pelvic organ prolapse (POP) is caused by defects in the pelvic floor support structure, resulting in a downward displacement of the uterus, bladder, and rectum. It is a common disease bothering all ages of women, especially older women, and greatly affects the quality of life. A population-based, cross-sectional study investigating 53,178 women in six provinces of mainland China showed that the prevalence of symptomatic POP was 9.56% (data unpublished). Current evidence suggests that both genetic and environmental factors contribute to POP. The most frequent environmental risk factors include age, body mass index, parity, and chronic increases in intra-abdominal pressure, whereas genetic risk factors include race/ethnicity, family history, Marfan syndromes or Ehlers-Danlos syndromes, and so on. The susceptibility loci of POP at the genetic level, however, remains to be elucidated since no definite conclusions have been reached so far.

The pelvic floor support system is essential in maintaining the normal anatomic position of pelvic organs. Certain risk factors can influence the structure and function of the pelvic floor connective tissues, muscles, and nerves, which can trigger POP. Collagen fibers are the main components of the connective tissue. Changes in the structure, content, and proportion of the collagen can lead to changes in the supporting force of the pelvic floor and control women (n = 48) without prolapse. A target region sequencing approach was used to identify the SNPs in collagen-related genes. The association between SNPs and POP was examined by Fisher exact tests for unadjusted model and logistic regression analysis adjusted for delivery and pregnancy. Although there was no significant association between the SNPs and POP, there was a trend toward significance for COL1A1 SNP (rs2305603), COL4A2 SNP (rs74941798), two COL1A1 SNPs (rs2586488 and rs2249492), and three COL18A1 SNPs (rs1050351, rs56355679, and rs55690336), and POP. We are the first to evaluate the relationship between COL1A1, COL3A1, COL3A2 polymorphisms and POP, besides COL3A1, COL1A1, and COL18A1, which have been reported previously. We found several candidate SNPs that were significantly associated with prolapse in Chinese women. Our results provide new evidence for further investigation of the involvement of these potential genes in the etiology of POP.

Key Words: Candidate gene – Collagen – Pelvic organ prolapse – Single-nucleotide polymorphisms.

Original Study

Genetic polymorphisms in collagen-related genes are associated with pelvic organ prolapse

Lei Li, PhD, Zhijing Sun, MD, Juan Chen, MD, PhD, Ye Zhang, PhD, Honghui Shi, MD, and Lan Zhu, MD

Abstract

Objectives: Pelvic organ prolapse (POP) is a common health issue that has a profound negative influence on women’s quality of life. Genetic susceptibility to POP has been increasingly investigated. In this study, we assessed the single-nucleotide polymorphisms (SNPs) of six collagen-related genes (COL1A1, COL1A1, COL4A2, COL3A1, COL1A1, and COL18A1) and the genetic association with POP in Chinese women.

Methods: We performed a candidate gene association study of case women (n = 48) with stage III and IV prolapse and control women (n = 48) without prolapse. A target region sequencing approach was used to identify the SNPs in collagen-related genes. The association between SNPs and POP was examined by Fisher exact tests for unadjusted model and logistic regression analysis adjusted for delivery and pregnancy.

Results: There was a significant association between COL1A1 SNPs (rs4870723, rs2305600, and rs2305598; P = 0.013, 0.019, and 0.028, respectively), a COL5A1 SNP (rs3827852; P = 0.016), and COL4A2 SNPs (rs76425569, rs388222, and rs2281968; P = 0.049 for the three, and rs445348, P = 0.040) and POP, respectively. Although there was no significant association between the COL3A1 SNP and POP, there was a trend toward significance for COL1A1 SNP (rs2305603), COL4A2 SNP (rs74941798), two COL1A1 SNPs (rs2586488 and rs2249492) and three COL18A1 SNPs (rs1050351, rs56355679, and rs55690336), and POP.

Conclusion: We are the first to evaluate the relationship between COL1A1, COL3A1, and COL4A2 polymorphisms and POP, besides COL3A1, COL1A1, and COL18A1, which have been reported previously. We found several candidate SNPs that were significantly associated with prolapse in Chinese women. Our results provide new evidence for further investigation of the involvement of these potential genes in the etiology of POP.

Key Words: Candidate gene – Collagen – Pelvic organ prolapse – Single-nucleotide polymorphisms.
This study, we aim to conduct an analysis of genes that may be associated with POP in Chinese women. In performed this study to verify the hypothesis that these allele frequencies are associated with POP among Chinese women. We also examined for the first time whether any are associated with POP. We also examined for the first time whether any are associated with POP. Given the important role of the collagen family genes, it is considered that POP is a common complex disease caused by genetic variants (SNPs) that are likely to occur in multiple susceptibility markers. In addition to COL3A1, COL1A1, and COL18A1, our previous work also screened COL14A1, COL5A1, and COL4A2 as possible candidate genes. Given the important role of the collagen family genes in pelvic floor connective tissues and the previous controversial findings on COL3A1, COL1A1, and COL18A1, we performed this study to verify the hypothesis that these genes may be associated with POP in Chinese women. In this study, we aim to conduct an analysis of COL3A1, COL1A1, and COL18A1 genes using a target region sequencing approach with the Illumina sequencing platform in a case-control association study of Chinese women, and to determine whether any polymorphisms of these genes are associated with POP. We also examined for the first time whether any COL14A1, COL5A1, and COL4A2 gene allele frequencies are associated with POP among Chinese women.

Study population

This was part of a case-control association study including 48 cases and 48 controls conducted at the Peking Union Medical College Hospital, Beijing, China, from October 2016 to May 2017. This study was approved by the Ethics Committee of the Peking Union Medical College Hospital. We previously recruited women, including participants diagnosed with stage III and stage IV prolapse and control participants with no prolapse (stage 0) and no prior history of prolapse surgery. All cases and controls were individuals of Chinese ancestry.

POP stages were defined by Pelvic Organ Prolapse Quantification (POP-Q) system as previously described by Bump et al. It is the most reliable grading system and widely applied in the world. Briefly, stage 0 (control women) is defined as no prolapse with each point to be at normal position. Stage I is defined when the most distal part of prolapse is more than 1 cm above the hymen. Stage II is defined when the most distal part of prolapse is less than or equal to +1 cm at the proximal end or distal end of the hymen. Stage III is identified when the most distal part of prolapse is more than +1 cm but less than +(+ total vaginal length − 2) cm distal to the hymen, and stage IV is defined when the most distal part of prolapse is bulging more than or equal to +(+ total vaginal length − 2) cm. All the measurements were taken by two trained clinical doctors.

We excluded women with known connective tissue or collagen diseases, such as Marfan syndromes or Ehlers-Danlos syndromes, gynecological malignancies, and neurological conditions, such as sclerosis or stroke. Demographic data were summarized as described in our previous study in which we matched cases and control groups by age and body mass index, and the majority of participants were postmenopausal women. Women with POP had approximately one or more deliveries and pregnancies compared to the control group.

Genotyping and SNP selection

DNA was isolated from peripheral venous whole-blood samples. Sequencing was efficiently performed with 1 to 2 μg DNA using the Agilent Liquid Capture System (Agilent SureSelect Custom Kit; Agilent Technologies, Palo Alto, CA). Paired-end sequencing of 2 × 150 bp reads was performed at Novogene (Novogene Co., Ltd., Beijing, China) using an Illumina HiSeq 4000 platform to provide a mean coverage of more than 200× on the target regions of every sample. Thus, the capture system enabled a comprehensive evaluation of the six genes.

Based on the reference genome (UCSC hg19), the valid sequencing data were mapped by Burrows-Wheeler Aligner software to obtain the original mapping files in a binary alignment/map (BAM) format. We used Samtools and Picard (http://broadinstitute.github.io/picard) to sort the files and note duplicates. Samtools mpileup and bcftools were applied to the marked BAM files to call variants and identify SNPs, insertions, and deletions (indels). CoNIFER was used
to identify genic copy number variants. ANNOVAR was used to annotate the variant position, variant type, conservative prediction, and other information.

We filtered the variants with a minor allele frequency greater than 5% in the 1,000 genome database (1000 Genomes Project Consortium). Single-nucleotide variants were then entered into PolyPhen-2, SIFT, MutationTaster, and CADD software for functional prediction.

### Statistical analysis

To analyze the associations of SNPs and POP, we performed Fisher exact tests for unadjusted model and logistic regression models for adjusted model using PLINK. For multivariable models in the adjusted model, we adjusted for pregnancy and delivery that were not matched between POP and control women. We presented OR and 95% CI for the altered allele, suggesting a protective effect when OR is less than 1 and a risk effect when OR is higher than 1.

### RESULTS

In this case-control study, we sequenced six genes encoding the chains consisting of different types of collagen reported in previous work using an Illumina sequencing platform. We have genotyped a total of 110 SNPs in these genes (SDC, http://links.lww.com/MENO/A497). Among these, 13 SNPs with P values less than 0.1 were considered to be significant or borderline significant in unadjusted model and 10 SNPs with P values less than 0.1 in adjusted model with adjustment for delivery and pregnancy. These SNPs were further analyzed in this study (Table 1).

In the analysis of COL14A1, there was a significant association between the COL14A1 SNP rs4870723 and POP in both unadjusted model and adjusted model (OR, 0.46; 95% CI, 0.25-0.86; P = 0.013 and OR, 0.48; 95% CI, 0.24-0.93; P = 0.030, respectively), and a significant association between rs2305600 and POP in both models (OR, 0.48; 95% CI, 0.26-0.89; P = 0.019 and OR, 0.51; 95% CI, 0.27-0.99; P = 0.045). rs2305598 was significantly associated with POP in the unadjusted model (OR, 0.50; 95% CI, 0.27-0.93; P = 0.028). There was also a trend toward significance for the SNP rs2305603 in the unadjusted model (OR, 0.54; 95% CI, 0.29-1.01; P = 0.055) (Table 1). Figure 1A shows a schematic of the gene structure and indicates exons and introns of the four SNP sites. rs4870723, rs2305600, and rs2305598 were in good LD with each other (r² > 0.9) (Fig. 1D). rs4870723 was a missense SNP that resulted in a substitution of asparagines for histidine located in the fibronectin type 3 domain (FN3) in COL14A1 (Fig. 2). rs2305600, rs2305598, and rs2305603 were synonymous SNPs that did not cause amino acid changes.

In the analysis of COL5A1, there was a significant association between the SNP rs3827852, which is an intronic SNP, and POP in the unadjusted model (OR, 0.40; 95% CI, 0.18-0.86; P = 0.016). Regarding the gene COL1A1, there was a trend toward significance for the SNP rs2586488 and POP in the unadjusted model (OR, 1.73; 95% CI, 0.94-3.20; P = 0.082), and SNP rs2249492 and POP in the adjusted model (OR, 1.70; 95% CI, 0.93-3.10; P = 0.082), which were both intronic SNPs (Table 1).

### TABLE 1. Allele frequencies for genes encoding chains of multiple types of collagen

| SNP       | Allele (frequency) | Unadjusted | Adjusted* | cytoBand | Function | Exonic function | AA change |
|-----------|--------------------|------------|-----------|----------|----------|----------------|-----------|
| COL14A1   |                    |            |           |          |          |                |           |
| rs4870723 | C (0.48)           | 0.013      | 0.030     | q2412    | Exonic   | Missense       | N563H     |
| rs2305600 | C (0.49)           | 0.019      | 0.030     | q2412    | Exonic   | Synonymous     | S07S      |
| rs2305598 | C (0.49)           | 0.028      | 0.030     | q2412    | Exonic   | Synonymous     | G204G     |
| rs2305603 | C (0.32)           | 0.055      | 0.055     | L794L    | Intron   | —              | —         |
| COL1A1    |                    |            |           |          |          |                |           |
| rs3827852 | G (0.67)           | 0.016      | 0.089     | q343     | Intron   | —              | —         |
| rs445348  | G (0.66)           | 0.237      | 0.200     | L941     | Intron   | —              | —         |
| rs64225569| A (0.33)           | 0.049      | 0.074     | P356P    | Exonic   | Synonymous     | —         |
| rs388222  | T (0.67)           | 0.049      | 0.070     | L941     | Intron   | —              | —         |
| rs2281968 | A (0.33)           | 0.049      | 0.070     | L941     | Intron   | —              | —         |
| rs74941798| T (0.32)           | 0.070      | 0.089     | L941     | Intron   | —              | —         |
| COL5A1    |                    |            |           |          |          |                |           |
| rs2586488 | G (0.61)           | 0.082      | 0.104     | L941     | Intron   | —              | —         |
| rs2249492 | T (0.66)           | 0.111      | 0.102     | L941     | Intron   | —              | —         |
| COL1A1    |                    |            |           |          |          |                |           |
| rs1050351 | A (0.40)           | 0.082      | 0.106     | A1326A   | Exonic   | Synonymous     | —         |
| rs63535679| C (0.40)           | 0.082      | 0.106     | L941     | Intron   | —              | —         |
| rs5569036 | A (0.40)           | 0.082      | 0.106     | L941     | Intron   | —              | —         |

Significant data (P < 0.05) are indicated in bold. A, Alanine; AA, amino acid; CI, confidence interval; G, glycine; H, histidine; I, isoleucine; L, leucine; N, asparagine; OR, odds ratio; P, proline; S, serine; SNP, single-nucleotide polymorphism.

*Adjusted by pregnancy and parity.
In the analysis of \(\text{COL4A2}\), there was a significant association between the \(\text{COL4A2}\) SNPs rs76425569 (OR, 2.02; 95% CI, 1.00-4.15; \(P = 0.049\)), rs388222 (OR, 0.50; 95% CI, 0.24-1.00; \(P = 0.049\)), and rs2281968 (OR, 2.02; 95% CI, 1.00-4.15; \(P = 0.049\)) and POP in unadjusted model, and rs445348 (OR, 2.15; 95% CI, 1.03-4.47; \(P = 0.040\)) and POP in adjusted model (Table 1). rs76425569 and rs445348 were synonymous SNPs that did not result in amino acid changes. rs388222 and rs2281968 were intronic SNPs. There was also a trend toward significance for the SNP rs74941798 in both unadjusted and adjusted models.

In the analysis of \(\text{COL4A2}\), there was a significant association between the \(\text{COL4A2}\) SNPs rs76425569 (OR, 2.02; 95% CI, 1.00-4.15; \(P = 0.049\)), rs388222 (OR, 0.50; 95% CI, 0.24-1.00; \(P = 0.049\)), and rs2281968 (OR, 2.02; 95% CI, 1.00-4.15; \(P = 0.049\)) and POP in unadjusted model, and rs445348 (OR, 2.15; 95% CI, 1.03-4.47; \(P = 0.040\)) and POP in adjusted model (Table 1). rs76425569 and rs445348 were synonymous SNPs that did not result in amino acid changes. rs388222 and rs2281968 were intronic SNPs. There was also a trend toward significance for the SNP rs74941798 in both unadjusted and adjusted models.

**FIG. 1.** \(\text{COL14A1}, \text{COL4A2},\) and \(\text{COL18A1}\) gene structures, indication of SNPs and the linkage disequilibrium (LD) between SNPs. (A), (B), and (C) are \(\text{COL14A1}, \text{COL4A2},\) and \(\text{COL18A1}\) gene structures, respectively, with exons presented in vertical lines. Candidate SNPs are marked by arrows and presented in different colors: exons (red) and introns (blue). (D), (E), and (F) are the LD graphics for \(\text{COL14A1}, \text{COL4A2},\) and \(\text{COL18A1}\), respectively, with \(r^2\) values in each box. The closer the value is to 1.0, the higher the correlation between two SNPs. Boxes without values indicate perfect LD (\(r^2 = 1\)).

**FIG. 2.** Schematic diagram indicating amino acid changes in the \(\text{COL14A1}\) transcript NM_021110. Protein domains for NM_021110 in \(\text{COL14A1}\) are shown in color boxes: collagen (orange), FN3 (fibronectin type 3 domain, blue) and vWA (collagen_alphaI-XII-like, green). The missense SNP rs4870723 is identified with a straight line. SNP, single-nucleotide polymorphism.
model and adjusted model, which is a synonymous SNP (OR, 1.93; 95% CI, 0.95-3.97; \( P = 0.070 \), and OR, 1.78; 95% CI, 0.92-3.46; \( P = 0.089 \)) (Table 1). These SNPs are indicated on the schematic in Figure 1B, and exons and introns are noted. rs76425569 and rs74941798 were in good LD with each other \( (r^2 = 0.97) \), and rs388222 and rs2281968 were in perfect LD with each other \( (r^2 = 1) \) (Fig. 1E).

Although there was no significant association between the \( \text{COL18A1} \) and \( \text{COL3A1} \) SNPs and POP, there was a trend toward significance for the synonymous \( \text{COL18A1} \) SNP rs1050351 (OR, 0.58; 95% CI, 0.31-1.07; \( P = 0.082 \)) and two intronic \( \text{COL18A1} \) SNPs, rs56335679 and rs55690336, and POP in unadjusted model (for both, OR, 0.58; 95% CI, 0.31-1.07; \( P = 0.082 \)) (Table 1). These SNPs are marked in Figure 1C. All three SNPs showed perfect LD with each other \( (r^2 = 1) \) (Fig. 1F).

**DISCUSSION**

It is commonly considered that POP occurrence is attributed to multiple risk factors that influence the pelvic floor support system. Potential candidate genes related to connective tissue and extracellular matrix (ECM) components have attracted special interests, and many researchers have searched for polymorphisms in these genes.21,24 In this case-control analysis of several genes encoding the chains of elastin-like polypeptide (ELP) components, we sequenced \( \text{COL1A1}, \text{COL5A1}, \) and \( \text{COL4A2} \) for the first time and further confirmed the previously studied genes \( \text{COL3A1}, \text{COL1A1}, \) and \( \text{COL18A1} \). As we did not match for delivery and pregnancy, which were key risk factors for POP, in case and control groups, we evaluated the association between SNPs and POP in unadjusted model and the adjusted model with adjustment for delivery and pregnancy. In the two models, we provided new evidence that in addition to \( \text{COL3A1}, \text{COL1A1}, \) and \( \text{COL18A1} \), the genes \( \text{COL14A1}, \text{COL5A1}, \) and \( \text{COL4A2} \) might be potential candidates for nonfamilial POP in Chinese populations.

The main collagen types in pelvic connective tissue are collagens I and III, with very little collagen V.24 Collagen I is well recognized for playing a role in tissue stiffness, whereas collagen III plays a role in tissue elasticity.36 Previous studies have mainly focused on rs1800012 in \( \text{COL1A1} \), and rs1800255 and rs111929073 in \( \text{COL3A1} \). rs1800012 was also identified as the polymorphism of the transcription factor Sp1 in \( \text{COL1A1} \). Five previous studies observed no association between rs1800012 and POP respectively in Israeli, Italian, Korean, Polish, and Brazilian women,16-20 consistent with our results in Chinese women. In addition, we found a trend toward a significant association between two SNPs, rs2586488 and rs2249492, both intronic SNPs, and POP, providing new polymorphism loci for the disease. Several studies also investigated the associations with the missense SNP rs1800255 in \( \text{COL3A1} \). Chen et al11 and Kluivers et al12 showed respectively that rs1800255-AA in the \( \text{COL3A1} \) gene was significantly associated with POP in Taiwanese and Dutch women. In addition, the two studies contributed to the meta-analysis by Ward et al,24 suggesting that the AA genotype of \( \text{COL3A1} \) rs1800255 was significantly associated with POP compared with the GG reference genotype. However, Lince et al13 put forward the opposite conclusion, which was consistent with our result. Regarding the analysis of \( \text{COL3A1} \) rs111929073, Jeon et al14 reported a higher frequency of the G allele in rs111929073 in Korean women. However, Martins et al15 demonstrated no significance in Brazilian women. In our case-control study, we also did not observe any significant associations between \( \text{COL3A1} \) SNPs and POP. The reason for the diverse and controversial results may be partially due to the population frequencies of the genetic variant which may vary between different ethnic groups and may also be partially due to the different inclusion criteria (POP-Q III and IV in our study) and different detection methods (target region sequencing for our study) used in the studies.

\( \text{COL18A1} \), which encodes the alpha chain of type XVIII collagen, and endostatin, which is cleaved from the C-terminal region of collagen XVIII, can act on growth factors and play a role in the structure composition of the basement membranes.37 We selected the \( \text{COL18A1} \) gene based on previous findings from the genome-wide association study by Allen-Brady et al22 that the rs2236479 polymorphism was significantly associated with POP. However, Giri et al38 and Dos Santos et al23 could not repeat the result. In our study, we sequenced \( \text{COL18A1} \) and still did not find a positive association between rs2236479 and POP. We, however, found three additional SNPs, one synonymous SNP rs1050351 and two intronic SNPs rs56335679 and rs55690336, that have borderline significant associations with POP and show that they may have potential protective effects for the disease.

We sequenced three genes, \( \text{COL14A1}, \text{COL5A1}, \) and \( \text{COL4A2} \), for the first time according to our microarray data. Collagen XIV is related to fibril formation and is commonly expressed in connective tissues, such as skin, tendons, and corneas, especially in areas under high mechanical stress.39 This suggests that collagen XIV may influence the mechanical nature of the tissue. Evidence has indicated an effect of collagen XIV in corneal stromal compaction40 and endothelial maturation.41 Mice null for \( \text{COL14A1} \) showed defects in interstitial collagen network construction in the tendons and skin.42 Mutations of \( \text{COL14A1} \) have been reported in patients with punctate palmoplantar keratodera,43 gastric cancer,44 and osteoarthritis.45 Given the important role of collagen XIV in ECM organization, its expression and function in the pelvic floor also need to be clarified. In our study, we found a significant association between the missense SNP rs4870723, and two synonymous SNPs rs2305600 and rs2305598, and POP, and a borderline significant association between the synonymous SNP rs2305603 and POP in Chinese women. Our study suggested that they may exhibit a protective role for POP development. rs4870723 was a missense SNP and produced a replacement of amino acid asparagines to histidine, which might have a benign effect predicted by PolyPhen-2.31 In addition, this amino acid change caused a
Collagen V is a kind of tiny fibrillar collagen that is embedded with collagen I to form collagen fibrils.\textsuperscript{47} Mutations in \textit{COL5A1} exist in approximately half of the patients with typical Ehlers-Danlos syndrome, which presents a phenotype of joint hypermobility and fragility.\textsuperscript{48} Collagen V is important in the organization and function of connective tissue. Our study reported an intronic SNP rs3827852 associated with POP, which should be included in future research.

\textit{COL4A2} is expressed in almost all of the basement membranes in every organ.\textsuperscript{49} Thus, mutations in \textit{COL4A2} contribute to multiple disorders, including hemorrhagic stroke,\textsuperscript{50} cardiac abnormalities, and muscular abnormalities.\textsuperscript{51} No research with respect to \textit{COL4A2} has, however, been conducted on the dysfunction of the pelvic floor. In our study, we found rs445348, rs76425569, and rs74941798, three synonymous SNPs, and rs2281968, an intronic SNP, to be risk factors of POP occurrence, whereas rs388222, an intronic SNP, may be a protective factor for POP development, indicating a potential role of \textit{COL4A2} in prolapse.

In this study, we used target region sequencing to analyze six collagen-related genes. All of the previous studies were, however, limited to only one or two SNPs in a gene. We confirmed the results of the previously reported SNPs in \textit{COL3A1}, \textit{COL1A1}, and \textit{COL18A1}, and we investigated the relationship between \textit{COL14A1}, \textit{COL5A1}, and \textit{COL4A2} polymorphisms and POP for the first time, resulting in several new candidate SNPs. In addition, in this candidate gene study, we investigated nonfamilial Chinese groups, which may be different in genetic background from other previous studies, as genetic variants may differ by different racial/ethnic groups. We recruited women from mainland China strictly limited to POP-Q stages III and IV, which increased the likelihood that the disease-contributing variants could be detected.

However, our study also has some limitations, the most important of which is the relatively small sample size. In our nationwide cross-sectional epidemiological survey in six populous provinces of mainland China, we demonstrated a prevalence of POP-Q stages limited to III and IV to be 2.04% (data unpublished). The strict restriction for the POP-Q stage screening also increased the difficulty for the recruitment of women within a certain period of time. Because we did not have enough power to assess the more moderate effects, more participants are needed to be enrolled in this association study for a more convincing result. Another limitation is that we only considered SNPs (minor allele frequency >5%) in this study by target region sequencing. More genetic variants, including copy number variants, insertions, and deletions, could also be expected in the upcoming study. Finally, we detected only collagen-related genes in Han Chinese. Other candidate genes involved in ECM metabolism could be investigated in large scale in other ethnic/race groups. In summary, our results provide preliminary evidence that these polymorphisms in the genes encoding chains of different collagen types may have an association with POP, and more studies should be performed in the future.

CONCLUSIONS

Collagen is an important ECM component; the alteration of collagen-related genes may be associated with the development of POP. Our study suggested several candidate SNPs that were associated with POP in the \textit{COL3A1}, \textit{COL1A1}, \textit{COL18A1}, \textit{COL14A1}, \textit{COL5A1}, and \textit{COL4A2} genes in a Chinese population, providing crucial information on the pathophysiology of POP.

REFERENCES

1. Barber MD. Pelvic organ prolapse. \textit{BMJ} 2016;354:i3853.
2. Ielovese JC, Maher C, Barber MD. Pelvic organ prolapse. \textit{Lancet} 2007;369:1027-1038.
3. Rortveit G, Brown JS, Thom DH, Van Den Eeden SK, Creasman JM, Subak LL. Symptomatic pelvic organ prolapse: prevalence and risk factors in a population-based, racially diverse cohort. \textit{Obstet Gynecol} 2007;109:1396-1403.
4. Budatha M, Roshanravan S, Zheng Q, et al. Extracellular matrix prostate contribute to progression of pelvic organ prolapse in mice and humans. \textit{J Clin Invest} 2011;121:2048-2059.
5. Moali PA, Shand SH, Zyczynski HM, Gordy SC, Meyn LA. Remodeling of vaginal connective tissue in patients with prolapse. \textit{Obstet Gynecol} 2005;106:953-963.
6. Kim J, Feng J, Jones CAR, et al. Stress-induced plasticity of dynamic collagen networks. \textit{Nat Commun} 2017;8:842.
7. Halper J, Kjer M. Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins. \textit{Adv Exp Med Biol} 2014;802:31-47.
8. Borajani A, Kow N, Harris S, Ridgeway B, Damaer MS. Transcriptional regulation of connective tissue metabolism genes in women with pelvic organ prolapse. \textit{Female Pelvic Med Reconstr Surg} 2017:23:44-52.
9. Dokmeci F, Telsken F, Cetinkaya SE, Ozkan T, Kaplan F, Kose K. Expressions of homeobox, collagen and estrogen genes in women with uterine prolapse. \textit{Eur J Obstet Gynecol Reprod Biol} 2019;233:26-29.
10. Liu C, Yang Q, Fang G, et al. Collagen metabolic disorder induced by oxidative stress in human uterosacral ligament derived fibroblasts: a possible pathophysiological mechanism in pelvic organ prolapse. \textit{Mol Med Rep} 2016;13:3999-3008.
11. Chen HY, Chung YW, Lin WY, Wang JC, Tsai FJ, Tsai CH. Collagen type 3 alpha 1 polymorphism and risk of pelvic organ prolapse. \textit{Int J Gynaecol Obstet} 2008;103:55-58.
12. Kluivers KB, Dijkstra JR, Hendriks JC, Linse SL, Vierhout ME, Van Kempen LC. COL3A1 2209G\textrightarrow{}A is a predictor of pelvic organ prolapse. \textit{Int Urogynecol J Pelvic Floor Dysfunct} 2009;20:1113-1118.
13. Linse SL, Van Kempen LC, Dijkstra JR, Int’lhtout J, Vierhout ME, Kluivers KB. Collagen type III alpha 1 polymorphism (rs1800255, COL3A1 2209 G\textrightarrow{}A) assessed with high-resolution melting analysis is not associated with pelvic organ prolapse in the Dutch population. \textit{Int Urogynecol J} 2014;25:1237-1242.
14. Jeon MJ, Chung SM, Choi JR, Jung HJ, Kim SK, Bai SW. The relationship between COL3A1 exon 31 polymorphism and pelvic organ prolapse. \textit{J Urol} 2009;181:1213-1216.
15. Martins Kde F, De Jamny-DirBella ZI, Da Fonseca AM, et al. Evaluation of demographic, clinical characteristics, and genetic polymorphism as risk factors for pelvic organ prolapse in Brazilian women. \textit{Neuroendocrinology} 2011;93:1325-1328.
16. Feiner B, Fares F, Azam N, Auslender R, David M, Abramov Y. Does COL1A1 SP1-binding site polymorphism predispose women to pelvic organ prolapse? \textit{Int Urogynecol J Pelvic Floor Dysfunct} 2009;20:1061-1065.
17. Cho HJ, Jung HJ, Kim SK, Choi JR, Cho NH, Bai SW. Polymorphism of a COL1A1 gene SP1 binding site in Korean women with pelvic organ prolapse. \textit{Yonsei Med J} 2009;50:564-568.
18. Ferrarri MM, Rossi G, Biandi ML, Vigano P, Dell’utri C, Meschia M. Type I collagen and matrix metalloproteinase 1, 3 and 9 gene
polymorphisms in the predisposition to pelvic organ prolapse. *Arch Gynecol Obstet* 2012;285:1581-1586.

19. Rodrigues AM, Girao MJ, Da Silva ID, Sartori MG, Martins Kde F, Castro Rde A. COL1A1 Sp1-binding site polymorphism as a risk factor for genital prolapse. *Int Urogynecol J Pelvic Floor Dysfunct* 2008;19:1471-1475.

20. Skorupski P, Mostl P, Jankiewicz K, Rechberger T. Polymorphism of the gene encoding alpha-1 chain of collagen type I and a risk of pelvic organ prolapse—a preliminary study. *Ginekol Pol* 2007;78:852-855.

21. Cartwright R, Kirby AC, Tikkinen KA, et al. Systematic review and metaanalysis of genetic association studies of urinary symptoms and prolapse in women. *Am J Obstet Gynecol* 2015;212:e191-e124199.

22. Allen-Brady K, Cannon-Albright L, Farnham JM, et al. Identification of six loci associated with pelvic organ prolapse using genome-wide association analysis. *Obstet Gynecol* 2011;118:1345-1353.

23. Dos Santos RGM, Pechicelli FCA, Battista NC, de Carvalho CV, Bortolini MAT, Castro RA. Collagen XVIII and LOXL-4 polymorphisms in women with and without advanced pelvic organ prolapse. *Int Urogynecol J* 2018;29:893-898.

24. Ward RM, Velez Edwards DR, Edwards T, Giri A, Jerome RN, Wu JM. Genetic epidemiology of pelvic organ prolapse: a systematic review. *Am J Obstet Gynecol* 2014;211:326-335.

25. Li L, Kang J, Zhang Y, et al. LAMC1, LAMA2 and LAMA3 gene polymorphisms and the risk for severe pelvic organ prolapse. *Sci Adv* 2019;7:466-468.

26. Bump RC, Mattiasson A, Bo K, et al. The standardization of terminology for female pelvic organ prolapse and pelvic floor dysfunction. *Am J Obstet Gynecol* 1996;175:10-17.

27. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754-1760.

28. Li H, Handsaker B, Wysoker A, et al. The sequence alignment/map format and SAMtools. *Bioinformatics* 2009;25:2078-2079.

29. Krumm N, Sudmant PH, Ko A, et al. Copy number variation detection and genotyping from exome sequence data. *Genome Res* 2012;22:1525-1532.

30. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164.

31. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet* 2013; Chapter 7:Unit7.20.

32. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003;31:3812-3814.

33. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. Mutation Taster evaluates disease-causing potential of sequence alterations. *Nat Methods* 2010;7:575-576.

34. Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* 2014;46:310-315.

35. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-575.

36. De Landheere L, Munaut C, Nusgens B, et al. Histology of the vaginal wall in women with pelvic organ prolapse: a literature review. *Int Urogynecol J* 2013;24:2011-2020.

37. Seppinen L, Sormunen R, Soini Y, Elamaa H, Heljasaara R, Pihlajaniemi T. Lack of collagen XVIII accelerates cutaneous wound healing, while overexpression of its endostatin domain leads to delayed healing. *Matrix Biol* 2008;27:535-546.

38. Giri A, Wu JM, Ward RM, et al. Genetic determinants of pelvic organ prolapse among African American and Hispanic Women in the Women’s Health Initiative. *PLoS One* 2015;10:e0141647.

39. Ansorge HL, Meng X, Zhang G, et al. Type XIV collagen regulates fibrillogenesis: premature collagen fibril growth and tissue dysfunction in null mice. *J Biol Chem* 2009;284:8427-8438.

40. Gordon MK, Foley JW, Lisenmayer TF, Fitch JM. Temporal expression of types XII and XIV collagen mRNA and protein during avian corneal development. *Dev Dyn* 1996;206:49-58.

41. Hemmavanh C, Koch M, Birks DE, Espana EM. Abnormal corneal endothelial maturation in collagen XII and XIV null mice. *Invest Ophthalmo Vis Sci* 2013;54:3297-3308.

42. Tao G, Levay AK, Peacock JD, et al. Collagen XIV is important for growth and structural integrity of the myocardium. *J Mol Cell Cardiol* 2012;53:626-638.

43. Guo BR, Zhang X, Chen G, et al. Exome sequencing identifies a COL14A1 mutation in a large Chinese pedigree with punctate palmo-plantar keratoderma. *J Med Genet* 2012;49:563-568.

44. Li X, Wu WK, Xing R, et al. Distinct subtypes of gastric cancer defined by molecular characterization include novel mutational signatures with prognostic capability. *Cancer Res* 2016;76:1724-1732.

45. Karlsson C, Dehne T, Lindahl A, et al. Genome-wide expression profiling reveals new candidate genes associated with osteoarthritis. *Osteoarthritis Cartilage* 2010;18:581-592.

46. Garnier J, Gibrat JF, Robson B. GOR method for predicting protein secondary structure from amino acid sequence. *Methods Enzymol* 2005;395:380-390.

47. Kadler KE, Baldock C, Bella J, Boot-Handford RP. Collagens at a glance. *J Cell Sci* 2007;120:1955-1958.

48. Malfait F, Wensing RJ, De Paepe A. Clinical and genetic aspects of Ehlers-Danlos syndrome, classic type. *Genet Med* 2010;12:597-605.

49. Khoshnoodi J, Pedchenko V, Hudson BG. Mammalian collagen IV. *Microsc Res Tech* 2008;71:357-370.

50. Jeanne M, Labelle-Dumais C, Gould DB. COL4A1 and COL4A2 mutations and genotyping from exome sequence data. *Am J Hum Genet* 2012;90:91-101.

51. Kuo DS, Labelle-Dumais C, Gould DB. COL4A1 and COL4A2 mutations and disease: insights into pathogenic mechanisms and potential therapeutic targets. *Hum Mol Genet* 2012;21:R97-R110.