

## Original Article

# Relationship of advanced glycation end products and their receptor to pelvic organ prolapse

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**Abstract:** Objective: The aims of this study are to detect levels of AGEs and RAGE and SNPs for RAGE in vaginal tissues of women with POP and rats in a repair location, and to explore the relationship between AGEs-RAGE pathway and POP. Methods: This study involved human vaginal tissues in fornix from 44 women with POP and 46 women without POP who were assigned to pelvic floor reconstruction or LAVH. The proteins of AGEs, collagen I, and RAGE were detected by immunohistochemistry and Western blot with appropriate primary antibodies. The entire RAGE gene of 24 women with POP and 25 controls were sequenced, and SNPs within were detected. Then, sixty 8-week-old female Sprague-Dawley rats subjected to abdominal defect were divided into three surgical pelvic floor reconstruction repair groups (n = 20/group): A, repair with non-absorbable prolene mesh; B, repair with absorbable SIS mesh; and C, a no repair control group. 3, 9, 15, and 21 months after operation, rats were sacrificed and the expression of AGEs, RAGE and collagen I in the tissues of repair location were detected in the various experimental groups. Statistical analysis included comparison of means (Student's t-test) and proportions (Chi-square test or Fisher test). Results: By both immunohistochemistry and Western blot, patients with POP showed higher protein expression of AGEs of POP than controls ( $P < 0.05$ ). In contrast, the expression of collagen I was lower in POP patients than in the control group ( $P < 0.05$ ). No differences in the expression of RAGE between the POP patients and controls were observed ( $P > 0.05$ ). In POP patients, the expression of collagen I decreased particularly in patients  $\geq 60$  years old ( $P < 0.05$ ), but there were no difference in the expression of AGEs and RAGE dependent on age ( $P > 0.05$ ). RAGE gene sequence variance analysis identified 18 variable loci, but only two of these were potential SNPs: rs184003 (1806), rs55640627 (2346) ( $P < 0.05$ ). Both rs184003 and rs55640627 are both intronic variants, indicating that they may not influence the structure of RAGE. In rat surgical repair model, group B showed a greater extent of abdominal prolapse than groups A and C ( $P < 0.05$ ). Consistent with this, the expression of AGEs in group B was higher than groups A and C ( $P < 0.05$ ), and collagen I in group B was lower than the two others, further supporting our notion that AGEs are inversely related to type I collagen content. Conclusions: In summary, this study demonstrates that AGEs and RAGE might play important roles in the physiopathology of POP. Further studies are required to explore mechanisms of how AGEs-RAGE pathway may contribute to tissue degeneration and fragility in POP.

**Keywords:** POP, AGEs, RAGE, collagen I, gene sequence

## Introduction

Pelvic organ prolapse (POP) is a common disease affecting women, frequently leading to significant reductions in quality of life [1-3]. Old age, obesity, multiparity, hereditary factors, chronic straining and coughing are the most known important risk factors [4-6], but the detailed pathophysiology of POP remains unknown. The quantity and quality of collagen and elastin determine the mechanical properties of connective tissue and are regulated by a

precise equilibrium between synthesis, maturation and degradation. This dynamic process of constant remodeling [7, 8] often underlies POP occurrence. When the equilibrium is broken by many factors, tissue weakness and POP may occur [9].

An understanding of the factors that can impact collagen metabolism in the pelvic floor will enhance our understanding of POP. Recently, it has been demonstrated that changes in advanced glycation end-products (AGEs) and

**Table 1.** Patients' demographics

Variables	POP	control	P value
Age, mean (SD), years	63.76 (±8.31)	62.11 (±8.27)	0.33*
Postmenopausal age, mean (SD), years	50.88 (±2.06)	50.68 (±2.15)	0.62*
BMI, mean (SD)	23.53 (2.09)	22.94 (±2.41)	0.2*
Parity, mean (SD), n	2.82 (±1.34)	2.53 (±1.39)	0.26*
Hypertension, n (%)	21, (47.7%)	13, (28.3%)	0.06#
Diabetes mellitus	2 (4.55%)	4 (0.87%)	0.43#
the degree of III, (POP-Q), n	34	0	
the degree of IV, (POP-Q), n	10	0	
cervical lesions (reason of LAVH), n	0	15	
endometrial lesions (reason of LAVH), n	0	11	
ovary lesions (reason of LAVH), n	0	20	

BMI (body mass index) = weight (kg)/height<sup>2</sup> (m<sup>2</sup>), \*Student's t test, #chi-square test.

their receptor (RAGE), and the SNP in the RAGE gene, are involved in dysregulated collagen metabolism in many human diseases [10]. AGEs are products of nonenzymatic glycation and oxidation of proteins and lipids, and are known to accumulate in diverse biological settings including aging, diabetes, neurodegeneration, and inflammation. RAGE is the receptor for advanced glycation end products, and functions as a central signal transduction receptor for these products. AGEs-RAGE interaction can activate a wide array of signal transduction cascades. For example, RAGE is strongly expressed in the gingiva of diabetic patients with severe periodontitis [11]. AGEs are formed intracellularly and extracellularly in skin, and AGEs and RAGE alter the physicochemical properties of dermal collagen proteins, decrease cell proliferation, increase apoptosis and senescence, and induce oxidative stress and proinflammatory mediators through the MAPK, JAK/Stat, ROS, and NF- $\kappa$ B signaling pathways, indicating that AGEs strongly contribute to skin aging [12-15]. Single nucleotide polymorphisms in the RAGE gene may participate in disease progression; rs2070600 and rs17493811 predicted increased risk of type 1 diabetes, whereas the rs9469089 SNP was related to decreased risk, on a high risk HLA background [16].

Patients with POP have low collagen content and a relatively high amount of advanced glycation end products (AGEs) with impaired mechanical strength [17, 18]. Estrogen treatment has profound effects upon pelvic collagen metabolism, leading to synthesis of new collagen as witnessed by increased immature cross-links and decreased mature cross-links and advanced glycation end-products [19]. Gly-

cated "old" collagen also may be difficult to degrade and susceptible to rupture, leading to POP. But there is little additional evidence supporting this hypothesis, and no further studies on the role of RAGE in pelvic tissue fibroblasts have been performed.

The roles of AGEs and RAGE in POP need to be elucidated further. We therefore hypothesize that AGEs and RAGE

might participate in POP progression, and that single nucleotide polymorphisms in RAGE might underlie disease susceptibility. In this study, the expression of AGEs, RAGE, collagen I were detected in tissues of humans and experimental animals. In addition, RAGE gene sequence in POP patients and controls was determined. These results allowed us to further strengthen the correlation of AGEs and RAGE to POP.

## Patients and methods

### Patients

Forty four patients admitted to our hospital for pelvic floor reconstruction surgeries for different types of POP were included in this study. According to POP-Q scoring system, all patients were stage 3 or above. The surgeries consisted of hysterectomy with normal-size uterus and vaginal repair for one or more of these conditions. Forty six women with normal-sized uterus undergoing hysterectomy for other diseases (such as cervical carcinoma in situ, atypical hyperplasia of endometrium, and benign ovarian lesions) served as the control group. All the patients (minimum 45 years old) were of Han nationality, and all had confirmed postmenopausal status. Patients were excluded if they had pelvic malignancy, fibroids, endometriosis, pelvic inflammation, hormone replacement therapy, and Caesarean section history. The age distribution, menstrual status, parity, and disease accompanying are matched and listed in **Table 1**.

### Treatment of biopsies

After obtaining ethical approval and informed consent for this study, the surgeries mentioned

above were carried out under general or spinal anesthesia. Samples of vaginal wall were collected from the vaginal residual of free uterus equivalent to the midline anterior portion of the pericervical cuff at hysterectomy. Each biopsy measured approximately a 5-10 mm cube, and was stored in liquid nitrogen immediately prior to analysis. Every sample was divided into tri-section for WB, IHC, and gene sequencing.

## Immunohistochemistry

After being washed, fixed, dehydrated, cleared, and embedded in paraffin, the sections were obtained and collected on positively charged microscope slides. Tissue sections were deparaffinized, rehydrated, and antigen retrieved prior to staining [20, 21]. Immunohistochemistry was performed on the sections using the Histostain-plus Kit (MRBiotec, Shanghai, China) and the following primary antibodies were used for immunostaining: anti-RAGE (sc-365154, Santa Cruz), anti-collagen I (NBP1-30054, Novus), and anti-AGEs (ab23722, Abcam). Incubation with immunoglobulin of the same species at the same concentration with no primary antibody was used as a negative control. We used an Olympus microscope and Image-Pro Plus (Media Cybernetics, Inc) to analyze results. For quantification of the representative areas of collagen, five visual fields were digitized using a 40× magnifying objective. Each digitized field contained resolution of 929×700 points, all in the region of connective tissues. The IOD (sum) of all fields were collected and analyzed by Image-Pro Plus.

## Western blotting

Lysates of vaginal tissues were prepared by glass homogenizer in lysis buffer, 50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 2 mM NaF, 2 mM EDTA, 0.1% SDS and a protease inhibitor cocktail tablet [22]. Protein concentration was quantified by BCA protein Assay Kit (Beyotime China) according to the manufacturer's instructions. Proteins (50 mg per well) and prestained molecular weight markers (Fermentas, SM0641) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membrane. The membranes were blocked for 1 h at room temperature with Protein Blocker (Bio-Rad) and were incubated overnight at 4°C with primary anti-

bodies as above mentioned, at a dilution of 1:500 in Tris-buffered saline with Tween-20 (TBST). After washing with PBST, the membranes were incubated with second antibody, then visualized by enhanced chemiluminescence (ECL), and quantified by Image J [23] (National Institutes of Health).

## Gene sequencing of RAGE

A total of 24 patients and 25 controls were randomly chosen from the entire study cohort for RAGE gene sequencing. After the DNA was extracted, RAGE was genotyped by PCR using the following primer sequences: AGER-1F: 5' AAAGGCATGAATTCCTAGCATT 3', AGER-1R: 5' GGTGAAGGCTTTTCTTAGGTA 3'; AGER-2F: 5' TCCAGGATGAGGGGATTTT C 3', AGE-2R: 5' AGER-2R: 5' ACCCTGTCTTTTGTCTCCATA 3', AGER-3F: 5' TAGGGTGAACCATACTAGCAAC 3', AGER-3R: 5' TGGTATAGTATGGTGAAGTATGGG 3'. PCR reactions were performed in 20-μL reaction volumes containing 10 mM Tris HCl (pH 8.9), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 μM of each primer, 250 μM of each dNTP, 50 ng of genomic DNA, and 0.5 units of Taq thermostable DNA polymerase (ABI, Foster city, CA, USA). Cycling parameters were 5 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at the annealing temperature (T<sub>m</sub>) of the primers (52°C-63°C, see Table S1, Additional File 1), and 45 seconds to 1 minute at 72°C, with a final 10minute extension at 72°C [24-26]. Finally, the sequence of the entire PCR product was determined by 3730 sequence analyzer (ABI, Foster city, CA, USA).

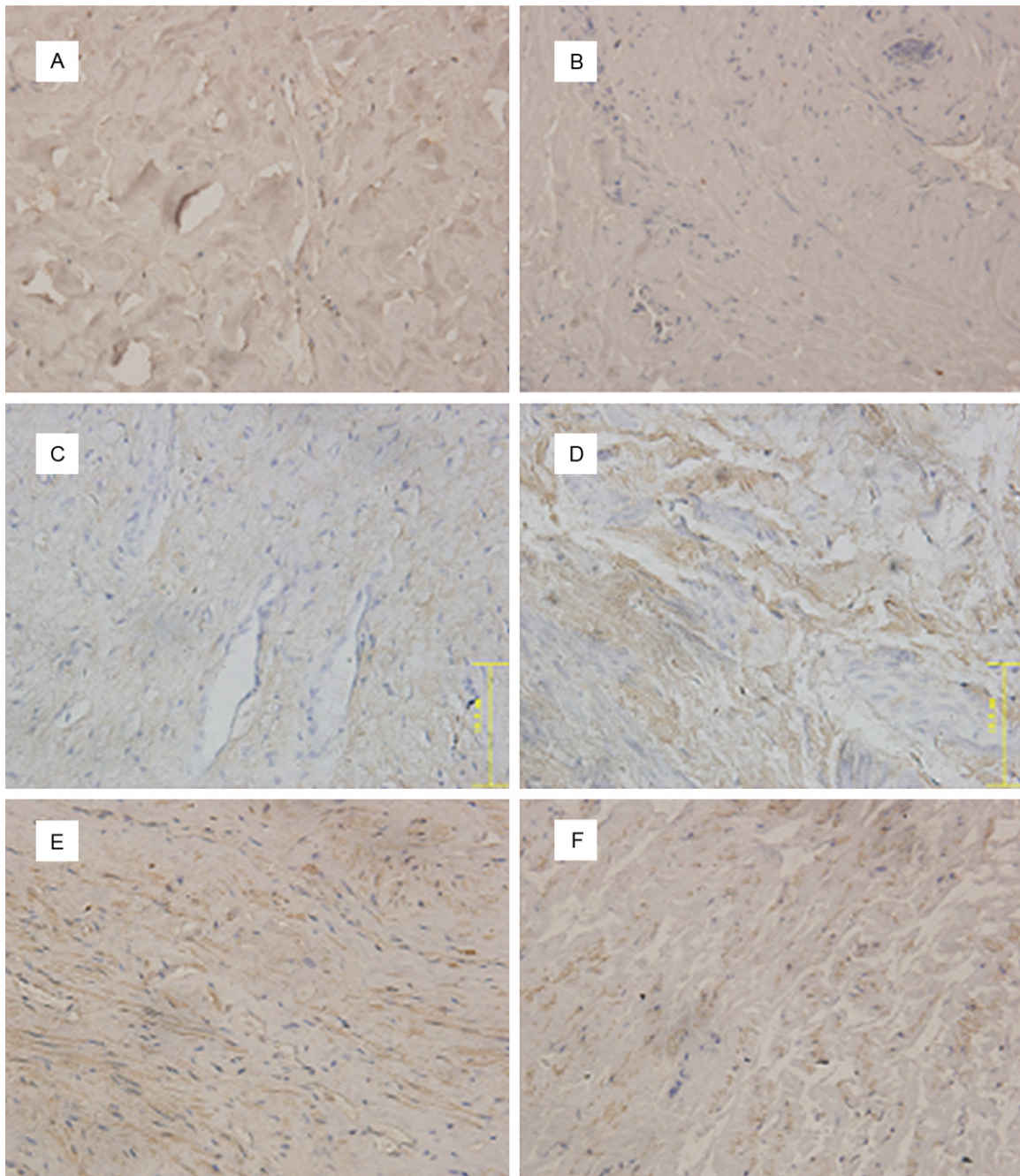
## Animals

Sixty 8-week-old female Sprague-Dawley rats between the weight ranges of 180-200 g were used in the study, and housed in the SPF animal laboratory [27]. Experimental protocols were approved by the Animal Ethics Committees of Gynaecology and Obstetrics Hospital of Fudan University.

## Animal grouping and abdominal wall defect treatment

Animals were grouped according to the mesh implanted at the artificial abdominal wall defect. Rats were randomized to three groups (n = 20/group): A, Prolene implants (non-absorbable mesh group; Ethicon, USA) [28]; B,



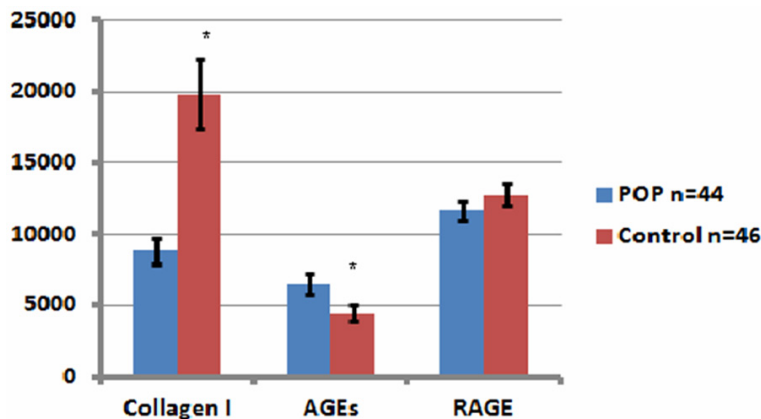


**Figure 1.** Immunohisto-chemical localization of AGEs, Collagen I and RAGE proteins in vaginal tissue is shown, (A, B) The distribution of AGEs in POP (A) and in control (B). AGEs protein staining was expressed faintly at vaginal matrices in POP. (C, D) The distribution of collagen I in POP vaginal tissue (C) and in control group (D), collagen I protein staining was prominent in control group than in POP group. (E, F) The distribution of RAGE POP (E) vaginal tissue and the control, RAGE-positive cells appear brown, and a light hematoxylin counter-stain was used to visualize nuclei. More positive cells were detected in POP than control. [original magnification, 4000 (A-F)].

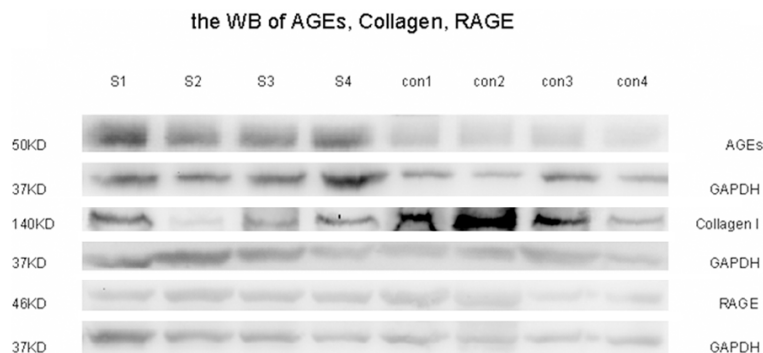
SIS implants (absorbable mesh group; Biodesign surgisis, Cook USA) [29]; C, no implants, as control.

Surgical Procedure was as follows: General anesthesia was induced by intraperitoneal injection of 3% pentobarbital sodium solution

(Sigma) at a dosage of 1.0 mL/kg, abdominal skin was shaved, a vertical incision was opened, then a 2 cm×2 cm wound including muscles and peritoneum was created. The implant size was trimmed to 2.5×2.5 cm<sup>2</sup> so that it extended 0.25 cm beyond the borders of the section area, and then sutured without tension to the



**Figure 2.** The IOD (SUM) of purpose protein by immunohistochemistry for POP and control. Data are exprssion as mean  $\pm$  SEM. \*Indicate the significant different ( $P < 0.05$ ).



**Figure 3.** The WB of AGEs, collagen I, RAGE of vaginal tissues is shown above. S1, S2, S3, S4 were the expression of four samples of POP. Con1, Con2, Con3, Con4 were the expression of foursamples of control. The expression of AGEs in POP group were higher than the control group; the collagen I in POP decreased obviously; and the expression of RAGE remain stable.

abdominal wall with interrupted sutures by 4-0 Mu Si line (Syneture; Ethicon, USA). Finally, the skin was closed and the rats were fed in SPF class lab, and given injection of penicillin (80000 U/rat/day, im, three days) [27].

## *Treatment of tissues at repair center*

At intervals of 3, 9, 15, and 21 months post-operation, five rats from each group were selected at random and were treated as below. First, the abdominal hair was taken out and the bulge extent of abdominal wall repaired was observed under the force of gravity. Then, the rats were killed by cervical vertebra dislocation after general anesthesia as above, and then the abdominal skin was opened to expose the embedded objects. The tissue specimens at

the repair location were obtained and kept in 10% neutral formalin aqueous solution, and analyzed by immunohistochemistry to detect expression of AGEs, RAGE and collagen I.

## *Statistical methods*

Data were analyzed with the software of SPSS 16.0, the level of significance for all tests was 0.05 ( $P < 0.05$ ). The results of semi-quantitative pathological evaluation were compared among two groups with the Variances (ANOVA) or Student's t test, and the rates of different groups were compared with Chi-square and Fisher exact tests.

## **Results**

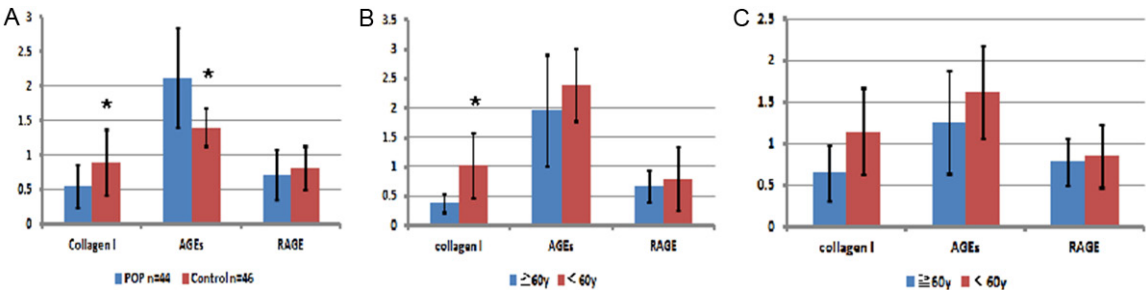
### *Expression of AGEs, RAGE and collagen I in the tissues of pelvic floor in POP patients*

Immunohistochemically, Levels of AGEs, collagen I and RAGE in vaginal connective tissues by immunohistochemistry are shown in **Figures 1, 2**. The expression of AGEs in POP patients is higher than in the control group (**Figure 1A, 1B, Figure 2,  $P < 0.05$** ); the expres-

sion of collagen I is lower in POP patients than in controls (**Figure 1C, 1D, Figure 2,  $P < 0.05$** ); but no differences in RAGE expression between the POP group and controls are observed (**Figure 1E, 1F, Figure 2**).

Western blotting showed that protein levels of AGEs, collagen I and RAGE in vaginal tissues as measured by western blotting are shown in **Figure 3**. Similar to our immunohistochemical results, the expression of AGEs in POP patients are higher than in controls, and POP patients show lower levels of collagen I ( $P < 0.05$ ); RAGE protein levels are similar in both groups, as was seen by immunohistochemistry. Amongst POP patients, collagen I levels in the subgroup  $\geq 60$  years old are higher than in those subjects younger than 60, but there are no age-depen-

AGEs and RAGE express in POP



**Figure 4.** Rate of gray level for purpose protein/GAPDH of the tissues of vaginal wall by Western Blotting between different groups. Data are expressed as the mean ± SEM. \*indicate the significant difference (P < 0.05). A illustrate the expression of collagen I, AGEs and RAGE between the POP and control groups. B illustrate the expression of collagen I, AGEs and RAGE between the group of ages above 60 and the left for POP group. C illustrate the expression for control group.

**Table 2.** Allele and genotype frequency of polymorphisms in control and POP groups

polymorphic site	POP					NPOP					P Value HWE (x <sup>2</sup> )
	n	genotypes		alleles, %		n	genotypes		alleles, %		
108	24	AA/AT	23/1	A/T	97.9/2.1	25	AA	25	A	100	1.00#
168	24	TT/CT/CC	19/4/1	T/C	87.5/12.5	25	TT/CT	19/6	T/C	88/12	0.41#
644	24	CC/CT	23/1	C/T	97.9/2.1	25	CC	25	C	100	1.00#
659	24	GG/AG/AA	15/8/1	G/A	79.2/20.8	25	GG/AG/AA	15/9/1	G/A	78/22	0.98#
880	24	CC/CT/TT	18/5/1	C/T	85.4/14.6	25	CC/CT	19/6	C/T	88/12	0.35#
1541	24	CC/CT	23/1	C/T	97.9/2.1	25	CC/CT	24/1	C/T	98/2	1.00#
1542	24	CC/AC	23/1	C/A	97.9/2.1	25	CC/AC	24/1	C/A	98/2	1.00#
1806	24	GG/GT/TT	13/9/2	G/T	72.3/27.7	25	GG/GT	21/4	G/T	92/8	0.04#
2286	24	AA/AG	16/8	A/G	80/20	25	AA/AG/GG	18/6/1	A/G	84/16	0.41#
2301	24	GG/AG/AA	15/6/3	G/A	75/25	25	GG/AG/AA	15/8/2	G/A	76/24	0.79#
2346	24	GG/AG/AA	16/5/3	G/A	77.1/22.9	25	GG/AG	23/2	G/A	96/4	0.04#
2357	24	CC	24	C	100	25	CC/CT	23/2	C/T	96/4	0.49#
2530	24	CC/CT	22/2	C/T	95.8/4.2	25	CC/CT	23/2	C/T	96/4	1.00#
2565	24	GG/CG	21/3	G/C	92.7/6.3	25	GG/CG	23/2	G/C	96/4	0.67#
2653	24	GG/AA	23/1	G/A	97.9/2.1	25	GG	25	G	100	0.49#
2842	24	GG/AG	23/1	G/A	97.9/2.1	25	GG/AG	24/1	G/A	98/2	1.00#
1962	24	GG/AG	23/1	G/A	97.9/2.1	25	GG/AG	24/1	G/A	98/2	1.00#
3404	24	CC/CT	20/4	C/T	91.7/8.3	25	CC/CT	22/3	C/T	94/6	0.70#

#chi-square test.

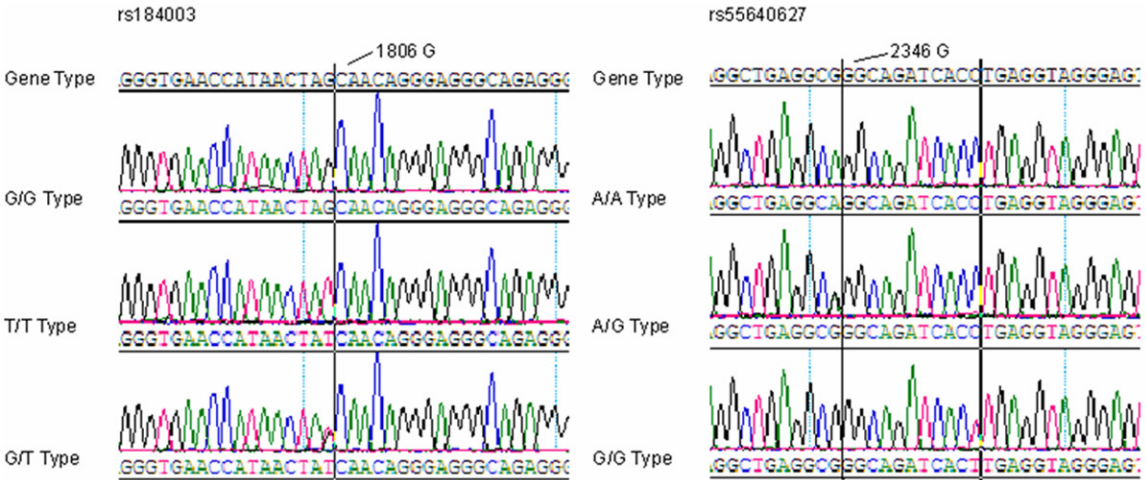




Figure 5. Gene variants of rs184003 and rs55640627 in vaginal tissues of POP and non-POP.

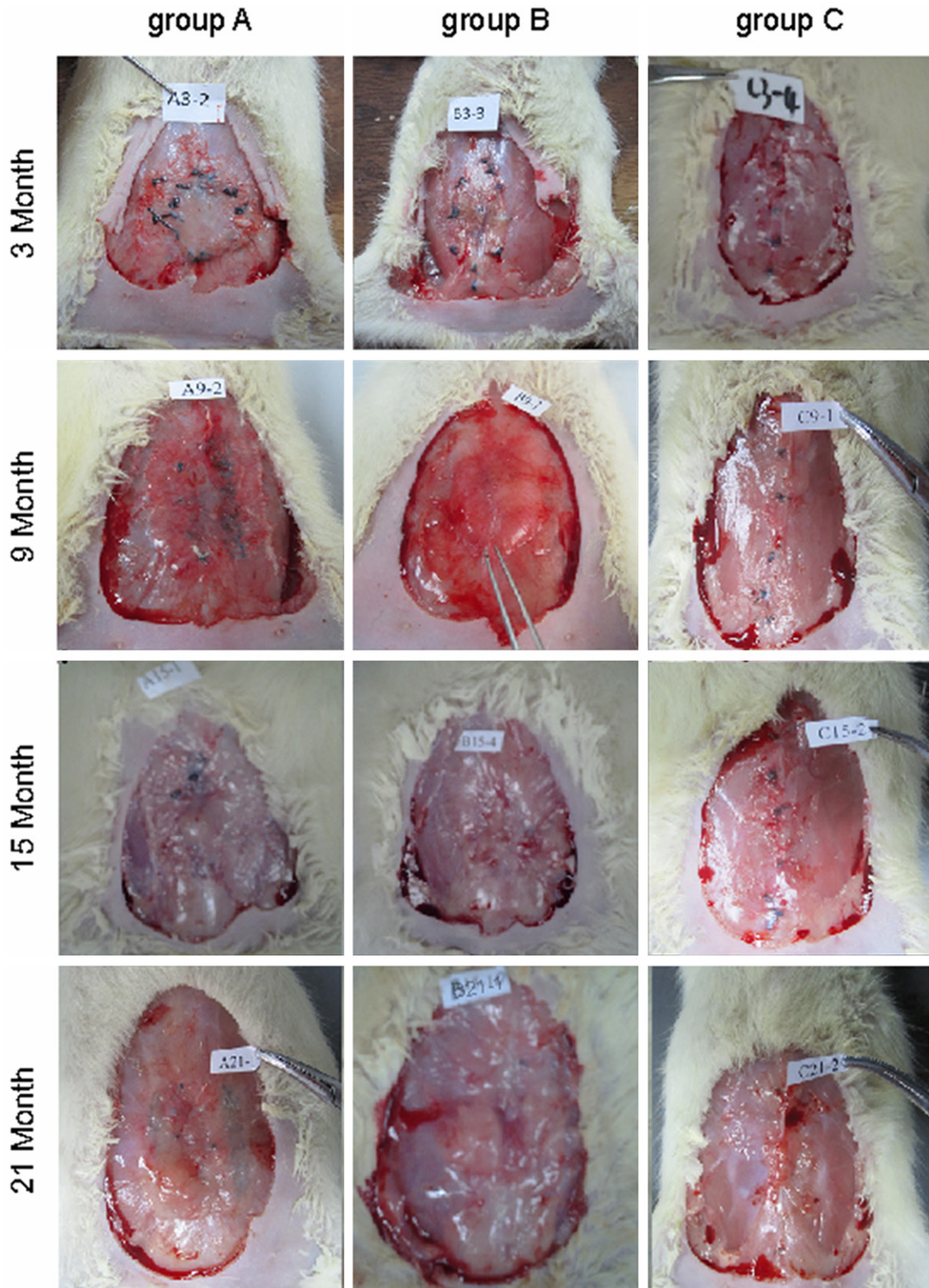
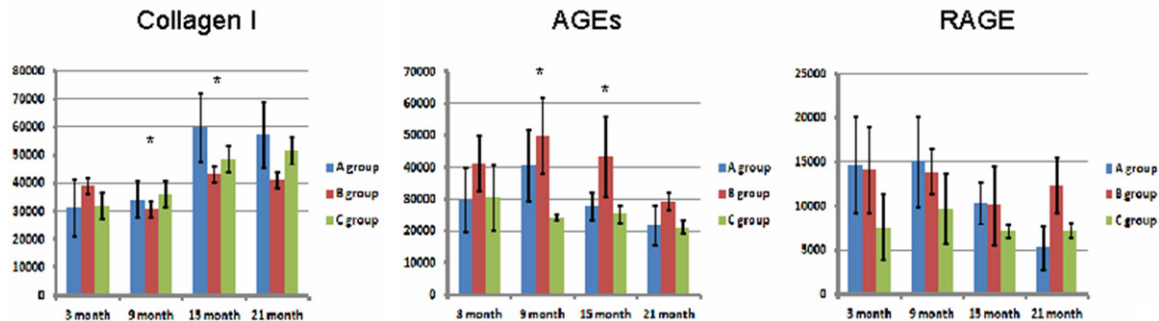


Figure 6. Repair location of SD rat after the operation of abdominal repair with mesh. A group was the mesh of polypropylene; B was the mesh of absorbate SIS; C was the operation without mesh.



**Figure 7.** Expression of collagen I, AGEs and RAGE in the tissues at repair center of S/D rats by immunohistochemistry at different time (3, 9, 15, 21 months) post operation. Data are expression as the mean  $\pm$  SEM, \*indicate significant different ( $P < 0.05$ ) among the different groups (Group A-C).

dent differences in AGEs and RAGE levels (Figure 4). For the control group, there are no age-dependent differences in any of the protein levels measured.

#### RAGE gene sequencing

In total, 18 variable loci in the RAGE gene were identified in the Table 2. But only two of these loci were potential SNPs: rs184003 (1806), rs55640627 (2346) ( $P < 0.05$ ) (Figure 5; Table 2). According to the genomic sequence of RAGE in the public database, rs184003 and rs55640627 are both intronic variants, and therefore may only disrupt RNA splicing. Therefore, we detected no functionally-significant RAGE polymorphic variants in POP patients, leading us to not pursue this area in more detail.

#### Expression of AGEs, RAGE and collagen I at abdominal repair sites in Sprague/Dawley rats

Several deaths (4 in group A, 3 in group B, and 3 in group C) occurred in our surgical cohort. One case of abdominal wall bulging occurred in group A, six cases in group B, and none in group C, and there were significant differences in this complication among the three groups ( $P < 0.05$ ). The abdominal repair location appearance at each time point is shown in Figure 6. Group A repairs typically had more performance of tension, but with increased stiffness and grittiness; the most prominent characteristic of group B was softy and simulation, the foreign body sensation improved 9 months post operation, but the weakness and the pallor of the repair location indicated poor blood supply; the appearance in group c return to normal approximately three months post operation.

The expression of AGEs, collagen I and RAGE in the tissues at repair site by immunohistochemistry at different post-operative times are shown in Figure 7. Collagen I expression in group A and B increased gradually and then remained constant 15 month post operation. Groups A and B appeared different from group C according to the different manner of repair. Collagen I expression in group A and B was higher than that of group C ( $P < 0.05$ ). AGEs expression in all groups first increased then decreased, with an expression nadir at 21 months post operation ( $P < 0.05$ ). Notably, AGEs expression in group B was higher than in others ( $P < 0.05$ ). RAGE expression in group A declined over time ( $P < 0.05$ ), but RAGE levels in groups B and C change showed no significant differences ( $P > 0.05$ ).

#### Discussion

By testing the protein expression of collagen I, AGEs, RAGE, and the gene sequencing of RAGE, the present study describes a possible role of AGEs and RAGE in the pathophysiology of POP.

The results were consulted as following: Collagen I and III are the common components of fasciae in pelvic floor, and collagen I contributes to the biomechanical properties of most connective tissues [30, 18]. The ultrastructure and content of collagen I in pelvic floor are fundamental to the pathophysiology of POP: undesirable changes of both aspects results in this disorder. The parametrium consists of shorter, thinner and more disorderly arranged collagen fibers in patients with uterine prolapse compared to healthy controls [31]. Consistent with previous studies [32], we also showed collagen I expression in vaginal tissues is decreased in POP patients, and was lower in the elderly



women. These findings directly explain the decrease of pelvic floor biomechanical properties. Previous studies reported discordant results about collagen I expression in POP, presumably because of different standards and methods [33-35]. These paradoxical results prompted us to repeat the detection of collagen I, and try to find the correlation of collagen I to AGEs and RAGE in the same subjects and conditions.

The subjects of AGEs in POP had not been revisited since Jackson tested their levels by detecting pyridinolines and pentosidine fluorescence [17]. He considered that AGEs are brittle and susceptible to rupture, resulting in tissue with an impaired mechanical strength. On the other hand, AGEs can affect the metabolism of collagen through RAGE, similar to the effects of AGEs in other diseases like diabetes [36, 37]. We detected AGEs by using a specific antibody, and found that AGEs was higher in POP patients than in controls. This was consistent previous results, and showed that AGEs maybe take part in the pathophysiology of POP. However, our data do not allow us to claim a causal role for AGEs in POP disease progression. We also compared the expression of AGEs in different ages and saw no difference, indicating that AGEs do not increase with aging, at least in this population studied. Compared to the change of collagen I levels with aging, AGEs levels remain stable, indicating that AGEs may be only one reason accounting for the change in collagen I levels, and therefore one cause of POP.

It has been demonstrated that RAGE is involved in many diseases through the change of its levels, structure, or both [38, 39]. Therefore we were disappointed to find that RAGE levels in vaginal tissues by immunohistochemistry and Western blotting were unchanged in pelvic floor of POP patients, and also were unchanged with aging ( $P > 0.05$ ). We asked if genetic variants in the RAGE gene itself might result in abnormal RAGE functions. The whole gene sequence of RAGE from 24 POP patients and 25 controls was determined. We found there were two potential SNPs: rs55640627, rs184003, but they are both intronic variants with little ability to affect the expression and function of RAGE, so at this point we do not believe that RAGE genetic variants may an important role in POP in our patient population.

Studying POP pathogenesis in experimental animals has been difficult due to the lack of an ideal animal model [40]. This study chose SIS mesh (in contrast to Prolene and blank control) to repair an artificial abdominal wall defect in Sprague Dawley rats. Then the relationship of AGEs and collagen I at the repair site was studied. SIS implants biodegrade three months after operation [41], and poorly-formed new connective tissue at repair sites occurs because of repeated injury from persistent abdominal pressure. This leads to increased bulging of the abdominal wall and more AGEs expression locally. In this study, abdominal wall bulging was greater in group B (SIS mesh repair) than in groups A and C. At the same time, the expression of AGEs in group B was also higher than that of group A and C. Furthermore, the collagen I content in group B was the lowest of the three groups. These results demonstrate that the expression of AGEs and collagen I were inversely related, hinting that AGEs might play a causal role to decrease collagen I levels. One alternative explanation is that the bugle occurred in group B mainly because of SIS biodegradation, not because of AGEs. Whether AGEs can directly impact collagen metabolism through the receptor of AGEs is currently unknown and must be the subject of future research.

Besides the change of the content and gene of RAGE, the function of RAGE depends on other factors, such as the number and functional status of tissue resident fibroblasts, all of which need to be studied further in POP tissue. Finally, POP development in humans takes place over a very long time. Therefore, the changes of collagen I, AGEs, and RAGE observed only described the final result of disease evolution. Prospective studies focusing on the role of the AGEs-RAGE pathway in POP disease processes and mechanisms need to be performed.

In summary, this study has demonstrated that the AGEs-RAGE pathway might play important roles in the pathophysiology of POP through the expression of AGEs, but it is not clear whether the change of AGEs is the reason or the result of POP. Further studies are required to elucidate mechanisms of the AGEs-RAGE pathway in POP, to determine the relative importance of this pathway to overall POP disease progression.

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## Disclosure of conflict of interest

None.

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## AGEs and RAGE express in POP

**Table S1.** Procedure of PCR

procedure	temperature	time
initial denaturation	95 °C	5 min
denaturation	95 °C	30 s
annealing	52-63 °C	30 s
extention	72 °C	60 S
final extention	72 °C	10 min
cycle	35 °C	