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Short Communication

LAMC1, *LAMA2* and *LAMA3* gene polymorphisms and the risk for severe pelvic organ prolapse

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Pelvic organ prolapse (POP) is the descent of the pelvic organs, including bladder, uterus, vagina and rectum, resulting in pelvic discomfort, urinary and fecal incontinence and sexual dysfunction [1]. The prevalence of symptomatic POP in China is 9.56% according to a cross-sectional study involving 54,000 adult women in six provinces in the mainland of China (unpublished data). The etiology of this disorder is multifactorial, including race, age, body mass index (BMI), parity and menopause [1]. The loss of the integrity of vaginal connective tissue has been demonstrated to weaken the pelvic floor support and promote the development of POP. Studies have suggested genetic polymorphisms of several genes involved in the biosynthesis of collagen and elastin and the metabolism of the extracellular matrix (ECM) [2]. Laminins are ECM glycoproteins of the basement membranes, composed of alpha, beta and gamma subunits [3]. Nikolova et al. [4] initially reported a single-nucleotide polymorphism (SNP) (rs10911193) in *LAMC1* that encodes the laminin gamma-1 chain in a linkage study. Subsequently, Chen et al. [5] assessed 3 *LAMC1* SNPs (rs10911193, rs20563 and rs20558) in nonfamilial Caucasians and African Americans, and Wu et al. [6] conducted a *LAMC1* gene association study in non-Hispanic white women. However, both studies found no association of *LAMC1* SNPs and nonfamilial prolapse. Given these previous findings on *LAMC1*, whether *LAMC1* was a potential candidate gene for severe POP remained unknown. *LAMA2*, encoding laminin alpha-2 chain, has been comprehensively studied in congenital muscular dystrophy (CMD) [3]. *LAMA3*, also encoding a laminin alpha subunit, is related to some epithelial-mesenchymal regulators and the related diseases [7]. Currently, there are no reports about the *LAMA2* and *LAMA3* genes in the etiology of POP.

In this study, we sought to perform an analysis of the *LAMC1*, *LAMA2* and *LAMA3* gene using a paired-end sequencing approach on the Illumina sequencing platform in a case-control association study. A total of 48 Chinese women with severe prolapse and 48 Chinese controls with no prolapse were available from Peking Union Medical College Hospital. The majority of cases (41, 85.42%) were POP stage III. Compared to controls, women with severe prolapse were significantly more likely to have had ≥1 delivery (2.35 ± 1.34 vs 1.58 ± 1.06, $P = 0.005$) and pregnancy (3.60 ± 1.89 vs 2.50 ± 1.50, $P = 0.023$). Cases and controls were matched in age

Table 1
Allele frequencies for *LAMC1*, *LAMA2* and *LAMA3*.

SNP	Allele (Frequency)	*P*	OR (95% CI)	cytoBand
LAMC1				
rs10911194	T (0.61)	0.059	1.80 (0.98–3.34)	1q25.3
rs2296288	C (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs3736888	G (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs2027082	A (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs20563	G (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs2296292	C (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs20557	C (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs20558	C (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs1886501	G (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs2296300	A (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs12095664	A (0.60)	0.082	1.72 (0.94–3.19)	1q25.3
rs20560	C (0.59)	0.111	1.65 (0.90–3.05)	1q25.3
LAMA2				
rs2297738	A (0.26)	0.109	1.90 (0.88–4.19)	6q22.33
rs2297742	C (0.27)	0.166	1.72 (0.82–3.69)	6q22.33
rs2244008	G (0.18)	0.166	0.58 (0.27–1.22)	6q22.33
rs7754560	A (0.14)	0.185	0.56 (0.24–1.27)	6q22.33
LAMA3				
rs9962023	C (0.80)	0.016	2.31 (1.16–4.74)	18q11.2
rs1154226	G (0.14)	0.045	0.45 (0.19–0.99)	18q11.2
rs1268716	A (0.14)	0.066	0.47 (0.20–1.05)	18q11.2
rs1154232	A (0.16)	0.109	0.53 (0.24–1.13)	18q11.2
rs1131521	T (0.16)	0.109	0.53 (0.24–1.13)	18q11.2
rs7232856	G (0.61)	0.110	1.66 (0.90–3.07)	18q11.2

CI, confidence interval.
OR, odds ratio.

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(61.85 ± 10.66 vs 62.73 ± 8.88 years, $P = 0.663$), BMI (25.11 ± 3.83 vs 24.52 ± 3.48 kg/m^2, $P = 0.434$) and the number of post-menopause women (44 vs 39, $P = 0.136$) (Table S1 online). We genotyped a total of 101 SNPs in the three genes: 38 SNPs for *LAMA2*, 37 SNPs for *LAMA3* and 26 SNPs for *LAMC1* (Table S2 online). Among these, 22 SNPs with P <0.2 were further analyzed (Table 1).

In the study of *LAMC1*, there was no significant association between the *LAMC1* SNPs and severe POP. However, there was a trend toward significance for the previously reported missense SNPs rs20563 and rs20558 (for both SNPs, odds ratio [OR], 1.72; 95% confidence interval [CI], 0.94–3.19; $P = 0.082$) (Table 1). There was also a trend toward significance for the following SNPs: rs10911194, a synonymous coding SNP (OR, 1.80; 95% CI, 0.98–3.34; $P = 0.059$); rs2296288, rs2296292 and rs20557, synonymous coding SNPs (for all three, OR, 1.72; 95% CI, 0.94–3.19; $P = 0.082$); and rs3736888, rs2027082, rs1886501, rs2296300 and rs12095664, intronic SNPs (for all, OR, 1.72; 95% CI, 0.94–3.19; $P = 0.082$) (Fig. 1a, Table 1). These assessed SNPs were in good linkage disequilibrium (LD) with each other ($r^2 \approx 0.8$, $r^2 = 0.95$) (Fig. 1b). However, the SNP rs10911193, which was reported in previous studies, was not identified in our study. In addition, for the two missense SNPs rs20563 and rs20558, there was a substitution of isoleucine to valine and leucine to proline, respectively (Table S2 online), which were altered in the fourth and eighth EGF-like domains respectively of the laminin gamma 1 chain (Fig. S1 online). The EGF-like domains mediate the polymerization of laminin network associated with cells when the basement membrane is assembling [8]. Mutations at the two sites may lead to a decrease of the random coil structure of the domain secondary structure; especially the proline in place of leucine would cause a corner in the peptide chain [9], which in turn might influence the function of the protein.

Although there was also no significant association between the *LAMA2* SNPs and severe POP, there was a trend toward significance for a synonymous SNP rs2297738 (OR, 1.90; 95% CI, 0.88–4.19;

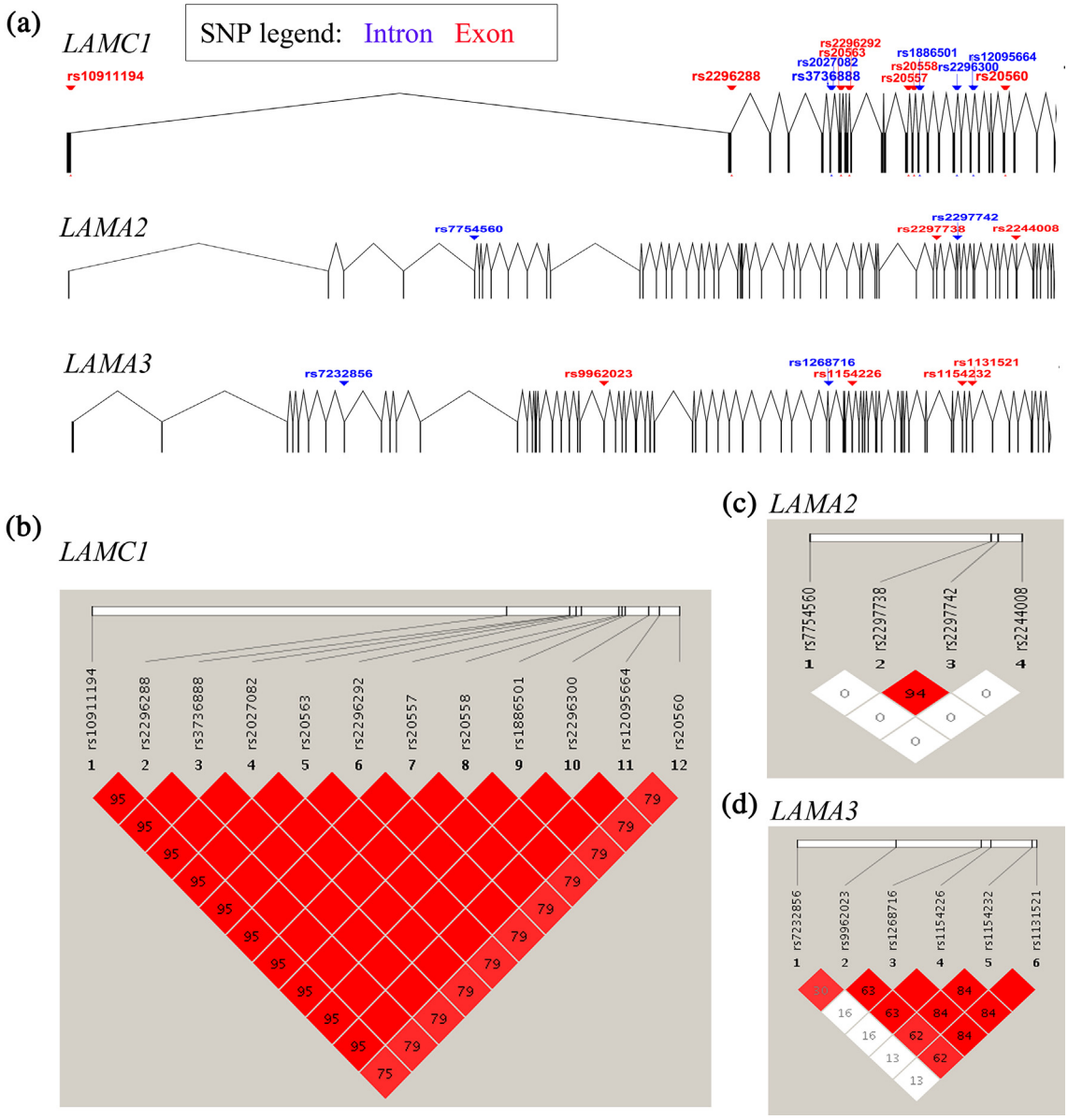

Fig. 1. *LAMC1*, *LAMA2* and *LAMA3* gene structures, assessed SNPs and linkage disequilibrium (LD) between SNPs. (a) The *LAMC1*, *LAMA2* and *LAMA3* gene structures are presented graphically with exons indicated in vertical lines. Genotyped SNPs are pointed out by arrows and color denoted: exons (red) and introns (blue). (b–d) LD graphics of *LAMC1*, *LAMA2* and *LAMA3*, respectively, with inter-SNP r^2 values in each box. The closer the r^2 value is to 1.0, the higher the correlation between the two SNPs. Squares with no value show perfect LD ($r^2 = 1$).

$P = 0.109$) (Fig. 1a, Table 1). There were 4 SNPs (rs2297738, rs2297742, rs2244008 and rs7754560) with P values <0.2, with rs2297738 and rs2297742 being in good LD with each other ($r^2 = 0.94$) (Fig. 1c).

In the study of *LAMA3*, there was a significant association between the *LAMA3* SNPs and severe POP, rs9962023 (OR, 2.31; 95% CI, 1.16–4.74; $P = 0.016$) and rs1154226 (OR, 0.45; 95% CI, 0.19–0.99; $P = 0.045$) (Table 1). They were both synonymous SNPs and did not result in amino acid changes (Table S2 online). There was also a trend toward significance for the following SNPs: rs1268716, an intronic SNP (OR, 0.47; 95% CI, 0.20–1.05; $P = 0.066$); rs1154232, a missense SNP (OR, 0.53; 95% CI, 0.24–1.13; $P = 0.109$); rs1131521, a synonymous SNP (OR, 0.53; 95% CI, 0.24–1.13; $P = 0.109$); and rs7232856, an intronic SNP (OR, 1.66; 95% CI, 0.90–3.07; $P = 0.110$) (Fig. 1a, Table 1). There was perfect LD between rs1268716 and rs1154226, rs1154232 and rs1131521 ($r^2 = 1.0$) (Fig. 1d). Additionally, for the missense SNP rs1154232, the substitution of C to A produces an amino acid substitution of asparagine to lysine that located in the laminin G domain (Table S2, Fig. S2 online). Laminin G domain possesses the binding sites for steroids, beta1 integrins, heparin, fibulin-1 and alpha-dystroglycans (https://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=238058). Mutation at this site leads to a moderate decrease of alpha helix and random coil structure of the domain secondary structure [9], which might influence signal transduction, adhesion, migration and differentiation via other cell adhesion molecules.

In this study, we used paired-end sequencing to conduct a comprehensive assessment of the *LAMA2*, *LAMA3* and *LAMC1* genes. In contrast, all of the previous studies [4–6] of *LAMC1* focused only on limited SNPs. Additionally, we examined *LAMA2* and *LAMA3* for the first time, and found two new *LAMA3* SNPs associated with prolapse in Chinese groups. Another important factor of this study is that we performed the candidate gene association study in nonfamilial Chinese groups. Because allele frequencies vary widely in diverse racial populations, that is, population stratification is observed, we recruited women from mainland China for both the case and control groups to avoid the complicated effect of the population stratification and to make sure the same source and genetic background of the two tested groups. In addition, the results provide several new opportunities for further study, and future genetic epidemiological studies should assess other race and ethnic groups or involve large-scale investigations of these genes or other ECM candidate genes of interest.

In summary, laminins, critical components of the ECM, play an important role in the pathophysiology of POP. *LAMC1*, *LAMA2* and *LAMA3* are interesting candidate genes, and in this study we found two *LAMA3* SNPs that were associated with POP and some other SNPs that had a tendency towards POP susceptibility in Chinese groups. These results not only provide crucial information for the etiology of the prolapse, but also aid to identify genetically high-risk women for the preventive strategies.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2019.02.007.

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