS

I want to send my gratitude to those who helped me through all this work on my belated course of graduate school. I deeply appreciate professor Sang Wook Bai, for all his teaching and encouraging until this thesis has been finished. Also I thank professor Sei Kwang Kim and professor Soon Jung Park for their unsparing support even in the midst of their busy hours, and professor Jong Rak Choi and doctor Eun Kyung Lee for the help in the laboratory.

I wish to share this inspiring moment with my parents who always encourage me with unlimited love, my loving wife Si Jung whose prayers became our guiding light, and my pretty daughter Celine who brought us joy by coming into the world.

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

<ABSTRACT>

The relationship between *COL3A1* exon 31polymorphism and pelvic organ prolapse

Jeon Myung Jae

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Bai Sang Wook)

Objective: Despite the high incidence of pelvic organ prolapse (POP), its pathogenesis is still poorly understood. Some biochemical studies have demonstrated decreased ratio of type I to III collagen due to increased expression of type III collagen in the vagina and its supportive structures of women with POP, but others have shown conflicting results. This may be due to the heterogeneity of the study population. Moreover, these studies have the limitation that it is impossible to distinguish whether the finding is a cause or an effect of POP. A recent study found that patients with floppy mitral valve (FMV)/mitral valve prolapse(MVP) had higher frequency of *COL3A1* exon 31 GG genotype than the control group. The aim of this study was to investigate

whether the polymorphism of *COL3A1* exon 31 (a single base substitution from Guanine to Adenine at +2209), resulting in the replacement of alanine with threonine at the 570th amino acid of *COL3A1*, is associated with POP.

Materials and Methods: A total number of 72 postmenopausal Korean women not taking hormonal replacement therapy, aged from 50 to 65 were enrolled in this study. The patient group consisted of 36 women diagnosed as POP of which stage was II or greater, irrespective of the presence of urodynamic stress incontinence. The control group consisted of 36 age- and vaginal parity-matched volunteers with pelvic organ prolapse-quantification stage 0 or I without urodynamic stress incontinence. The genomic DNA was extracted from peripheral blood leukocytes. The polymorphism of exon 31 of *COL3A1* was typed by the restriction fragment length polymorphism (RFLP) method, *Alu I* RFLP and confirmed by direct sequencing.

Results: A significantly higher frequency in G allele was noted in patients with POP than in controls (0.8 vs. 0.6, p=0.002). A significant difference in the genotype was also noted (p=0.001). An odds ratio for POP in women with G allele was 3.2 (95% confidence interval 1.4-7.3) and in women with GG genotype 4.3 (95% confidence interval 1.4-13.3).

Conclusion: This study shows that *COL3A1* exon 31 polymorphism is associated with increased risk of POP.

Key words: *COL3A1*, exon 31 polymorphism, pelvic organ prolapse

The relationship between *COL3A1* exon 31 polymorphism and pelvic organ prolapse

Jeon Myung Jae

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Bai Sang Wook)

I. INTRODUCTION

Pelvic organ prolapse (POP) is the herniation of uterus, bladder, small bowel, or rectum into the vaginal cavity. It is common among older women and its incidence has been reported as high as 39.8%.[1] In Korea, it has been reported that 28.1% of women older than 50 years have POP stage II or greater.[2] Despite the high incidence of this disease, its pathogenesis is still poorly understood. Many risk factors have been implicated in the etiology of POP. However, most of them remain controversial except for aging, vaginal childbirth and hypoestrogenism.[3] Moreover, not all women with these risk factors will develop POP and some develop POP without presenting risk factors. This suggests that there may be other intrinsic factors responsible for this condition.

Abnormalities in the connective tissue of pelvic floor supportive structures have been considered as one of the mechanisms that predispose prolapse.[4] Type I and III collagens are the main constituent of pelvic connective tissue. However, they have distinctive physical properties and their relative proportions may influence the mechanical strength of the connective tissue.[5] Some biochemical studies have demonstrated decreased ratio of type I to III collagen due to increased expression of type III collagen in the vagina and its supportive structures of women with POP,[6-9] but others have shown conflicting results.[10-12] This may be due to the heterogeneity of the study population as well as differences in biopsy sites and techniques, variation in biochemical tests applied for the analysis, etc. Moreover, these studies have the limitation that it is impossible to distinguish whether the finding is a cause or effect of POP.

Single nucleotide polymorphisms (SNPs) are the most abundant types of DNA sequence variation in the human genome and popularly researched to identify complex or multifactorial gene-associated diseases in various area.[13] Recently, Skorupski et al. have identified that G-T polymorphism at the Sp1 binding site of the gene encoding α-1 chain of type I collagen is associated with stress urinary incontinence, which coexists with POP frequently.[14] Women with GT and TT genotype were more likely to experience stress urinary incontinence. However, this SNP is rare or absent in Asian women, therefore the high incidence of POP in Korean women cannot be explained.[15,16]

Type III collagen is a homotrimer of α-1 (III) collagen chains, which are

encoded at the locus *COL3A1* on chromosome 2q24.3-q31.[17] Until now, there have been five SNPs found in *COL3A1* gene,[18] and only exon 31 polymorphism is proven to be associated with collagen-associated disorder. In one study, patients with floppy mitral valve (FMV)/mitral valve prolapse(MVP) had higher frequency of *COL3A1* exon 31 GG genotype than controls (59% vs. 31%).[19]

The aim of this study was to investigate whether the polymorphism of *COL3A1* exon 31 (a single base substitution from Guanine to Adenine at +2209), resulting in the replacement of alanine with threonine at the 570th amino acid of *COL3A1*, is associated with POP.

II. MATERIALS AND METHODS

1. Study population and sample collection

A total number of 72 postmenopausal Korean women not taking hormonal replacement therapy, aged from 50 to 65 in Department of Obstetrics and Gynecology, Yonsei University Health System between October 2007 and February 2008, were enrolled in this study. Sample size was determined on the basis of the results from previous studies.[19] More than 18 subjects are needed in each group to detect a 43% difference in prevalence of the exon 31 GG genotype between groups with 80% of statistical power and 0.05 of significance level, however, the sample size was increased to 36 in each group to detect a 30% difference, considering ethnic difference and medical condition. The patient group consisted of 36 women diagnosed as POP of which stage was II or greater, irrespective of the presence of urodynamic stress incontinence. The control group consisted of 36 age- and vaginal parity-matched volunteers with pelvic organ prolapse-quantification (POP-Q) stage 0 or I without urodynamic stress incontinence. Women who had undergone hysterectomy or pelvic reconstructive surgery were excluded in this study. The study was approved by the Institutional Review Board of Yonsei University Health System and all participants gave informed consent.

They were assessed with a standard history taking, physical examination including the evaluation on the degree of prolapse and urodynamic study including uroflowmetry, multi-channel cystometry, measurements of Valsalva

leak-point pressure, and urethral pressure profilometry using the Dante-5000 (Menuet, Copenhagen, Denmark). A standard history taking included age, vaginal parity, body mass index (BMI), menopausal and hormone replacement therapy status, presence of previous pelvic surgery and underlying comorbidities such as diabetes mellitus, chronic obstructive pulmonary disease, lumber disc herniation, etc. POP was quantified at a 45° upright sitting position while performing the Valsalva maneuver with maximal effort according to POP-Q system by the same examiner.

Blood samples were taken into tubes containing anticoagulant EDTA. The genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA blood Mini Kit (Qiagen GmbH, Hilden, Germany).

2. Polymorphic site analysis for exon 31 of *COL3A1*

Polymerase chain reactions (PCRs) were carried out in a total volume of 20 μl containing genomic DNA, 20 pmol of primer, 1U of HF DNA polymerase, 300 μM of dNTP, and 10X reaction buffer (AccuPower HF PCR PreMix, Bioneer Inc., Daejeon, Korea). The polymorphism of exon 31 of *COL3A1* was typed by the restriction fragment length polymorphism (RFLP) method, *Alu I* RFLP.

Alu I RFLP. A polymorphism in the first codon 531 for the proα1 (III) chain with G and A alleles was assayed using

5’-AAGTATACAAATTTCTAGATTG-3’ (in IVS 30) as a forward primer and 5’-ATAAATGATCAGAAGGAAATCA-3’ (in IVS 31) as a reverse primer and 40 cycles of amplifications at 94^{o}C for 1.5 min, 46^{o}C for 1 min, and 74^{o}C for 1 min. After digestion of the 294-bp PCR products with the *Alu I*, the restriction fragments were separated by capillary electrophoresis (ScreenTape analysis, Lab901 Ltd., Loanhead, UK). The G allele was recognized by the presence of a 208-bp fragments, whereas the A allele was recognized by the presence of 113- and 95-bp fragments (Figure 1). The results were confirmed by direct sequencing of the PCR product (Figure 2).

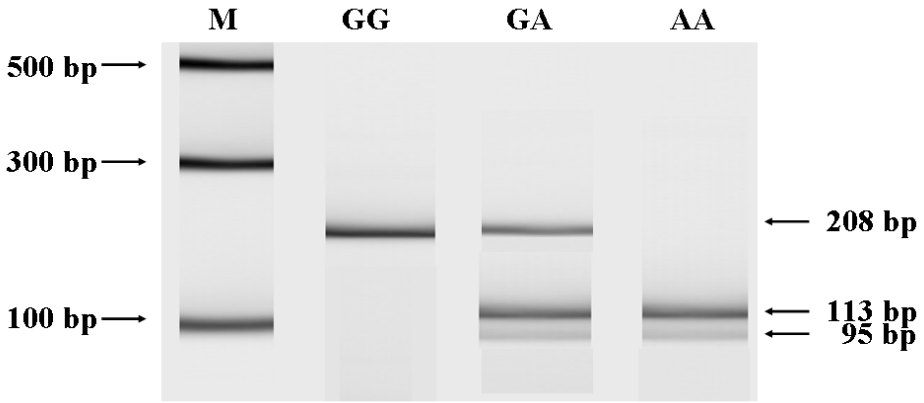

Figure 1. Polymorphism of the exon 31 in *COL3A1* gene shown by ScreenTape analysis. M=molecular-weight markers

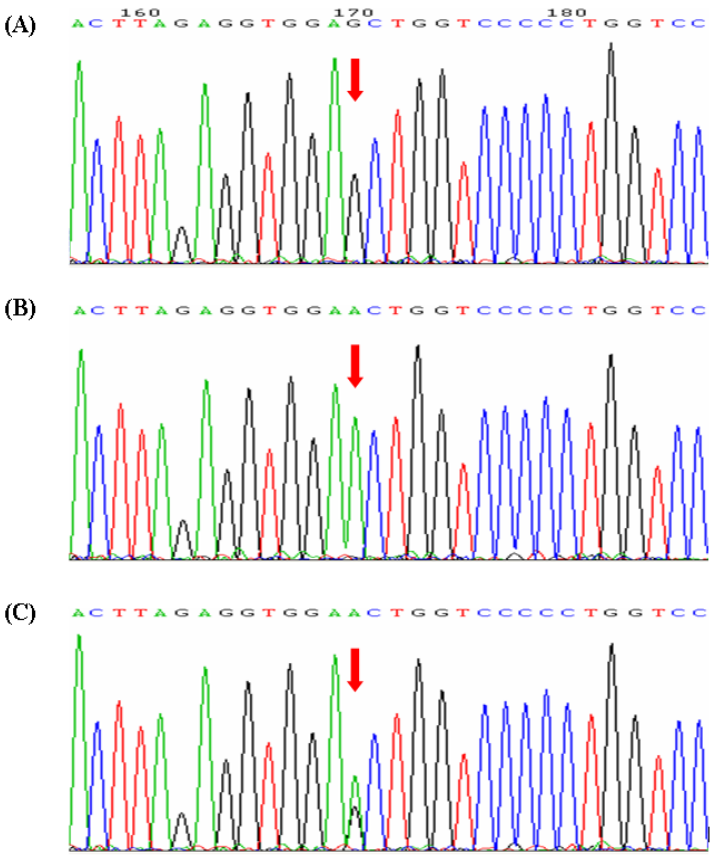

Figure 2. Exon 31 polymorphism of *COL3A1* gene shown by direct sequencing of PCR products; (A) homozygous G allele, (B) homozygous A allele, and (C) heterozygous allele of exon 31

3. Statistical analysis

The Student's t test, chi-square test, and multivariate logistic regression were used for statistical analysis using SPSS software 14.0 (SPSS Inc, Chicago, IL, USA). A *p*-value of less than 0.05 was considered to be statistically significant.

III. RESULTS

1. Demographic and clinical characteristics of the study population

The demographic and clinical characteristics of the study population were presented in Table 1.

TABLE 1. Characteristics of the study population

	Patients (n=36)	Controls (n=36)	p-value
Age (years, mean ± SD)	57.6 ± 5.5	58.3 ±5.3	0.571
Parity (median, range)	3 (1-6)	3 (1-6)	0.641
BMI (kg/m^2, mean ± SD)	25.2 ± 3.1	24.2 ± 2.4	0.131
Years since menopause (mean ± SD)	9.4 ± 6.2	8.8 ± 5.0	0.659
Diabetes mellitus (n, %)	4 (11.1)	4 (11.1)	1.000
COPD (n, %)	2 (5.6)	0	0.493
HLD (n, %)	1 (2.8)	0	1.000
Smoking (n, %)	2 (5.6)	0	0.493
POP-Q stage (n, %)			<0.001
0	0	24 (66.7)	
I	0	12 (33.3)	
II	2 (5.6)	0	
III	23 (63.9)	0	
IV	11 (30.6)	0	
Urodynamic stress incontinence (n, %)	24 (66.7)	0	<0.001

BMI, body mass index; HRT, hormone replacement therapy; COPD, chronic obstructive pulmonary disease; HLD, herniated lumbar disc

Age (57.6 vs. 58.3 years, p=0.571) and vaginal parity (3 vs. 3, p=0.641) were well matched between the patients and the control group. Other characteristics were not significantly different except for POP-Q stage and the presence of urodynamic stress incontinence.

2. Distribution of *COL3A1* exon 31 polymorphism

The frequencies of the G and A allele in this study population (72 women) were 0.7 and 0.3, respectively, similar to the results of other studies.[18-20] However, a significantly higher frequency in G allele was noted in patients with POP than in controls (0.8 vs. 0.6, p=0.002). A significant difference in the genotype was also noted (p=0.001) (Table 2).

TABLE 2. Allelic frequencies and genotype distribution in patients with POP and controls

	Patients (n=36)	Controls (n=36)	p-value
Allelic frequency			
G/A	0.8/0.2	0.6/0.4	0.002
Genotype distribution (n, %)			0.005
GG	23 (64)	11 (31)	
GA	13 (36)	20 (55)	
AA	0	5 (14)	

Allelic and genotype frequencies are indicated in fractions and absolute values, respectively.

An odds ratio for POP in women with G allele was 3.2 (95% confidence interval 1.4-7.3) and in women with GG genotype 4.3 (95% confidence interval 1.4-13.3) (because of a small number of AA genotype, GA and AA genotypes were collapsed for the calculation of odds ratio).

IV. DISCUSSION

In addition to the levator ani muscles, the vagina and its supportive connective tissue (ligaments and fascia) of the pelvic floor play an important role in the maintenance of pelvic organ support. When the levator ani muscles function properly, the pelvic floor is closed and the ligaments and fascia are under no tension during the periods of increased intraabdominal pressure. However, if the levator ani muscles are relaxed or damaged, the pelvic floor opens with the ligaments and fascia placed under tension which are thought to stretch and eventually fail to support the pelvic organs. Damage to the levator ani muscles occurs as a result of direct injury or through denervation, mainly from labor and vaginal delivery.[3]

Type I and III collagen takes a large proportion in the vagina and pelvic supportive ligaments and fascia like other connective tissues, type III collagen being predominant.[5,7-9] Because type III collagen has smaller fibers than type I collagen and plays a role in tissue elasticity and extensibility rather than mechanical strength, the predominance of type III collagen may allow these tissues to accommodate sudden increases in intra-abdominal pressure and the passage of fetus, making the tissues more vulnerable to injury and subsequent prolapse.[8] In several biochemical studies, decreased ratio of type I to III collagen, mainly from increased expression of type III collagen, have been found in pelvic connective tissues of women with POP compared to controls having normal pelvic support.[6-9] However, these studies have the limitation that

it is impossible to distinguish if the finding is a cause or an effect of POP.

Therefore, I tried to investigate that *COL3A1*, encoding α-1 chains of type III collagen, is involved in the pathogenesis of POP. A recent study showed that Ala570Thr polymorphism of *COL3A1* exon 31 is associated with risk of FMV/MVP, a disease which is clearly associated with abnormal synthesis of type III collagen, in the Chinese population.[19] In this study, we found a significant difference in the genotype distribution and allelic frequencies between patients with POP and controls for *COL3A1* exon 31 polymorphism even though the difference was smaller than FMV/MVP. This finding supports a role of the *COL3A1* exon 31 polymorphism in determining the risk of POP.

The major limitation of this study is the small sample size. Nonetheless, overall allelic frequencies are similar to other studies and a statistical power of this study reaches more than 80%. The second limitation is the age of the study population. We confined the subject to women aged from 50 to 65 years because several articles showed younger women with prolapse may have inferior tissue quality.[21-23] POP may progress with aging. In one 4-year observational study including 259 postmenopausal women, overall prolapse incidence was increased with aging and 1-year regression rates in maximal vaginal descent exceeded progression rates when the baseline value was 0 cm or greater, whereas progression rates exceeded regression rates when the baseline maximal vaginal descent was -1 cm or less.[24] Therefore, some of the women who were classified into the control group in this study period may experience

POP in the future. However, it can be thought that Ala570Thr polymorphism of *COL3A1* exon 31 is at least associated with the risk of an early onset of POP.

I confined this study subjects to postmenopausal women not taking hormonal replacement therapy. The expression of collagen may have been influenced by the hormonal status.[5,7] Therefore, the impact of *COL3A1* exon 31 polymorphism on the development of POP can be modified by hormonal replacement therapy. To verify the role of *COL3A1* exon 31 polymorphism in the development of POP, a larger population-based study will be needed.

V. CONCLUSIONS

This study shows that patients with POP have higher frequency of *COL3A1* exon 31 GG genotype which supports a role of *COL3A1* exon 31 polymorphism in determining the risk of POP, at least early-onset of POP.

Therefore, the pathogenesis of type III collagen abnormality in POP might be partially explained by this finding.

REFERENCES

1. Hendrix SL, Clark A, Nygaard I, Aragaki A, Barnabei V, McTiernan A. Pelvic organ prolapse in the Women's Health Initiative: gravity and gravidity. Am J Obstet Gynecol 2002;186:1160-6.

2. Seo JT, Kim JM. Pelvic organ support and prevalence by Pelvic Organ Prolapse-Quantification (POP-Q) in Korean women. J Urol 2006;175:1769-72.

3. Schaffer JI, Wai CY, Boreham MK. Etiology of pelvic organ prolapse. Clin Obstet Gynecol 2005;48:639-47.

4. Norton PA, Baker JE, Sharp HC, Warenski JC. Genitourinary prolapse and joint hypermobility in women. Obstet Gynecol 1995;85:225-8.

5. Moalli PA, Talarico LC, Sung VW, Klingensmith WL, Shand SH, Meyn LA, et al. Impact of menopause on collagen subtypes in the arcus tendineous fasciae pelvis. Am J Obstet Gynecol 2004;190:620-7.

6. Kökçü A, Yanik F, Cetinkaya M, Alper T, Kandemir B, Malatyalioglu E. Histopathological evaluation of the connective tissue of the vaginal fascia and the uterine ligaments in women with and without pelvic relaxation. Arch Gynecol Obstet 2002;266:75-8.

7. Ewies AA, Al-Azzawi F, Thompson J. Changes in extracellular matrix proteins in the cardinal ligaments of post-menopausal women with or without prolapse: a computerized immunohistomorphometric analysis. Human Reprod 2003;18:2189-95.

8. Moalli PA, Shand SH, Zyczynski HM, Gordy SC, Meyn LA. Remodeling of

vaginal connective tissue in patients with prolapse. Obstet Gynecol 2005;106:953-63.

9. Gabriel B, Denschlag D, Gobel H, Fittkow C, Werner M, Gitsch G, et al. Uterosacral ligament in postmenopausal women with or without pelvic organ prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2005;16:475-9.

10. Jackson SR, Avery NC, Tarlton JF, Eckford SD, Abrams P, Bailey AJ. Changes in metabolism of collagen in genitourinary prolapse. Lancet 1996;347:1658-61.

11. Takano CC, Girao MJ, Sartori MG, Castro RA, Arruda RM, Simoes MJ, et al. Analysis of collagen in parametrium and vaginal apex of women with and without uterine prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2002;13:342-5.

12. Lin SY, Tee YT, Ng SC, Chang H, Lin P, Chen GD. Changes in the extracellular matrix in the anterior vagina of women with or without prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2007;18:43-8.

13. Kwok PY, Gu Z. Single nucleotide polymorphism libraries: why and how are we building them? Mol Med Today 1999;5:538-43.

14. Skorupski P, Król J, Starega J, Adamiak A, Jankiewicz K, Rechberger T. An α-1 chain of type I collagen Sp1-binding site polymorphism in women suffering from stress urinary incontinence. Am J Obstet Gynecol 2006;194:346-50.

15. Han KO, Moon IG, Hwang CS, Choi JT, Yoon HK, Min HK, et al. Lack of an intronic Sp1 binding-site polymorphism at the collagen type I α1 gene in

healthy Korean women. Bone 1999;24:135-7.

16. Lau EM, Choy DT, Li M, Woo J, Chung T, Sham A. The relationship between COLI A1 polymorphisms (Sp 1) and COLI A2 polymorphisms (Eco R1 and Puv II) with bone mineral density in Chinese men and women. Calcif Tissue Int 2004;75:133-7.

17. Dalgleish R. The human collagen mutation database 1998. Nucleic Acids Res 1998;26:253-5.

18. Välkkilä M, Melkoniemi M, Kvist L, Kuivaniemi H, Tromp G, Ala-Kokko L.Genomic organization of the human COL3A1 and COL5A2 genes: COL5A2 has evolved differently than the other minor fibrillar collagen genes.Matrix Biol 2001;20:357-66.

19. Chou HT, Hung JS, Chen YT, Wu JY, Tsai FJ. Association between COL3A1 collagen gene exon 31 polymorphism and risk of floppy mitral valve/mitral valve prolapse. Int J Cardiol 2004;95:299-305.

20. Zafarullah K, Kleinert C, Tromp G, Kuivaniemi H, Kontusaari S, Wu YL, et al. G to A polymorphism in exon 31 of the COL3A1 gene. Nucleic Acids Res 1990;18:6180.

21. Nieminen K, Huhtala H, Heinonen PK. Anatomic and functional assessment and risk factors of recurrent prolapse after vaginal sacrospinous fixation. Acta Obstet Gynecol Scand 2003;82:471-8.

22. Whiteside JL, Weber AM, Meyn LA, Walters MD. Risk factors for prolapse recurrence after vaginal repair. Am J Obstet Gynecol 2004;191:1533-8.

23. Diez-Itza I, Aizpitarte I, Becerro A. Risk factors for the recurrence of pelvic organ prolapse after vaginal surgery: a review at 5 years after surgery. Int Urogynecol J Pelvic Floor Dysfunct 2007;18:1317-24.

24. Bradley CS, Zimmerman MB, Qi Y, Nygaard IE. Natural history of pelvic organ prolapse in postmenopausal women. Obstet Gynecol 2007;109:848-54.