

# Sp1 collagen I A1 polymorphism in women with stress urinary incontinence

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## Abstract

**Introduction and hypothesis** Stress urinary incontinence (SUI) is in part attributed to qualitative and quantitative changes in connective tissue of the urogenital tract. We examined the association of collagen type I  $\alpha 1$  (COLIA 1) Sp1 polymorphism with the risk of SUI.

**Methods** Forty-five postmenopausal women suffering from urodynamically verified SUI (study group) were compared to 45 healthy volunteers (control). DNA was extracted from peripheral blood. The genotyping concerning the type 1  $\alpha 1$  collagen gene Sp1 polymorphism was performed with polymerase chain reaction.

**Results** The polymorphic T allele was overrepresented in the SUI patients (63.2% versus 36.8%,  $p=0.016$ ). Odds ratio for SUI in women harboring the T allele was 2.19 (95% CI 1.149–4.176) compared to women with the wild-type genotype.

**Conclusions** The COLIA1 Sp1 polymorphism is associated with increased prevalence of stress urinary incontinence in postmenopausal women.

**Keywords** Collagen I A1 gene · Sp1 polymorphism · Stress urinary incontinence

## Introduction

Urinary incontinence and pelvic floor disorders are frequent problems among women, with a serious impact on health and quality of life. Urinary incontinence is the involuntary loss of urine. It affects about 30% of the female population. Among the kinds of incontinence (stress, urge, and mixed urinary incontinence), stress urinary incontinence (SUI) is the most frequent, with a prevalence of 50%. Urge and mixed type incontinence prevalence are 11% and 36%, respectively [1].

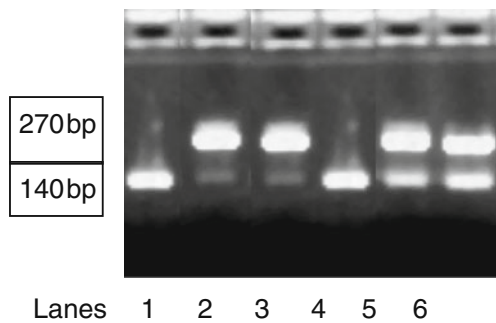
The prevalence of SUI is significantly increased after menopause, particularly in women with coexisting risk factors such as vaginal and instrumental deliveries, multiparity, obesity, and metabolic syndrome [2]. SUI can be attributed to insufficient bladder base support and neck or urethral sphincter dysfunction [3]. Continence also depends on the adequate function of the muscles of the pelvic floor as well as the connective tissue supporting structures of the paraurethral fascia and pubocervical ligaments [4]. Recent studies show an association between qualitative and quantitative changes in connective tissue and the presence of genuine SUI [5]. The main structural protein of connective tissue is type 1 collagen. The quality and organization of collagen fibers can affect the tensile strength of the pubocervical fascia which provides support to the bladder neck and base [6, 7].

Collagen type 1 is a protein consisting of two  $\alpha 1$  (I) and one  $\alpha 2$  (I) chain { $\alpha 1$  (I) $\alpha 2$ (I)} [8]. The expression of genes encoding the collagen  $\alpha 1$  chain (COLIA1) and  $\alpha 2$  chain (COLIA2) is coordinated and efficiently regulated. Polymorphisms of the COLIA1 gene can affect its expression rate [9]. COLIA1 Sp1 is a single nucleotide polymorphism at the regulatory region of the COLIA 1, where guanine is substituted by thymine at the first intron of the gene. This

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**Fig. 1** Gel electrophoretic results. Lanes 1, 3, and 5 (Mix 1 lanes), Lanes 2, 4, and 6 (Mix 2 lanes). Lanes 1 and 2 refer to a homozygote (s/s), Lanes 3 and 4 refer to a wild-type genotype (S/S) and finally, lanes 5 and 6 refer to a heterozygote (S/s)

polymorphism results in three different groups (homozygotes GG, heterozygotes G/T, and homozygotes T/T). These variations can affect a recognition site for the transcription factor Sp1, changing the affinity of Sp1 binding to the DNA chain. This leads to an increased production of COLIA1 relative to COLIA2 protein. Sp1 polymorphism may be responsible for reduced bone mechanical strength and mineral density [10]. There are also recent data implicating the COLIA1 polymorphism in the pathogenesis of SUI [11] or pelvic organ prolapse [12]. The aim of the present study was to examine the association between COLIA1 Sp1 polymorphism and the presence of stress urinary incontinence.

## Materials and methods

Ninety postmenopausal women, aged 47 to 69 years old, were enrolled in the study. Patients were followed on a regular basis in the Menopause Clinic of the Second Department of Obstetrics and Gynecology in Aretaieio Hospital. Exclusion criteria were: medical history of urge or mixed type of incontinence, hysterectomy, neurological disorders, or cancer. All patients signed an informed consent form. The study was approved by the institution's ethics committee.

The patients were divided into two groups: the first group involved 45 women suffering from SUI, while the second group involved 45 healthy women who served as controls. SUI was diagnosed by medical history and verified by urodynamic examination (uroflowmetry, cystometry, abdominal leak point pressure, and profilometry), according to the International Continence Society standards [13]. An ICS standardized 1-hour pad test was used to assess SUI severity [14]. All SUI patients had a positive (pad weight gain >2 gm) 1-hour pad test and were scheduled for operational treatment with tension-free vaginal tape.

All patients were examined in the morning in light clothing. Weight and height were recorded and body mass index (BMI) was calculated by the formula: weight (kg)/height<sup>2</sup> (m). Fasting blood was drawn in all participants and samples were stored at  $-80^{\circ}\text{C}$  until assessment. Bone

**Table 1** Demographic characteristic of the study and control groups

Characteristic	SUI group (n=45) Mean (SD)	Control group (n=45) Mean(SD)	p value
Age, years	58.5 (5.4)	55.8 (3.8)	0.008 <sup>a</sup>
Body mass index, kg/m <sup>2</sup>	27.8 (3.3)	25.2 (3.3)	<0.001 <sup>a</sup>
BMD, g/cm <sup>2</sup>	0.99 (0.2)	0.97 (0.20)	0.575
Age at menopause, years	49.2 (3.5)	49.2 (3.7)	0.953 <sup>a</sup>
Years since menopause	9.3 (6.3)	6.6 (4.6)	0.021 <sup>a</sup>
Menarche, years	12.82 (1.4)	12.4 (1.4)	0.109 <sup>a</sup>
Parity	Number (%)	Number (%)	
None	1 (2.2)	2 (4.4)	0.013 <sup>c</sup>
1 delivery	5 (11.1)	12 (26.7)	
≥2 deliveries	39 (86.7)	31 (68.9)	
Smoking			0.071 <sup>c</sup>
No	25 (55.6)	33 (73.3)	
Yes	18 (40.0)	8 (17.8)	
Ex-smoker	2 (4.4)	4 (8.9)	
Alcohol			0.530 <sup>c</sup>
No	16 (35.6)	19 (42.2)	
Substantially	24 (53.3)	24 (5.3)	
Daily	5 (11.1)	2 (4.4)	
Physical exercise			0.117 <sup>b</sup>
No	9 (20)	11 (24.4)	
1 h/week or less	12 (26.7)	6 (13.3)	
1–4 h/week	18 (40.0)	14 (31.1)	
>3 h/week	6 (13.3)	14 (31.1)	
1 h/week or less	12(26.7)	6 (13.3)	

SUI Stress urinary incontinence, BMD bone mineral density, SD standard deviation

<sup>a</sup> p value was calculated using *t* test

<sup>b</sup> p value was calculated using  $\chi^2$  test

<sup>c</sup> p value was calculated using Fisher's exact test

**Table 2** Allele frequencies and genotype distribution in SUI and control patients

	Genotype/allele	SUI group (%) (n=45)	Control group (%) (n=45)	p value
	GG	14 (31.1)	25 (55.6)	0.041 <sup>a</sup>
	GT	26 (57.8)	19 (42.2)	
	TT	5 (11.1)	1 (2.2)	
<sup>a</sup> p value was calculated using Fisher's exact test	G allele	54 (60)	69(76.7)	0.016 <sup>b</sup>
<sup>b</sup> p value was calculated using chi-square test	T allele	36 (40)	21 (23.3)	

mineral density (BMD) of the lumbar spine was measured using a Norland-Excell Plus-XR-36 densitometer (Norland Medical Systems, Fort Atkinson, WI, USA) by dual energy X-ray absorptiometry. Within-subject coefficient of variation was 1.1%.

DNA was extracted from blood samples collected in EDTA, using Macherey–Nagel NucleoSpin<sup>®</sup> blood kit. The detection of the regulatory variant (G→T mutation, Sp-1 polymorphism) in intron 1 of the type 1 alpha 1 collagen gene (COL1A1) encoding for the alpha 1 (I) protein chain of type I collagen was performed using MutaGel<sup>®</sup> Collagen (AS) kit of Immunodiagnostik AG. Two sets of primers, (Mix 1, Mix 2) for both (wild type or variant) allele specific sequences within the human collagen gene COL1A1, were used directly for PCR with extracted DNA (150 µg), using the following amplification protocol (94°C for 5 min, 94°C, 30 s/58°C, 30 s/72°C, 90 s for 37 cycles, and final hold at 74°C for 5 min) and generate amplicates only in case of presence of one from both sequence possibilities (wild type or variant). The resulting amplification products were subsequently identified with gel electrophoretic methods using E-Gel<sup>®</sup> 4% agarose (HR) from Invitrogen<sup>™</sup>. The presence of normal gene variant (S) was indicated by detection of the DNA fragment (270 bp) of Mix 1 whereas the presence of pathogen gene variant (s) was indicated by the presence of the DNA fragment (270 bp) of Mix 2 (Fig. 1).

Based on a previous study of Skorupski et al. [11], a sample size of 45 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 (G/T or T/T), with power of 80% and a p value <0.05. The  $\chi^2$  test was used to compare the prevalence of various genotypes among patients from the study and control groups. The association between each genotype and the presence of SUI was calculated using odds ratios and 95% confidence intervals. A p value <0.05 was considered statistically significant for all comparisons.

## Results

Demographic characteristics of the 90 postmenopausal women participating in the study are shown in Table 1.

Patients with SUI had a higher mean BMI (27.75±3.29) compared to the control group (25.18±3.32). Similarly, patients with SUI were older (58.5±5.5) compared to women without SUI (55.8±3.8, p=0.008). Finally, patients with SUI had a higher frequency of multiparity compared to controls (33.3% versus 8.9%, p=0.013).

The distribution of genotypes of the studied polymorphism in the 90 investigated women was GG 43.33%, GT 50%, and TT 6.7%. The comparison between the SUI and control groups showed higher prevalence of heterozygous state GT in the study group when compared to the control group (Table 2). The difference was statistically significant (p value=0.041).

The odds ratio for SUI in patients with TT genotype was almost nine times higher when compared to women with the GG genotype (Table 3). However, the wide confidence limits indicate that this estimate has low precision. Patients with GT genotype had an odds ratio of 2.4 for SUI compared to women with the GG genotype (95% confidence interval 1.011 to 5.903). The T allele in the SP1 binding site of the COL1A1 gene was overrepresented in SUI group. Concerning the gene–dose effect, the overall odds ratio for SUI was 2.19 per copy of the T allele.

## Discussion

SUI is a frequent disabling problem of postmenopausal women. Several studies attribute SUI to functional and

**Table 3** Odds ratios (95% confidence intervals) for stress urinary incontinence

Genotype/allele	Odds ratio (95% CI)
GG	1
GT	2.44 (1.01, 5.90) <sup>a</sup>
TT	8.93 (0.95, 84.24) <sup>a</sup>
G allele	1
T allele	2.19 (1.15, 4.18) <sup>b</sup>

GG wild type, GT heterozygous polymorphic, TT homozygous polymorphic

<sup>a</sup> Odds ratios were calculated for patients with GG genotype

<sup>b</sup> Odds ratio was calculated for G allele

quality changes of the fibrous connective tissue of the pelvic floor ligaments. Significant changes in the extracellular matrix may result to a stiffer and less supportive paraurethral connective tissue [6, 7]. Possible changes of the ratio between collagen I and III may lead to deterioration of the supporting properties of connective tissue. In a previous study, we demonstrated a significant reduction of collagen type I in about 53% patients with SUI, irrespectively of the presence or absence of genital prolapse [15]. This reduction can lead to inadequate support of the bladder neck, causing unequal transmission of the intraabdominal pressure between bladder neck and urinary bladder and a consequent development of SUI.

Numerous studies investigate genetic polymorphisms in the genes encoding the  $\alpha 1$  and  $\alpha 2$  chains of collagen type I and their influence in various pathological conditions. According to the Genetic Markers for Osteoporosis consortium (Genomos) framework project, COLIA1 Sp1 polymorphism was associated with reduced bone mineral density and increased fracture risk [16]. A meta-analysis, aiming to quantitate the association between collagen type I alpha 1 (COLIA1) Sp1 polymorphism and osteoporotic fracture risk in Caucasian postmenopausal women, showed that patients with vertebral fractures had a significantly higher frequency of the Ss genotype and a lower frequency of the SS genotype than controls. Additionally, patients with non-vertebral fractures had a significantly higher frequency of the ss genotype and a lower frequency of the SS genotype than controls (SS odds ratio [OR] 0.72; Ss OR 1.18; ss OR 1.97) [17]. These findings confirmed the results of a previous study, which showed that the “s” allele, associated with low BMD and increased fracture risk in Caucasians, is non-existent or very rare in the southern Chinese population. The absence of this “high risk” allele may in part account for the reduced fracture risk observed in the Chinese in comparison to western populations [18].

Data on the association of Sp1 polymorphism and pelvic floor disorders are sparse. A recent study found an increased prevalence of the heterozygous (GT) genotype of COLIA1 Sp1 polymorphism among women with pelvic organ prolapse (POP) compared to the control group (33.3% and 19.3%, respectively). The age-adjusted odds ratio for POP was two times higher for patients carrying the T allele compared to women who had the wild-type GG genotype. This difference, however, did not reach statistical significance [12]. Furthermore, Rodrigues et al. conducting a case–control study in 107 patients with POP and 209 control women could not demonstrate a statistically significant association between Sp1 polymorphism and POP [19]. Finally, two small studies in Korean and Polish women failed to show an association between Sp1 polymorphism and POP [20, 21].

In contrast to POP, Sp1 polymorphism may be associated with SUI. Our study demonstrated that the G to T substitution in transcription factor Sp1-binding site is associated with the development of stress urinary incontinence, as the prevalence of the T allele was statistically significantly higher in women with SUI in comparison to healthy women. To the best of our knowledge, there is only one study in the literature examining the association between Sp1 polymorphism and SUI. Skorupski et al. [11] evaluated 50 women with SUI with a mean age of 53.4 and compared them to 50 healthy women with a mean age of 52.7. In accordance with our study, the odds ratio for SUI was 4.98 in women with the GT genotype and 2.23 for the TT genotype, while the T allele-specific odds ratio for SUI was 2.0. Supporting the hypothesis that COLIA1 polymorphism associates with SUI rather than POP, Goepel et al. showed a decreased immunohistochemical staining of types I, III, and VI collagen in periurethral tissue of patients with both POP and SUI over patients with POP alone. This finding suggests that collagen deficiency is more pronounced in SUI than in POP [22].

Regarding the results of our study, there are certain limitations; the small sample size can result in bias. It was also expected that women suffering from SUI have higher values of weight, BMI, and parity. This difference could also result into bias of the results, however to the best of our knowledge there is no genotype association to collagen polymorphism.

In conclusion, COLIA1 Sp1 polymorphism is associated with increased prevalence of stress urinary incontinence. Since genetic predisposition represents a considerable part of SUI etiology, further prospective studies involving larger populations should be conducted in order to confirm the contribution of COLIA1 Sp1 polymorphism in the etiology of SUI.

**Conflicts of interest** None.

## References

- Hannestad YS, Rortveit G, Sandvik H, Hunskaar S (2000) Norwegian EPINCONT study. Epidemiology of incontinence in the county of Nord-Trøndelag. A community-based epidemiological survey of female urinary incontinence: the Norwegian EPINCONTstudy. Epidemiology of incontinence in the county of Nord-Trøndelag. *J Clin Epidemiol* 53(11):1150–1157
- Melville JL, Katon W, Delaney K, Newton K (2005) Urinary incontinence in US women: a population-based study. *Arch Intern Med* 165(5):537–542
- DeLancey JO, Starr RA (1990) Histology of the connection between the vagina and levator ani muscles. Implications for urinary tract function. *J Reprod Med* 35(8):765–771

4. Petros PE, Ulmsten UI (1993) An integral theory and its method for the diagnosis and management of female urinary incontinence. *Scand J Urol Nephrol Suppl* 153:1–93
5. Rechberger T, Postawski K, Jakowicki JA, Gunja-Smith Z, Woessner JF Jr (1998) Role of fascial collagen in stress urinary incontinence. *Am J Obstet Gynecol* 179(6 Pt 1):1511–1514
6. Ulmsten U, Ekman G, Gieritz G, Malmström A (1987) Different biochemical composition of connective tissue in continent and stress incontinent women. *Acta Obstet Gynecol Scand* 66(5):455–457
7. Falconer C, Ekman G, Malmström A, Ulmsten U (1994) Decreased collagen synthesis in stress-incontinent women. *Obstet Gynecol* 84(4):583
8. Keane DP, Sims TJ, Abrams P, Bailey AJ (1997) Analysis of collagen status in premenopausal nulliparous women with genuine stress incontinence. *Br J Obstet Gynaecol* 104(9):994–998
9. Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH (1996) Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. *Nat Genet* 14(2):203–5
10. Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP et al (2001) A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J Clin Invest* 107(7):899–907
11. Skorupski P, Król J, Starega J, Adamiak A, Jankiewicz K, Rechberger T (2006) An alpha-1 chain of type I collagen Sp1-binding site polymorphism in women suffering from stress urinary incontinence. *Am J Obstet Gynecol* 194(2):346–350
12. Feiner B, Fares F, Azam N, Auslender R, David M, Abramov Y (2009) Does COL1A1 SP1-binding site polymorphism predispose women to pelvic organ prolapse? *Int Urogynecol J Pelvic Floor Dysfunct* 20(9):1061–1065
13. Schäfer W, Abrams P, Liao L, Mattiasson A, Pesce F, Spangberg A et al (2002) International Continence Society. Good urodynamics practices: uroflowmetry, filling cystometry, and pressure-flow studies. *Neurourol Urodyn* 21(3):261–274
14. Ghoniem G, Stanford E, Kenton K, Achantari C, Goldberg R, Mascarenhas T, Parekh M, Tamussino K, Tosson S, Lose G, Petri E (2008) Evaluation and outcome measures in the treatment of female urinary stress incontinence: International Urogynecological Association (IUGA) guidelines for research and clinical practice. *Int Urogynecol J Pelvic Floor Dysfunct* 19(1):5–33
15. Liapis A, Bakas P, Pafiti A, Hassiakos D, Frangos-Plemenos M, Creatsas G (2000) Changes in the quantity of collagen type I in women with genuine stress incontinence. *Urol Res* 28(5):323–326
16. Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, Lips P, Lorenc R et al (2006) GENOMOS investigators. Large-scale evidence for the effect of the COL1A1 Sp1 polymorphism on osteoporosis outcomes: the GENOMOS study. *PLoS Med* 3(4):e90
17. Ji GR, Yao M, Sun CY, Zhang L, Han Z (2009) Association of collagen type I alpha1 (COL1A1) Sp1 polymorphism with osteoporotic fracture in Caucasian post-menopausal women: a meta-analysis. *J Int Med Res* 37(6):1725–1732
18. Lambrinoudaki I, Kung AW (2001) Absence of high-risk “s” allele associated with osteoporosis at the intronic SP1 binding-site of collagen Ialpha1 gene in Southern Chinese. *J Endocrinol Invest* 24(7):499–502
19. Rodrigues AM, Girão MJ, da Silva ID, Sartori MG, Martins Kde F, Castro Rde A (2008) COL1A1 Sp1-binding site polymorphism as a risk factor for genital prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 19(11):1471–1475
20. Cho HJ, Jung HJ, Kim SK, Choi JR, Cho NH, Bai SW (2009) Polymorphism of a COL1A1 gene Sp1 binding site in Korean women with pelvic organ prolapse. *Yonsei Med J* 50(4):564–568
21. Skorupski P, Miotła P, Jankiewicz K, Rechberger T (2007) Polymorphism of the gene encoding alpha-1 chain of collagen type I and a risk of pelvic organ prolapse—a preliminary study. *Ginekol Pol* 78(11):852–5
22. Goepel C, Hefler L, Methfessel HD, Koelbl H (2003) Periurethral connective tissue status of postmenopausal women with genital prolapse with and without stress incontinence. *Acta Obstet Gynecol Scand* 82(7):659–664