ARTICLE

Matrix Metalloproteinase-1 Expression in Women With and Without Pelvic Organ Prolapse: A Systematic Review and Meta-analysis

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This meta-analysis was conducted to estimate the association between matrix metalloproteinase-1 (MMP-1) expression and pelvic organ prolapse (POP) in women. Relevant studies published before 6 December 2015 were identified by searching PubMed, Ovid, EBSCO, and EMBASE. A total number of five case–control studies, including 182 POP cases and 192 controls, were identified. The results indicated that women without POP had a lower MMP-1 level of expression compared with women with POP (odds ratio = 0.54, 95% confidence interval: 0.43–0.67, P = 0.000). After stratification by biopsy site, ethnicity, or menopausal status, this finding was also confirmed in the subgroup analysis with no significant changes. Egger's linear regression test revealed a potential publication bias (P = 0.028). The findings of our study indicate that women who suffer from POP have a higher expression level of MMP-1 than women without POP.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ Matrix metalloproteinase-1 has important functions in collagen disassembly. Therefore, its enhanced activity may explain the reason for the reduced collagen content in the pelvic connective tissues, which eventually causes POP. However, the present research results regarding this issue are not completely consistent.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ This meta-analysis was conducted to estimate the association between matrix metalloproteinase-1 expression and pelvic organ prolapse in women by pooling the results of relevant studies.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE?
✔ Women who suffer from pelvic organ prolapse have a higher expression level of matrix metalloproteinase-1 compared with women without pelvic organ prolapse.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
✔ Data from our study provide new insight into the molecular mechanisms underlying the development of pelvic organ prolapse in women.

Pelvic organ prolapse (POP) affects ~50% of parous females and has a lifetime prevalence of 30–50%, which decreases the quality of life in aging women and is one of the most common reasons for gynecological surgery.[1–4] Previous studies have estimated that the lifetime risk is 11.1% for a woman undergoing at least one operation for pelvic organ prolapse and urinary incontinence, with a 10-year reoperation rate of 17%.[5,6] Reportedly, the genesis of POP can be caused by abnormalities of the connective tissues of the pelvic support system.[7] However, until now, the epidemiologic and pathophysiologic risk factors for POP have not been completely understood. Admittedly, numerous predisposing conditions may lead to abnormalities in the connective tissues, including lifestyle factors, such as heavy lifting, smoking, and adverse working environment; furthermore, some chronic medical conditions, such as obesity, anemia, estrogen deficiency, malnutrition, pulmonary disease, and constipation have been found to also contribute to the occurrence of this disorder.[8–10] Hence, to elucidate the underlying causes, the impact of gene polymorphisms and genetically predisposed susceptibility on the occurrence of POP have also been widely studied.[11–13]

Structurally, the female pelvic organs are supported by pelvic muscles, bony pelvis, and ligaments. The ligaments consist of connective tissues that contain matrix metalloproteinases (MMPs),[14] calcium-dependent zinc-containing endopeptidases,[15] regulated by tissue inhibitors of metalloproteinases (TIMPs), and involved in the breakdown

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of collagen. Pursuing further research in this direction, some authors suggested that reduced collagen content or increased collagen degradation may predispose an individual to a prolapse by decreasing the mechanical strength of the ligaments.[16] MMPs can be subdivided by their substrate affinities, and matrix metalloproteinase-1 (MMP-1) is also regarded as a collagenase with important functions in collagen disassembly.[17,18] Therefore, its enhanced activity may explain the reason for the reduced collagen content in the pelvic connective tissues, which eventually causes POP.

Several investigations evaluated the expression of MMP-1 in different parts of the supportive pelvic structures in women with and without POP.[19–21] However, the results are not completely consistent. Thus, to overcome the limitations of the single studies, we conducted this meta-analysis, combining evidence to provide a more comprehensive and precise understanding of this issue by comparing the immunohistochemical expression of MMP-1 in different parts of the supportive pelvic structures in women with and without POP.

METHODS
Data sources
Initially, four databases were electronically searched for studies evaluating the correlation between MMP-1 expression in women with and without POP until 6 December 2015, including PubMed, Ovid, EBSCO, and EMBASE. The searching terms were "MMP" or "matrix metalloproteinase" in combination with "POP" or "pelvic organ prolapse." Only human investigations were included. Moreover, we checked the reference lists of all retrieved studies to ensure the sensitivity of the searching procedure.

Since all analyses were based on previously published studies, no ethical approval and patient consent were required.

Inclusion and exclusion criteria
We performed an initial screening of titles and abstracts, followed by a second screening based on full-text reviews. A study was considered eligible if it met the following criteria: (i) It had the design of a case–control study; (ii) It compared the MMP-1 expression in women with and without POP; (iii) POP stages were classified by the Pelvic Organ Prolapse Quantification (POPQ) system[22]; (iv) MMP-1 immunohistochemical expression was classified into different grades by staining intensity, and for each grade the numbers or percentage of cases and controls were respectively reported.

Exclusion criteria
An investigation was excluded if it met any of the following criteria: (i) Literature sources were published repeatedly; (ii) Data were extracted from reviews or abstracts; (iii) The results were reported only as means ± standard deviation of staining scores.

Data extraction and quality assessment
Two reviewers independently searched and selected the literature, and then extracted relevant data according to a data extraction form. Disagreements were solved by discussion until consensus was reached. We extracted the following information: the first author's name, year of publication, country of origin of the subjects, ethnicity of the study population, age and menopausal status of cases and controls, staining method, source of control, sample size, and the number of cases and controls in each staining level.

Instead of establishing classified scores, we examined the quality of individual studies by providing the key components of study designs, including characteristics of participants, study information, and number of cases and controls in different staining grades.[23]

Statistical analysis
Data were analyzed using STATA 11.0 (Stata Statistical Software, College Station, TX, www.stata.com) software. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to evaluate MMP-1 expression in women with and without POP. Further, heterogeneity among included studies was checked by chi-square-based Q test and I^2 test. If the data showed significant heterogeneity ($P < 0.10$, $I^2 > 50\%$), we resorted to the DerSimonian–Laird random-effect model; otherwise, the Mantel–Haenszel fixed-effect model was used. Because the characteristics of the participants and biopsy sites were not consistent among the studies, we further conducted a subgroup analysis according to these factors to explore the potential differences between the subgroups. Moreover, to investigate the influence of every single study on the overall risk estimation, sensitivity analysis was performed by omitting each study in turn. Publication bias was quantitatively assessed by Egger's linear regression test and visual inspection of Begg's funnel plots.

RESULTS
Literature search
We initially identified 165 potentially eligible studies, but most were excluded after the screening of titles and abstracts because they were duplications or irrelevant to MMP-1 expression in women with POP. After assessing the full texts of nine potentially relevant articles, we determined five eligible case–control studies. Of the four excluded studies, one was irrelevant to MMP-1 expression,[24] one was excluded because it was a repeatedly published literature source,[20] and two investigations were excluded because the numbers or percentages of cases and controls in the different staining groups were not reported.[25,26] Although two of the studies were by the same first author and conducted in the same country, the included participants were different. Thus, we included both of them.[21,27] Consequently, five examinations were included in our meta-analysis,[19,21,27–29] which contained 182 POP cases and 192 controls. A flow chart of the data collection process is presented in **Figure 1**.

Study characteristics
The basic characteristics of the included studies are presented in **Table 1**, containing the name of the first author, year of publication, country of origin, ethnicity of the study population, age and menopausal status of cases and controls, staining method, source of control, sample size, and number of cases and controls in each staining level. Out of these investigations, three were conducted in Croatia,[21,27,29] one in Israel,[28] and one in Turkey.[19] The sample sizes of the cases with POP ranged from 20 to 46, and those of the controls
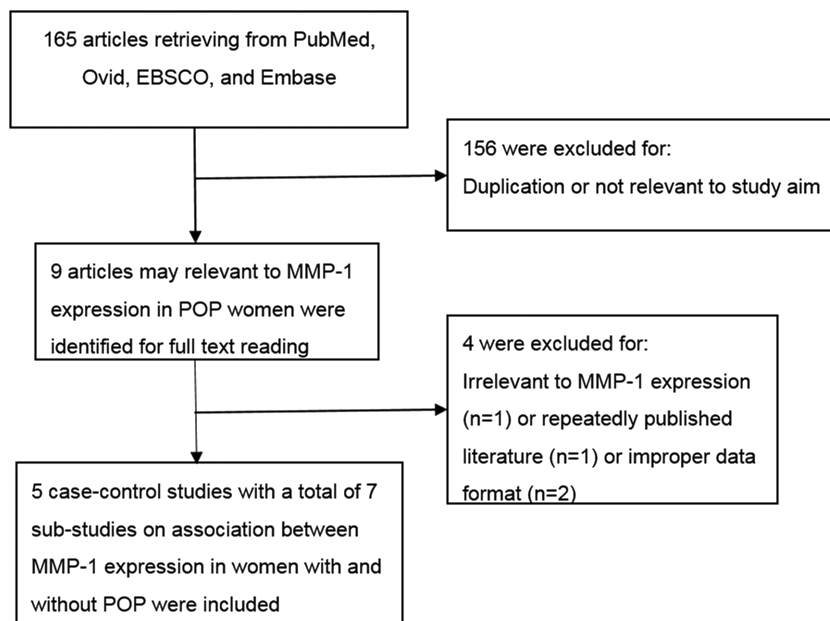
Figure 1 Flow chart of data collection.

were from 20 to 49. In four of the studies,[19–21,27] cases and controls were all postmenopausal, and in one investigation[28] 85% of the POP cases and 20% of the controls were postmenopausal. The mean age ranged from 55 to 61.4 years for the cases with POP and from 49.7 to 59 for the controls. The controls in all included studies were from hospitals, and all biopsies were stained by the immunohistochemical method.

Main analysis
In all studies, the staining level grade 1 was classified as weak expression, grade 2 as moderate expression, and grades 3 and 4 as strong expression. As illustrated in **Figure 2**, four of the seven substudies showed a statistical significance, and the ORs of the association varied from 0.44 to 0.65 among the substudies. Overall, the combined OR revealed that women without POP had a lower MMP-1 level of expression compared with women with POP (OR = 0.54, 95% CI: 0.43–0.67, $P = 0.000$). Nonetheless, substantial heterogeneity was not observed ($I^2 = 0.0\%$, $P = 0.920$). Model codes and data set information are presented in the **Supplementary Data**.

Subgroup and sensitivity analysis
The results of subgroup analyses are displayed in **Table 2**. Generally speaking, no significant changes were detected after stratification by biopsy site (uterosacral ligaments, vaginal mucosa, and round ligaments), ethnicity, or menopausal status. Further, to investigate the influence of a single study on the overall estimation, sensitivity analysis was conducted by omitting each study sequentially. The results indicated that no single study could alter the overall combined OR materially. The combined ORs and corresponding 95% CIs were similar to the results of the main analysis, with a narrow range from 0.52 to 0.55 (**Figure 3**).

Publication bias
Substantial asymmetry was established by visual inspection of Begg's funnel plot (**Figure 4**). The Begg's rank correlation test indicated no evidence of publication bias among the studies included ($P = 0.072$). However, evidence of publication bias ($P = 0.028$) was detected by the Egger's linear regression test.

DISCUSSION

The etiology of POP is possibly multifactorial. Previous studies have suggested that factors, including obstetric history, lifestyle, some unmodifiable components, comorbidities, social conditions, pelvic floor specificities, and surgical interventions may influence the etiology of POP.[30] For example, longer vaginal delivery time has been widely investigated as a risk factor for POP.[31,32]

It has been estimated that for each decade of life, there is a 10% increase of the risk of POP, and the incidence of primary operation for POP is 0.1% in 20–29 years, which is elevated to 11.1% in 70–79 years. Moreover, the uterosacral ligament resilience is significantly decreased in menopausal and older women.[5,33–35] Although ~50% of parous women suffer from POP, knowledge of its pathophysiology is still limited. In addition to the above-mentioned risk factors, recent evidence suggests that changes in the composition of the connective tissue may participate in its pathophysiology.[31,36] Collagen is the most abundant constituent of the connective tissue in the supportive pelvic structures, but the quantity is difficult to measure due to its triple helix structure.[20]

MMP-1 cleaves type 1 interstitial collagen, which may play a crucial role in the decline in the strength of the fibrous collagen and the subsequent tissue integrity loss in POP.[21,37] Previous studies have revealed the difference between the expression of MMP-1 in women with and without POP and

Table 1 Characteristics of the datasets included in meta-analysis on MMP-1 expression in women with and without POP

First author	Year	Country	Ethnicity	Age and menopausal status of cases	Age and menopausal status of control	Source of control	Staining method	Number of cases and distribution in different staining grades	Number of controls and distribution in different staining grades
Dvire	2011	Israel	Asian	Mean age: 61.4y (range: 40–75), 85% of the cases were postmenopausal	Mean age: 49.7y (range: 39–75), 20% of the controls were postmenopausal.	Hospital	IHC	$N = 20$ cases Uterosacral ligaments: 11-grade 1, 2-grade 2, 5-grade 3, 2-grade 4 Vaginal mucosa: 10-grade 1, 2-grade 2, 5-grade 3, 3-grade4	$N = 20$ controls Uterosacral ligaments: 17-grade 1, 3-grade 2 Vaginal mucosa: 18-grade 1, 2-grade 2
Strinic	2009	Croatia	Caucasian	Age: 57.6±3.5, all postmenopausal	Age: 56.9±3.8, all postmenopausal	Hospital	IHC	$N = 40$ cases Uterosacral ligaments: 9-grade 1, 17-grade 2, 14-grade 3	$N = 40$ controls Uterosacral ligaments: 20-grade 1, 13-grade 2, 7-grade 3
Strinic	2010	Croatia	Caucasian	Age: 58.7±3.2, all postmenopausal	Age: 57.5±3.7, all postmenopausal	Hospital	IHC	$N = 34$ cases Uterosacral ligaments: 9-grade 1, 13-grade 2, 12-grade 3	$N = 34$ controls Uterosacral ligaments: 18-grade 1, 12-grade 2, 4-grade 3
Usta	2014	Turkey	Asian and Caucasian	Mean age: 55y (range: 45–73), all postmenopausal	Mean age: 54y (range: 45–69), all postmenopausal	Hospital	IHC	$N = 42$ cases Uterosacral ligaments: 9-grade 1, 17-grade 2, 16-grade 3 Round ligament: 8-grade 1, 18-grade 2, 16-grade 3	$N = 49$ controls Uterosacral ligaments: 19-grade 1, 24-grade 2, 6-grade 3 Round ligament: 21-grade 1, 19-grade 2, 9-grade 3
Vulic	2010	Croatia	Caucasian	Mean age: 60 (range 50–70)	Mean age: 59 (range 51–69,)	Hospital	IHC	$N = 46$ cases Uterosacral ligaments: 14-grade 1, 17-grade 2, 15-grade 3	$N = 49$ controls Uterosacral ligaments: 24-grade 1, 19-grade 2, 6-grade 3

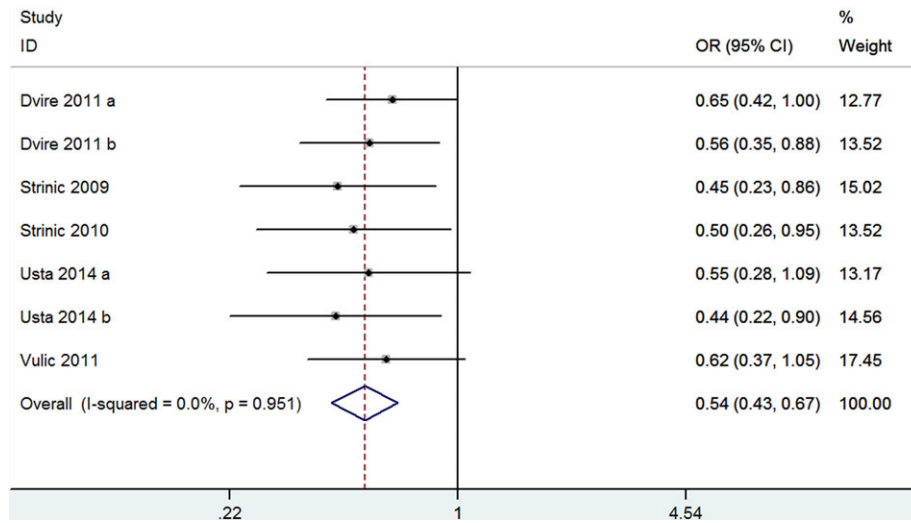MMP, matrix metalloproteinase; POP, pelvic organ prolapse, IHC, immunohistochemical.

Figure 2 Meta-analysis of MMP-1 expression in women with and without POP.

Table 2 Subgroup analysis of MMP-1 expression in women with and without POP

Group	No. of substudies	OR (95%CI)	*P*-heterogeneity	I^2 (%)
Total	7	0.54 (0.43–0.67)	0.951	0.0
Menopausal status				
Postmenopausal	5	0.52 (0.39–0.69)	0.928	0.0
Both pre- and postmenopause	2	0.60 (0.44–0.82)	0.638	0.0
Ethnicity				
Asian	2	0.60 (0.44–0.82)	0.638	0.0
Caucasian	3	0.53 (0.38–0.75)	0.730	0.0
Both Asian and Caucasian	2	0.50 (0.31–0.81)	0.662	0.0
Biopsy site				
Uterosacral ligament	5	0.56 (0.43–0.72)	0.886	0.0
Vaginal mucosa	1	0.56 (0.35–0.88)		
Round ligament	1	0.44 (0.22–0.90)		

tried to use it as an explanation for susceptibility to the condition. However, the results are not all the same. The results of some investigations indicate that there is a significant difference between the expression levels of MMP-1 in women suffering from POP compared with those in women lacking the disorder, while others suggest no association. Reasons for the discrepancies mentioned above have remained unclear until now. The possible explanations include the presence of differences in age, menopausal status, some lifestyle and environmental factors, and the availability of experimental conditions that could interact with MMP-1 expression.

In our research, to avoid the limitations of individual studies, we conducted this meta-analysis to combine the findings of multiple investigations comparing the differences between the expression levels of MMP-1 in women with and without POP. There are several strengths of this research. First of all, the five studies included in our meta-analysis were of a high quality. Most of the cases and controls were matched for age, body mass index (BMI), parity, and menopausal status, which to some extent, reduced the possibility of bias due to the influence of sex hormones on MMP-1 activities.[38,39] It is noteworthy that all the studies clearly stated the sample sizes of participants, characteristics of cases and controls, as well as the criteria for inclusion of subjects and the staining methods. Moreover, it was shown that no heterogeneity was present among the included studies. More specifically, two of the seven substudies providing data on the differences between the expression levels of MMP-1 in women with and without POP were based on Asian subjects and, of them,[28] one substudy provided statistical significance. In addition, three investigations concerned the relationship between MMP-1 expression and POP occurrence in Caucasians,[20,21,27] and two of them suggested a significant association. One of another two substudies, enrolling both Asian and Caucasian subjects, that were conducted in Turkey provided statistical significance.[19] As a result, after combining the findings of the single studies, our meta-analysis showed that women without POP had a lower MMP-1 level of expression compared with women with POP (OR = 0.54, 95% CI: 0.43–0.67). This result was stable and statistically robust; it did not change after stratification by menopausal status, ethnicity, or biopsy site, although the findings of a previous study suggested that menopause is associated with the development of POP.[19] Moreover, when we removed each study sequentially in the sensitivity analysis, no single study could alter the overall result.
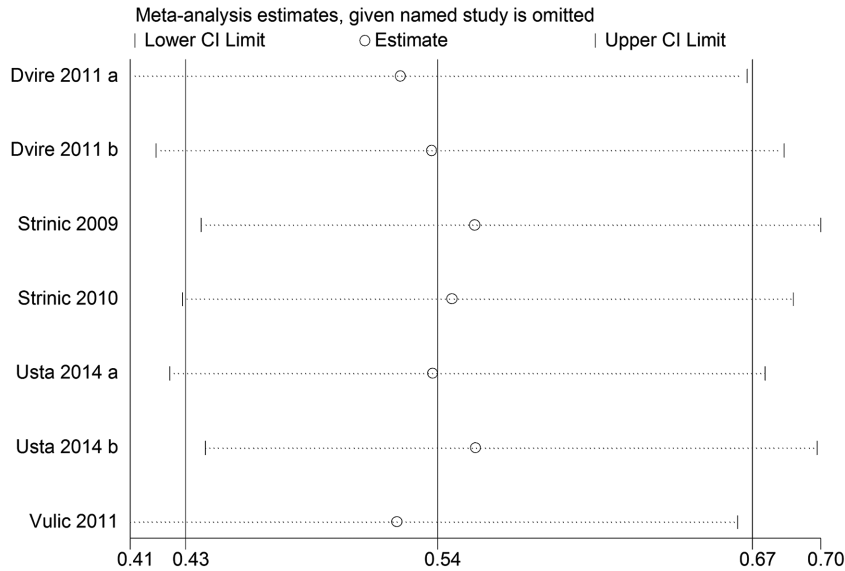
Meta-analysis estimates, given named study is omitted

| Lower CI Limit   ○ Estimate | Upper CI Limit

Dvire 2011 a

Dvire 2011 b

Strinic 2009

Strinic 2010

Usta 2014 a

Usta 2014 b

Vulic 2011

0.41 0.43   0.54   0.67 0.70

Figure 3 Sensitivity analysis by omitting each study sequentially.

Begg's funnel plot with pseudo 95% confidence limits
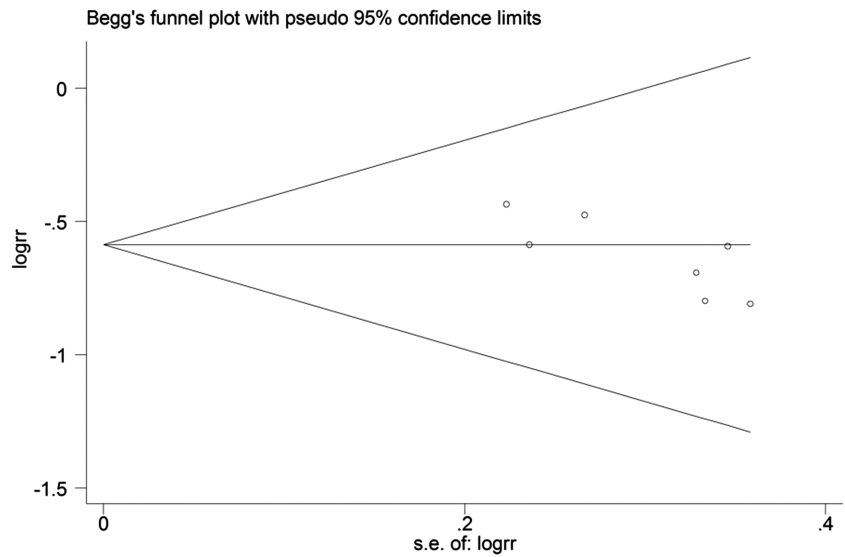
Figure 4 Begg's funnel plot testing potential publication bias among the included studies.

However, it is important to note the several limitations of this study, which might have affected the results. First, only published English reports from four databases were included in our meta-analysis. Relevant studies in other languages, databases, and unpublished papers might have been omitted. Second, in this meta-analysis, most original investigations were based on Asian and Caucasian participants. Thus, additional research in other ethnic categories should be conducted to generalize the results. Third, publication bias was found among the included studies, which might have been caused by our relatively strict inclusion criteria, and this might have influenced the findings. Considering that meta-analysis is a kind of retrospective research and may easily be affected by methodological deficiencies of the included studies, we developed a detailed protocol before conducting this analysis to ensure the quality of our meta-analysis.

The results of our study suggest that the level of MMP-1 expression significantly increases in the supportive pelvic structures of women with POP compared with women without POP. This finding confirms the pathophysiological hypothesis that MMP-1 takes part in the degradation process of collagen in the supporting pelvic structures, which leads to weakening of the connective tissues and eventually causes POP. However, it is also possible that the increased MMP-1 expression in the supporting pelvic structures is induced by the biomechanical changes after POP. Therefore, further work is needed to confirm this statement. Moreover, because of the complex etiology of POP, further studies may also pay more attention to the lifestyle and environmental factors contributing to the occurrence of the disorder.

Overall, to the best of our knowledge, our research is the first meta-analysis evaluating the differences between the

levels of expression of MMP-1 in women with and without POP. Importantly, our study provided evidence that the MMP-1 expression level in women suffering from POP was higher than that in women without POP. Nevertheless, due to the several limitations of our research, additional well-designed, large case–control studies with more subjects are required to generalize the results.

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Conflict of Interest.   The authors report no conflicts of interests.

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