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Association study of five functional polymorphisms in matrix metalloproteinase-2, -3, and -9 genes with risk of primary ovarian insufficiency in Korean women

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ABSTRACT

Objective: Primary ovarian insufficiency (POI) is diagnosed clinically by increased follicle-stimulating hormone (FSH) levels and estradiol (E_2) deficiency. A previous report suggests a possible association matrix metalloproteinase (MMP) and estrogen signaling pathway; however, there are no reports of *MMP* genetic associations with POI.

Study design: Blood samples were collected from 374 karyotypically normal study participants consisting of 138 patients with POI (46, XX; mean age ± standard deviation [SD], 31.7 ± 3.51 years) and 236 control subjects (46, XX; 32.2 ± 3.50 years). Five functional polymorphisms in *MMP-2* (-1575G > A [rs243866] and -1306C > T [rs243865]), *MMP-3* (-1612 5A/6A [rs3025058]), and *MMP-9* (-1562C > T [rs3918242] and 2678G > A [rs17576]) genes were genotyped using polymerase chain reaction-restriction fragment length polymorphism assays in a cohort of 236 controls and 138 POIs.

Results: *MMP-2* -1306CT + TT was associated with POI occurrence. Moreover, relatively lower serum estradiol levels were detected in healthy women with the *MMP-2* -1575GA + AA/*MMP-2* -1306CT + TT and *MMP-2* -1306CT + TT/*MMP-9* 2678GG combination genotypes.

Conclusions: *MMP-2* -1306C > T polymorphism may contribute to an increased POI prevalence in Korean women. Further studies are needed to confirm the genetic associations in other ethnic populations.

1. Introduction

Primary ovarian insufficiency (POI) is a complex disorder that produces amenorrhea lasting at least four months and increases gonadotropin levels in younger women under the age of forty [1–3]. Overall POI prevalence is 1.1% worldwide; however, the disorder is less prevalent in Asian women (Chinese women, 0.5%; Japanese women, 0.1%) [4]. POI is diagnosed clinically by increased follicle-stimulating hormone (FSH) levels (>40 mIU/mL in two consecutive measurements taken at least one month apart), and estradiol (E_2) deficiency (<15 pg/mL, indicating hypoestrogenism) [5]. A previous report suggests a possible association matrix metalloproteinase (MMP) genetic variation and estrogen signaling pathway [6]. *MMP-2* -1306TT genotype correlated with lower concentrations of estrogen receptor (ER) and better survival probability of ER positive breast cancer patients [6]. Over 50 reports have described diverse POI susceptibility genes including plasminogen activator inhibitor (PAI-1), functionally similar to MMP family [7]; however, there are no reports of *MMP* genetic associations with POI.

MMPs, a family of zinc-dependent proteinases, play an important role in reproductive physiology by remodeling the extracellular matrix (ECM) during ovarian follicular growth and ovulation [8]. Specific aspects of follicular development, ovulation, and the subsequent formation and regression of the corpus luteum

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are largely performed by MMPs [9]. MMPs are controlled by hormones, growth factors, and cytokines. They are involved in critical structural and functional changes within reproductive organs [10]. In human ovaries MMP production and their mechanisms of action are not well characterized. However, their presence in the follicular microenvironment has been shown to be vital for follicular development. In a previous study, intrafollicular MMP expression data was collected following *in vitro* fertilization (IVF) treatment with continued FSH administration [11]. This report found significantly reduced MMP expression and increased tissue inhibitors of metalloproteinase (TIMP)-1 levels in the follicular fluid of IVF treated-women compared with normally ovulating women [11].

There are several functional polymorphisms of *MMP* genes (*MMP-2* -1575G > A [rs243866], *MMP-2* -1306C > T [rs243865], *MMP-3* -1612 5A/6A [rs3025058], *MMP-9* -1562C > T [rs3918242], and *MMP-9* 2678G > A [rs17576]) [12–16]. *MMP-2*, *-3*, and *-9* are located on chromosomes 16q13-21, 11q22, and 20q11-13, respectively. The *MMP-2* -1575G allele functions as a transcriptional enhancer, whereas the -1575A allele reduces transcription activity significantly in estrogen receptor-positive MCF-7 cells [12]. The common C > T transition in *MMP-2* -1306 disrupts an Sp1-type promoter site (CCACC box), and the T allele displays strikingly lower promoter activity [13]. The *MMP-3* -1612 6A allele increases affinity for a transcriptional repressor leading to a 2-fold decrease in promoter activity [14]. The *MMP-9* -1562C allele binds a transcriptional repressor, decreasing promoter activity [15]. The *MMP-9* 2678G allele reduces expression in comparison with the A allele [16].

We hypothesized that aberrant extracellular matrix (ECM) remodeling by functional *MMP* gene polymorphisms may produce POI etiologies. We designed a genetic epidemiological study of five functional *MMP* gene polymorphisms to investigate their association with POI. This is the first study to identify an association between five *MMP* gene polymorphisms and POI in Asian women.

2. Materials and methods

2.1. Study subjects

Blood samples were collected from 374 karyotypically normal study participants consisting of 138 patients with POI (46, XX; mean age ± standard deviation [SD], 31.7 ± 3.51 years) and 236 control subjects (46, XX; 32.2 ± 3.50 years). All POI patients had been diagnosed with POI (cessation of menses for 6 months prior to 40 years of age and two serum FSH measurements of >40 mIU/mL, taken at least 1 month apart). None of the patients had a history of pelvic surgery, cancer, radiation exposure, positive for autoantibodies (antiovarian, antithyroid, and antinuclear antibodies), or genetic syndrome. The control group was recruited from CHA Bundang Medical Center and met the following enrollment criteria. All the participants had regular menstrual cycles, screened negative for autoantibodies including antiovarian, antithyroid, and antinuclear antibodies, and had at least one live birth performed in the Department of Obstetrics and Gynecology at CHA Bundang Medical Center between March 2004 and February 2013. The Institutional Review Board of CHA Bundang Medical Center approved this study and informed consent was obtained from all the participants.

2.2. Genotyping

DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to evaluate the *MMP* genotypes due to the efficiency and cost-effectiveness compared to gene sequencing.

The *MMP-2* -1575G > A polymorphism was detected using the sense primer (5′-GTC TGA AGC CCA CTG AGA CC-3′) and the antisense primer (5′-CTA GGA AGG GGG CAG ATA GG-3′). The 175 bp PCR product was then digested with 3 U *Nla*III for 16 h at 37 °C. The GG genotype produced a 175 bp digestion product; the GA genotype, 175 bp, 112 bp, and 63 bp fragments; and AA, 112 bp and 63 bp fragments.

The *MMP-2* -1306C > T polymorphism was detected using the following primers: forward, 5′-CTT CCT AGG CTG GTC CTT ACT GA-3′ and reverse, 5′-CTG AGA CCT GAA GAG CTA AAG AGC T-3′. The 193 bp product was digested with 3 U *Bfa*I for 16 h at 37 °C. The CC genotype produced a 193 bp digestion product; the CT genotype, 193 bp, 167 bp, and 26 bp fragments; and the TT genotype, 167 bp and 26 bp fragments.

The *MMP-3* -1612 5A/6A genotyping was performed using the forward primer (5′-GTT CTC CAT TCC TTT GAT GGG GGG AAA GA-3′) and reverse primer (5′-TTC CTG GAA TTC ACA TCA CTG CCA CCA CT-3′). The length of the amplified fragment was 128 bp. PCR products were digested with 3 U *Pfl*FI for 16 h at 37 °C. The 6A6A genotype yielded a 128 bp digestion product, whereas the 5A6A genotype produced of 128 bp, 95 bp, and 34 bp fragments and the 5A5A genotype produced 95 bp and 34 bp fragments.

The *MMP-9* -1562C > T genotype was analyzed with the sense primer (5′-GCC TGG CAC ATA GTA GGC CC-3′) and the antisense primer (5′-GGG TTC AAG CAA TTC TCC TG-3′). The length of the amplified fragment was 246 bp. PCR products were digested with 3 U *Sph*I for 16 h at 37 °C. The CC genotype had a 246 bp digestion product; the CT genotype, 246 bp, 194 bp, and 52 bp fragments; and the TT genotype 194 bp and 52 bp fragments.

The *MMP-9* 2678G > A genotype was detected with a sense primer (5′-ACC ATC CAT GGG TCA AAG AA-3′) and an antisense primer (5′-GAT TGG CCT TGG AAG ATG AA-3′). The length of the amplified fragment was 161 bp. PCR products were digested with 3 U *Sma*I for 16 h at 25 °C. The GG genotype restriction products were 103 bp and 58 bp in length; the GA genotype, 161 bp, 103 bp, and 58 bp; and the AA genotype, 161 bp.

For each polymorphism 30% of the PCR assays were randomly selected for a replicate PCR assay followed by DNA sequencing to validate the RFLP findings. Sequencing was performed using an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The quality control sample concordance was 100%.

2.3. Hormone assay

Blood was collected from the control group by venipuncture on the second or third day of the menstrual cycle to determine the FSH, luteinizing hormone (LH), and E_2 levels. POI group blood samples were collected without reference to menses. The serum was separated and the hormones were measured using a radioimmunoassay kit (E_2; Beckman Coulter, Brea, CA, USA) or an enzyme immunoassay kit (FSH and LH; Siemens, Malvern, PA, USA), according to the manufacturers' instructions, as previously described [17].

2.4. Statistical analysis

MMP genotype frequency differences between POI patients and controls were compared using logistic regression. Allele frequencies were calculated to investigate Hardy-Weinberg equilibrium (HWE) deviations. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to examine the association between *MMP* gene polymorphisms and POI prevalence, and were calculated using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA) and MedCalc version 12.1.4 (MedCalc Software bvba, Ostend, Belgium). Because the present study was a retrospectively designed case–control study and the disease incidence rate data was not available, the actual relative risk cannot be determined [18]. *MMP*