

**Verification of the chromosome region 9q21 association with pelvic organ prolapse
using RegulomeDB annotations**

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Supplementary Materials

SupplementaryTable 1. Summary on primer sequences^a, amplicons, and PCR cycling
conditions for individual SNPs analyzed in the study

| rs# of SNP | Primer sequences 5'-3', external forward/reverse, internal allele-specific forward/reverse | PCR ^b | Amplicon length (bp) |
|------------|--------------------------------------------------------------------------------------------------|------------------|-------------------------|
| rs1455311 | F aagtcctacccatcgaggt | 95°C (15s) | 303 |
| | R ccggtataatgggatgtgg | 62°C (15s) | |
| | F(t) ccatttctgaattacagagggt | 72 °C (20s) | 150 |
| | R(c) caggaaccttaatgaaaaatcaatag | 32 × | 200 |
| rs1036819 | F aaagcccatgctctgacctta | 95°C (15s) | 404 |
| | Rttttagtctctgggagagaatgct | 60°C (15s) | |
| | F(c)cacttttctgtctgtagcataaac | 72°C (20s) | 288 |
| | R(a) ggttctgttttaattgaatgcctt | 31 × | 165 |
| rs4077632 | F ggctagctcctgggaacaac | 95 °C (15s) | 560 |
| | R tgcaagctgtcatcagcata | 60.6°C(14s) | |
| | F(a) gagcaaggtacctttgcttga | 72°C(15s) | 369 |
| | R(g) cgataagaattagagaatcgc | 31 × | 232 |
| rs2807303 | F cgtcccactccaacttttt | 95 °C (15s) | 344 |
| | R gtggtgttcgagagtttga | 60 °C (20s) | |

| | | | |
|------------|-----------------------------------|---------------|-----|
| | F(c) cttttaccacatctcatttccttatttc | 72 °C (20s) | 153 |
| | R(t)gacaagaaaacaggaaggaagaa | 31 × | 241 |
| rs2777781 | F tgacctaagttgataaatgaggaa | 94 °C (15s) | 343 |
| | R tcctcttcaccttgtcagaa | 62.3 °C (20s) | |
| | F(a) ggcatgaacaaggctccta | 72 °C (22s) | 227 |
| | R(t) ctccattgtatctttgtcctgta | 32 × | 159 |
| rs11139451 | F aaggaccattctaaagttgtggac | 95 °C (15s) | 341 |
| | R actgatgcaataggacatgagata | 61 °C (20s) | |
| | F(t) agggaagaaagctgtct | 72 °C (20s) | 151 |
| | R(c) caagagtctgtgtttctg | 15 × | 226 |
| | | 95 °C (15s) | |
| | | 50.8 °C (20s) | |
| | | 72 °C (15s) | |
| | | 16 × | |
| rs12237222 | Fggatttaaccaggggccgttt | 95 °C(15s) | 370 |
| | Rgcatccaagctccgcttct | 66°C(15s) | |
| | F(g)cttctgtgggttcccactgg | 72 °C (15s) | 149 |
| | R(t)aaggacgaggctgtcca | 30 × | 260 |
| rs12551710 | F aggactccagcccctagact | 95 °C(15s) | 334 |
| | R cttggagtttacttggtgggaaa | 63°C(15s) | |
| | F(t) tccagtcctccactcacctat | 72 °C (30s) | 150 |
| | R(c)cacagaacctgctgtggaagg | 31 × | 225 |
| rs430794 | F ggtagtaagcctgcactctgc | 95 °C (15s) | 332 |
| | R caactgagtggggagcattt | 65.3°C (14s) | |
| | F(c) catcagcttccagtgccacc | 72 °C (14s) | 216 |

| | | | |
|-----------|-----------------------------------|---------------|-----|
| | R(a) gcagaacatcaagcaagttacagt | 29 × | 159 |
| rs8027714 | F ccagcatcttctctctttagc | 95 °C (15s) | 277 |
| | R acacatatgggcaaatgcag | 61.2 °C (15s) | |
| | F(g) tcaaggaatcctaattctttatttag | 72 °C (25s) | 191 |
| | R(a) ccagaactttgttcctataactattaat | 32 × | 140 |
| rs1810636 | F ggtgacagatcagcaaccac | 95 °C (15s) | 557 |
| | R ctcaaccaggaggaagag | 59 °C (15s) | |
| | F(g) cctctaaacaattttttaaagcg | 72 °C (25s) | 236 |
| | R(t) gacacactcaccaagcacata | 30 × | 365 |
| rs2236479 | F gggctctagagggaacagtg | 95 °C (15s) | 379 |
| | R cctctgacctccctgtcg | 59.6 °C (20s) | |
| | F(a) cgtgtcctcagaacgaa | 72 °C (30s) | 168 |
| | R(g) ctgtcctgcatgtcatcc | 31 × | 246 |

^aThe Primer3 algorithm was used to design external primer sequences.

^bPCR conditions are provided as temperature (duration) of denaturation, annealing and extension phase, respectively, followed by the number of cycles for a given SNP. Each PCR reaction was finished by a final extension phase lasting 5 min.

Supplementary Table 2. Characteristics of the POP and control groups involved in the study

| Characteristics | Control | POP | <i>P</i> ^e |
|---------------------------------------------------------------------------------------------|---------------|---------------|------------------------------|
| | N (%) | N (%) | |
| | Mean ± SD | Mean ± SD | |
| Total number | 292 | 210 | |
| POP-Q stage | | | |
| Without POP | 292 (100) | 0 (0.00) | |
| Stage I | 0 (0.00) | 0 (0.00) | |
| Stage II | 0 (0.00) | 0 (0.00) | |
| Stage III | 0 (0.00) | 194 (92.38) | |
| Stage IV | 0 (0.00) | 16 (7.62) | |
| Age ^a | 57.25 ± 12.70 | 57.65 ± 10.80 | 0.68 |
| BMI (kg/m ²) ^b | 27.46 ± 6.56 | 29.17 ± 5.85 | 5.6 × 10⁻⁴ |
| Vaginal parity ^c | | | |
| 0 | 57 (20.73) | 4 (1.96) | 0.014^f |
| 1 | 112 (40.73) | 79 (38.73) | |
| 2 | 100 (36.36) | 96 (47.06) | |
| ≥3 | 6 (2.18) | 25 (12.25) | |
| Perineal trauma in childbirth (episiotomy and spontaneous perineal laceration) ^d | 93 (32.75) | 107 (52.45) | 1.7 × 10⁻⁵ |

Data are missing for: ^a8 controls, 6 POP patients; ^b11 controls, 12 POP patients; ^c17 controls, 6 POP patients; ^d91 controls, 51 POP patients.

^eSignificant results are in bold.

^fWe compared two groups: 0-1 vaginal birth vs. ≥ 2 vaginal birth.

Supplementary Table 3. The distribution of genotypes among cases and controls

| SNPs and genotypes | Control | | POP | | Crude P- value, OR, (95% CI) | Adjusted P- value ^a , OR, (95% CI) |
|--------------------|------------|------|------------|------|---------------------------------------|--------------------------------------------------------|
| | Number (%) | HWP | Number (%) | HWP | | |
| rs1455311 | n=287 | | n=210 | | | |
| T/T | 187 (65.2) | | 146 (69.5) | | 0.24 (add) | 0.10 (add) |
| T/C | 91 (31.7) | 0.70 | 60 (28.6) | 0.61 | 0.82 | 0.72 |
| C/C | 9 (3.1) | | 4 (1.9) | | (0.58 – 1.15) | 0.48 – 1.07 |
| rs1036819 | n=290 | | n=211 | | | |
| A/A | 232 (80.0) | | 156 (74.3) | | 0.13 (dom) | 0.11 (dom) |
| A/C | 54 (18.6) | 0.55 | 50 (23.8) | 1.00 | 1.38 | 1.49 |
| C/C | 4 (1.4) | | 4 (1.9) | | (0.91 – 2.11) | 0.92 – 2.41 |
| rs4077632 | n=291 | | n=209 | | | |
| A/A | 128 (44.0) | | 79 (37.8) | | 0.094 (add) | 0.22 (add) |
| A/G | 134 (46.0) | 0.51 | 101 (48.3) | 0.77 | 1.26 | 1.20 |

| | | | | | | | |
|------------|-----|------------|------|------------|------|---------------|---------------|
| rs2807303 | G/G | 29 (10.0) | | 29 (13.9) | | (0.96 – 1.64) | (0.89 – 1.62) |
| | | n=285 | | n=210 | | | |
| | C/C | 102 (35.8) | | 80 (38.1) | | 0.19 (rec) | 0.38 (rec) |
| | C/T | 142 (49.8) | 0.53 | 108 (51.4) | 0.13 | 0.79 | 0.76 |
| rs2777781 | T/T | 41 (14.4) | | 22 (10.5) | | (0.40 – 1.21) | (0.41 – 1.40) |
| | | n=292 | | n=210 | | | |
| | A/A | 154 (52.7) | | 111 (52.9) | | 0.25 (rec) | 0.56 (rec) |
| | A/T | 122 (41.8) | 0.23 | 82 (39.0) | 0.73 | 1.52 | 1.25 |
| rs11139451 | T/T | 16 (5.5) | | 17 (8.1) | | (0.75 – 3.08) | (0.59 – 2.67) |
| | | n=291 | | n=210 | | | |
| | T/T | 185 (63.6) | | 131 (62.4) | | 0.78 (dom) | 0.74 (dom) |
| | T/C | 88 (30.2) | 0.11 | 70 (33.3) | 1.00 | 1.05 | 1.07 |
| rs12551710 | C/C | 18 (6.2) | | 9 (4.3) | | (0.73 – 1.52) | (0.71 – 1.62) |
| | | n=291 | | n=210 | | | |
| | C/C | 233 (80.1) | | 171 (81.4) | | 0.58 (add) | 0.70 (add) |
| | C/T | 56 (19.2) | 0.75 | 39 (18.6) | 0.23 | 0.88 | 0.91 |

| | | | | | | | |
|-----------|-----|------------|------|------------|------|---------------|---------------|
| rs430794 | T/T | 2 (0.7) | | 0 (0.0) | | (0.57 – 1.37) | (0.56 – 1.47) |
| | | n=289 | | n=210 | | | |
| | C/C | 148 (51.2) | | 122 (58.1) | | 0.13 (dom) | 0.11 (dom) |
| | C/A | 113 (39.1) | 0.39 | 72 (34.3) | 0.27 | 0.76 | 0.72 |
| rs8027714 | A/A | 28 (9.7) | | 16 (7.6) | | (0.53 – 1.08) | (0.49 – 1.08) |
| | | n=290 | | n=210 | | | |
| | G/G | 278 (95.9) | | 197 (93.8) | | 0.30 (dom) | 0.27 (dom) |
| | G/A | 12 (4.1) | 1.00 | 13 (6.2) | 1.00 | 1.53 | 1.66 |
| rs1810636 | A/A | 0 (0.0) | | 0 (0.0) | | (0.68 – 3.42) | (0.67 – 4.09) |
| | | n=290 | | n=210 | | | |
| | G/G | 119 (41.0) | | 101 (48.1) | | 0.12 (dom) | 0.25 (dom) |
| | G/T | 144 (49.7) | 0.09 | 91 (43.3) | 0.75 | 0.75 | 0.79 |
| rs2236479 | T/T | 27 (9.3) | | 18 (8.6) | | (0.53 – 1.07) | (0.53 – 1.18) |
| | | n=287 | | n=207 | | | |
| | G/G | 131 (45.6) | | 81 (39.1) | | 0.14 (add) | 0.097 (add) |
| | A/G | 120 (41.8) | 0.29 | 94 (45.4) | 0.66 | 1.22 | 1.28 |

| | | | | |
|-----|-----------|-----------|---------------|---------------|
| A/A | 36 (12.5) | 32 (15.5) | (0.94 – 1.57) | (0.96 – 1.71) |
|-----|-----------|-----------|---------------|---------------|

HWP, Hardy– Weinberg probability; OR, odds ratio; CI, confidence interval; rec, recessive model; dom, dominant model; add, additive model.

^aAdjusted by age, body mass index (BMI), perineal trauma in childbirth and vaginal parity. In the multivariate analysis, the number of cases and controls may be less than in the crude analysis since some subjects had insufficient data on covariates.

Supplementary Table 4. Linkage disequilibrium analysis of six SNPs in the region 9q21

| D' | rs11139451 | rs12237222 | rs12551710 | rs2777781 | rs2807303 |
|------------------|------------|-----------------------|-----------------------|-----------|-----------|
| <i>P</i> - value | | | | | |
| rs4077632 | 0.149 | 0.034 | 0.323 | 0.009 | 0.022 |
| | 0.073 | 0.367 | 4.17×10^{-6} | 0.895 | 0.515 |
| rs11139451 | | 0.257 | 0.129 | 0.236 | 0.0007 |
| | | 1.45×10^{-6} | 0.009 | 0.019 | 0.988 |
| rs12237222 | | | 0.266 | 0.052 | 0.098 |
| | | | 0.001 | 0.252 | 0.035 |
| rs12551710 | | | | 0.043 | 0.373 |
| | | | | 0.456 | 0.002 |
| rs2777781 | | | | | 0.177 |
| | | | | | 0.008 |

D' and *P* – value for linkage are given for every pair of SNPs.

Supplementary Table 5. Fst pairwise values between populations

| Pops | CHD | GIH | JPT | TSI | YRI | RUS | MEX | LWK |
|------|--------|--------|--------|--------|--------|--------|--------|--------|
| CEU | 0.1108 | 0.0324 | 0.0753 | 0 | 0.1574 | 0 | 0.0236 | 0.1654 |
| CHD | | 0.0492 | 0.0148 | 0.1302 | 0.1951 | 0.1104 | 0.0712 | 0.1822 |
| GIH | | | 0.029 | 0.0435 | 0.1548 | 0.0297 | 0.0159 | 0.1341 |
| JPT | | | | 0.079 | 0.1749 | 0.0689 | 0.0499 | 0.1562 |
| TSI | | | | | 0.1681 | 0.0017 | 0.0281 | 0.1638 |
| YRI | | | | | | 0.1482 | 0.1148 | 0.0093 |
| RUS | | | | | | | 0.0256 | 0.1575 |
| MEX | | | | | | | | 0.101 |