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GENETIC ASSOCIATION STUDY IN WOMEN WITH PELVIC ORGAN PROLAPSE

Hypothesis / aims of study

Pelvic organ prolapse (POP) is a common yet distressing pelvic floor dysfunction affecting one-third of adult women worldwide. However, the underlying pathophysiology is largely unknown. Its etiology is multifactorial, including direct mechanical injury, partial denervation of pelvic floor, and hormonal changes caused by pregnancy. Findings from epidemiologic studies suggest that occurrence of POP can be attributed to genetic factors. Endothelin-1 (*EDN-1*) is a potent vasoconstrictor and accumulating evidence showed that endothelin-1 plays an important role in vascular pathophysiology and wound repair. Among the polymorphisms reported in *EDN1*, clinical studies demonstrated that Lys allele carrier status at codon Lys198Asn (G/T, rs5370) was related to higher levels of plasma EDN1 among women during pregnancy. Another sequence variant, the 3A/4A polymorphism (-134delA; rs10478694) located in the 5'-untranslated region, has also been found to be significantly different in plasma EDN1 levels between individuals with the 3A/3A and 3A/4A genotypes. Because EDN1 induces collagen matrix contraction and stimulates mitogenesis of myofibroblast and fibroblast in vitro, we postulate that genetic variations in *EDN1* could lead to alterations in *EDN1* protein expression and affect the mechanical and contractile properties of pelvic floor myofibroblasts in women with pelvic organ prolapse (POP).

Study design, materials and methods

To delineate the possible roles of *EDN1* sequence variants in POP, we investigated the allelic frequency of non-synonymous polymorphisms/functional variants that effects the expression of EDN1 among women with POP and age-matched female controls. POP patients with at least second degree prolapse and normal age-matched controls attending the out-patient gynaecology clinic were recruited. 10ml EDTA blood was collected from each participant and genomic DNA was extracted by Qiagen blood kit according to manufacturer's description. The *EDN1* rs5370 sequence variants were detected by PCR of exon 5 with the sense primer 5'CTTTTGCCAAAGGGTGATTT-3' and antisense primer 5'-AGGGTGGAGAGTGCAGAGTC-3' followed by direct sequencing. The rs10478694 genotypes were detected by PCR with the sense primer 5'-GCTGCTTTTCTCCCCGTTAA-3; and antisense primer 5'-CAAGCCACAAACAGCAGAGA-3' and digestion with restriction enzyme BsiYI. The genotypes are 4A/4A (195 base pairs), 4A/3A (176+195+19 base pairs), and 3A/3A (176+19 base pairs) being analyzed and visualized on a 7% PAGE-gel.

Results

By direct DNA sequencing, we found that there were no statistical differences in the distribution of rs5370 genotypes (GG, GT and TT alleles) between POP patients and age matched female controls (Table 1). Interestingly for rs10478694, the frequency of 4A/3A genotype was significantly higher (Chi-square test; *P*=0.0000) in control women compared to POP subjects (60.3% versus 23.9%, Table 2). Except the frequency of rs10478694 genotypes among control subjects, there is no significant difference between the genotype frequencies of rs10478694 and rs5370 among our Chinese control or POP populations comparing to the genotype distribution reported from the International HapMap project (http://www.hapmap.org).

Interpretation of results

Our data suggest that presence of 4A/3A genotype or 4A allele may be considered as a protective factor in pelvic floor prolapse. Based on these observations and the results form Poncet S *et al*, our preliminary small scale pilot study revealed a genetic association between rs10478694 polymorphism with POP in our Chinese populations. However, as the genotype frequency of rs10478694 among control group did not follow Hardy-Weinberg equilibrium. A large scale genetic analysis of rs10478694 by direct sequencing is undergoing to confirm our observations

Concluding message

Our data indicated that the genetic variations affecting the protein level of the inflammatory mediator endothelin-1 may be involved to the development of pelvic organ prolapse.

EDN1 rs5370	**Control**	**POP**	**HapMap-HCB**
G/G	**68 (55.7%)**	**30 (50%)**	**20 (44.4%)**
G/T	**43 (35.2%)**	**25 (41.7%)**	**22 (48.9%)**
T/T	**11 (9%)**	**5 (8.5%)**	**3 (6.7%)**
Total number	**122**	**60**	**45**

Table 1. Genotype distributions of Lys198Asn (SNP rs5370) *EDN1* polymorphism among pelvic organ prolapse (POP) patients, normal age matched controls and HapMap.

EDN1 rs10478694	**Control**	**POP**	**HapMap-Global**
4A/4A	**4 (6.3%)**	**0 (0%)**	**4 (5.1%)**
4A/3A	**#38 (60.3%)**	**16 (23.9%)**	**18 (23.1%)**
3A/3A	**#21 (33.3%)**	**51 (76%)**	**56 (71.8%)**
Total number	**63**	**67**	**78**

Table 2. Genotype distributions of -134delA (SNP rs5370) *EDN1* polymorphism among pelvic organ prolapse (POP) patients, normal age matched controls and HapMap. #(Chi-square test $p<0.05$).

Reference:

1. Poncet S, Meyer S, Richard C, Aubert JD, Juillerat-Jeanneret L The expression and function of the endothelin system in contractile properties of vaginal myofibroblasts of women with uterovaginal prolapse. Am J Obstet Gynecol. 2005;192:426-32.

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