Association of five common polymorphisms in the plasminogen activator inhibitor-1 gene with primary ovarian insufficiency

Young Joo Jeon, M.S.,[a] Young Ran Kim, M.D.,[b] Bo Eun Lee, M.S.,[a] Sun Hee Cha, M.D., Ph.D.,[b] Myoung-Jin Moon, M.D., Ph.D.,[b] Doyeun Oh, M.D., Ph.D.,[c] Woo Sik Lee, M.D., Ph.D.,[d] and Nam Keun Kim, Ph.D.[a]

[a] Institute for Clinical Research, [b] Department of Obstetrics and Gynecology, and [c] Department of Internal Medicine, CHA Bundang Medical Center, School of Medicine, CHA University, Seongnam 463-712, South Korea; and [d] Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul 135-081, South Korea

Objective: To investigate the association between potentially functional plasminogen activator inhibitor-1 (PAI-1) genetic polymorphisms and primary ovarian insufficiency (POI).
Design: Case–control study.
Setting: Urban university-based hospital.
Patient(s): A cohort of 137 POI patients and 227 controls.
Intervention(s): None.
Main Outcome Measure(s): Genotyping of five PAI-1 polymorphisms (−844G>A [rs2227631], −675 4G/5G [rs1799889], 43G>A (Ala>Thr) [rs6092], 9785G>A [rs2227694], and 11053T>G [rs7242]) was assessed by polymerase chain reaction–restriction fragment length polymorphism assay.
Result(s): *PAI-1* polymorphisms 9785GA+AA, −844A/9785A, 4G/9785A, and 9785A/11053G were associated with POI occurrence. Moreover, −844GA+AA and 11053TG+GG were associated with lower serum E_2 levels in controls.
Conclusion(s): We have identified an association between five *PAI-1* polymorphisms and POI occurrence. However, the mechanism underlying the function of these polymorphisms in POI remains to be determined. Further studies are needed to improve understanding of the roles of *PAI-1* polymorphisms and genes in related pathways, using a larger and more heterogeneous cohort. (Fertil Steril® 2014;101: 825–32. ©2014 by American Society for Reproductive Medicine.)
Key Words: Plasminogen activator inhibitor-1, polymorphism, primary ovarian insufficiency

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/kimnk-polymorphism-plasminogen-activator-inhibitor-1/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Primary ovarian insufficiency (POI) is a complex disorder in which amenorrhea lasting for 4 months or more, accompanied by elevated levels of gonadotropins, occurs before the age of 40 years (1–3). The overall prevalence of POI is 1.1% worldwide; however, prevalence is lower in Asian women (Chinese women, 0.5%; Japanese women, 0.1%) (4). Follicle-stimulating hormone is a glycoprotein, the function of which is dependent on glycosylation, and alterations in FSH level are seen in POI (5, 6). Primary ovarian insufficiency is clinically manifested by increased levels of FSH (usually >40 IU/L, in two measurements obtained at least 1 month apart) and decreased levels of E_2 (<20 IU/L, indicating hypoestrogenism) (1). The etiology of POI is not fully understood, and several mechanisms have been proposed, including altered gene expression or aberrant function of hormones or receptors due to abnormalities in glycosylation (7).

Received May 27, 2013; revised October 25, 2013; accepted November 11, 2013; published online December 17, 2013.
Y.J.J. has nothing to disclose. Y.R.K. has nothing to disclose. B.E.L. has nothing to disclose. S.H.C. has nothing to disclose. M.-J.M. has nothing to disclose. D.O. has nothing to disclose. W.S.L. has nothing to disclose. N.K.K. has nothing to disclose.
Y.J.J. and Y.R.K. contributed equally to this work.
This study was supported by grants from the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2009-0093821 and NRF-2012R1A1A2007033).
Reprint requests: Nam Keun Kim, Ph.D., CHA University, CHA Bundang Medical Center, Institute for Clinical Research, 351, Yatap-dong, Bundang-gu, Seongnam 463-712, South Korea (E-mail: namkkim@naver.com).

Fertility and Sterility® Vol. 101, No. 3, March 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.11.015

More than 50 reports have described a variety of genetic variants associated with POI (Supplemental Table 1, available online). Of the genetic risk factors, vascular endothelial growth factor, endothelial nitric oxide synthase, kinase insert domain receptor, methylenetetrahydrofolate reductase, and angiotensin II receptor type 1 are associated with thrombotic disease (8–12). Moreover, a recent report showed higher levels of diverse thrombotic risk factors (D-dimer, white blood cell count, mean platelet volume, prothrombin time, total cholesterol, and low-density lipoprotein cholesterol) in POI patients (13). These findings may indicate that thrombotic responses are potential predisposing factors to development of POI. Therefore, it may be valuable to investigate associations between thrombosis-associated genes, including plasminogen activator inhibitor-1 (PAI-1), and POI susceptibility.

Plasminogen activator inhibitor-1 is a serine protease inhibitor (SERPIN), and the *PAI-1* gene is located on chromosome 7 (7q21.3-22). Plasminogen activator inhibitor-1 is a principal inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA), which are the main plasminogen activators that convert plasminogen to the proteolytic enzyme plasmin (14). Fibrinolysis is the process of breaking down fibrin and is primarily controlled by PAI-1. Fibrinolysis maintains vessel patency, degrades extracellular matrix (ECM), and regulates cell adhesion, migration, and tissue remodeling (15–17). Circulating PAI-1 is involved in extracellular proteolytic processes during ovarian follicle growth, ovulation, and embryo implantation (18, 19). A recent study found expression of SERPIN messenger RNA, including that of PAI-1, in healthy and atretic follicles (20). In atretic follicles, expression levels of PAI-1 and SERPING1 were increased, whereas those of SERPINA6, SERPINB6, SERPINE2, and SERPINF2 were decreased (20).

According to a previous study (21), the *PAI-1* gene has 10 tag polymorphisms (rs2227631 [−844G>A], rs6092 [43G>A], rs2227708, rs2227662, rs2227666, rs2227667, rs2227672, rs2227683, rs2227694 [9785G>A], and rs7242 [11053T>G]), and rs1799889 [−675 4G/5G] has been widely studied in *PAI-1* genetic studies. Increased promoter activity, messenger RNA expression, and IgE production are linked to the *PAI-1* 4G allele (22–24). Another extensively studied *PAI-1* genetic mutation is the −844G>A polymorphism. The *PAI-1* −844A allele is associated with increased transcriptional activity and PAI-1 protein levels (21, 25). The polymorphisms 43G>A, 9785G>A, and 11053T>G may also affect plasma PAI-1 level (21). However, of the other six *PAI-1* polymorphisms, five (rs2227708, rs2227662, rs2227667, rs2227672, and rs2227683) do not influence PAI-1 protein levels, and one (rs2227666) does not occur in the Asian population. Therefore, we designed a genetic epidemiological study of five *PAI-1* polymorphisms to investigate the association between *PAI-1* and POI. This is the first study to identify an association between five *PAI-1* polymorphisms and POI in Asian women.

MATERIALS AND METHODS

Study Subjects

Blood samples were collected from 364 karyotypically normal study participants consisting of 137 patients with POI (46,XX; mean age ± standard deviation [SD], 31.67 ± 3.51 years) and 227 control subjects (46,XX; mean age ± SD, 32.21 ± 3.45 years). All POI patients had been diagnosed with POI (cessation of menses for 6 months before the age of 40 years, and two serum FSH measurements of >40 IU/L, taken at least 1 month apart). None of the patients had a history of pelvic surgery, cancer, radiation exposure, autoimmune disorder, or genetic syndrome. The women in the control group, who had regular menstrual cycles, were recruited from CHA Bundang Medical Center and met the following enrollment criteria. All had negative results for an autoantibody screening test that included antiovarian, antithyroid, and antinuclear antibodies, and had had at least one live birth at the Department of Obstetrics and Gynecology of CHA Bundang Medical Center between March 1999 and February 2008. Of 500 initial participants (Non-POI: 250; POI patients: 250) evaluated, 136 women (Non-POI: 23; POI patients: 113) who had chromosomal abnormalities, cancer, autoimmune disorders, and/or history of pelvic surgery or other infertility diseases were excluded from the study. The Institutional Review Board of CHA Bundang Medical Center approved this study, and informed consent was obtained from all participants.

Genotyping

Deoxyribonucleic acid was extracted from leukocytes using a G-DEX II Genomic DNA Extraction Kit (Intron Biotechnology) according to the manufacturer's instructions. To analyze *PAI-1* genotypes, we chose polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis because this procedure is more economical and cost-effective than sequencing of entire genes. The *PAI-1* polymorphism −844G>A was detected using a forward primer (5′-CAG GCT CCC ACT GAT TCT AC-3′) and a reverse primer (5′-GAG GGC TCT CTT GTG TCA AC-3′). The 510-bp PCR product was then digested with *Xho*I. A digestion product of 510 bp represented the AA genotype; fragments of 510 bp, 364 bp, and 146 bp represented the GA genotype; and 364-bp and 146-bp products represented the GG genotype.

The *PAI-1* −675 4G/5G polymorphism was detected by PCR-RFLP analysis using forward (5′-CCA ACA GAG GAC TCT TGG TC-3′) and reverse (5′-CAC AGA GAG AGT CTG GCC ACG-3′) primers. The 99-bp product was digested with 3U *Bsl*I for 16 hours at 55°C. A restriction fragment of 99 bp represented the 4G4G genotype; fragments of 99 bp, 77 bp, and 22 bp represented the 4G5G genotype; and 77-bp and 22-bp products represented the 5G5G genotype.

To detect the *PAI-1* 43G>A genotype, PCR-RFLP analysis was performed with forward (5′-TGT CTT CCA GAA CGA TTC CTT CAC C-3′) and reverse (5′-GTT GTC AGC TGG AGC ATG GCC-3′) primers. The length of the amplified fragment was 266 bp. The PCR products were digested with 3U *Psh*AI for 16 hours at 37°C. Restriction products of 266 bp identified the GG genotype; products of 266 bp, 172 bp, and 94 bp represented the GA genotype; and 172-bp and 94-bp products represented the AA genotype.

The *PAI-1* 9785G>A genotype was amplified using forward (5′-ATG AAG GTG CCA CTG CAC TCG C-3′) and