ORIGINAL ARTICLE: GENETICS

Maternal common variant rs2305957 spanning *PLK4* is associated with blastocyst formation and early recurrent miscarriage

Qian Zhang, M.D.,[a,b,c,d] Guangyu Li, M.S.,[a,b,c,d] Lei Zhang, M.D.,[a,b,c,d] Xiaohe Sun, M.D.,[a,b,c,d] Dandan Zhang, M.B.,[a,b,c,d] Juanjuan Lu, M.D.,[a,b,c,d] Jinlong Ma, M.D., Ph.D.,[a,b,c,d] Junhao Yan, M.D., Ph.D.,[a,b,c,d] and Zi-Jiang Chen, M.D., Ph.D.[a,b,c,d,e,f]

[a] Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University, Jinan; [b] National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Jinan; [c] The Key Laboratory of Reproductive Endocrinology (Shandong University), Ministry of Education, Jinan; [d] Shandong Provincial Key Laboratory of Reproductive Medicine, Jinan; [e] Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai; and [f] Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai, People's Republic of China

Objective: To investigate whether the common variant rs2305957 spanning *PLK4* (Polo-like kinase 4) confers risk to embryo development in Northern Chinese Han (CHN) women.
Design: Genetic association study.
Setting: University hospital.
Patient(s): A total of 2,015 infertile women who underwent in vitro fertilization (IVF), 530 women with early recurrent miscarriage (ERM), and 600 fertile control women in the CHN population.
Intervention(s): Genotyping of rs2305957 was performed by means of high-resolution melting analysis.
Main Outcome Measure(s): Blastocyst formation, implantation, early miscarriage, and live birth rates in infertile women; genotype distribution at rs2305957 in ERM case and control subjects.
Result(s): In the first cohort of this study, infertile women with AA genotype had a lower blastocyst formation rate than those with AG or GG genotype. No significant differences were observed in implantation rate, early miscarriage rate, or live birth rate among AA, AG, and GG subgroups. In the second cohort, common variant rs2305957 was related to ERM. Genotype frequency differences were also significant in both additive model and dominant model.
Conclusion(s): Common variant rs2305957 is associated with blastocyst formation and ERM in CHN women. Further investigations of *PLK4* gene during embryo development could be worthwhile. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, rs2305957, blastocyst formation, infertility, early recurrent miscarriage

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/14161-22922

Human reproduction is an inefficient process and ~50% of conceptions end in reproductive failure (1). Human reproductive failure ranges from the inability to conceive through the incapacity to maintain pregnancy after successful conception. Infertility, a common consequence of human reproductive failure, is defined by the failure to achieve a clinical pregnancy after ≥12 months of regular unprotected intercourse and affects 9%–18% of the general population (2, 3). Despite many advances in assisted reproductive technologies (ART) for infertile couples, >50% of in vitro fertilization (IVF)–generated embryos suffer from mosaic aneuploidy at cleavage stage (4). And these aneuploid embryos will probably experience cleavage arrest before blastocyst formation, implantation failure after embryo transfer, or pregnancy loss (4). Early recurrent miscarriage (ERM), traditionally

Received August 15, 2016; revised and accepted January 5, 2017.
Q.Z. has nothing to disclose. G.L. has nothing to disclose. L.Z. has nothing to disclose. X.S. has nothing to disclose. D.Z. has nothing to disclose. J.L. has nothing to disclose. J.M. has nothing to disclose. J.Y. has nothing to disclose. Z.-J.C. has nothing to disclose.
Supported by the Major Program of National Natural Science Foundation of China (81490743), General Program of National Natural Science Foundation of China (81671522), National Health and Family Planning Commission of China (201402004), and National Key Research and Development Program (2016YFC10002).
Reprint requests: Junhao Yan, M.D., Ph.D., Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University, 157 Jingliu Road, Jinan 250001, People's Republic of China (E-mail: yyy306@126.com).

Fertility and Sterility® Vol. ■, No. ■, ■ 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2017.01.006

defined as at least two spontaneous pregnancy losses before 12 weeks' gestation, is another common reproductive disorder (5, 6). Historically, ERM has been attributed to either genetic disorders, uterine malformations, endocrine dysfunctions, autoimmune diseases, thrombophilias, or unexplained causes (6). Recently, aneuploid embryos have been discovered to account for >50% of early miscarriages (7, 8).

Aneuploidy, defined as structural and numeric aberrations of chromosomes, is prevalent in human embryonic development (9). It is considered to be a primary genetic origin of implantation failure, miscarriage, and congenital abnormalities, both naturally and after IVF (9–12). Despite extensive investigation, little is known about the complex origins and mechanisms underlying aneuploidy. Although most studies support the considerable role of oocyte meiotic-origin error in aneuploidy formation, accumulated evidence has highlighted the importance of post-zygotic mitotic-origin aneuploidy in reproductive losses (13, 14). The phenomenon that aneuploidy incidences differ even among age-similar women indicates that parental genome variants may be involved in aneuploidy formation and account for variations in aneuploidy rates occurring among individuals (15).

Recently, McCoy et al. first reported that a common maternal genetic variant, rs2305957, encompassing the *PLK4* gene contributed to mitotic-origin aneuploidy risk during human early embryo development (10). The single-nucleotide polymorphism (SNP) rs2305957 is located in chromosome 4 region q28.1, and the frequency of minor allele (A) in diverse human populations ranges from 20% to 45%. Based on a genome-wide association study, McCoy et al. disclosed that the SNP rs2305957 was most strongly associated, with the minor allele (A) conferring a significantly increased rate of mitotic error and consequent embryonic mortality. Women with the high-risk genotype (AA) at rs2305957 formed significantly more aneuploid embryos at cleavage stage and fewer blastocysts. Further linkage disequilibrium (LD) analysis revealed that seven genes in a low-recombination region spanning from chromosome 4 regions q28.1 to q28.2 were tightly linked to the high-risk variant, including *INTU* (inturned planar cell polarity [PCP] protein), *SLC25A31* (solute carrier family 25 member 31), *HSPA4L* (heat shock protein family A member 4 like), *PLK4* (Polo-like kinase 4), *MFSD8* (major facilitator superfamily domain containing 8), *LARP1B* (La ribonucleoprotein domain family member 1B), and *PGRMC2* (progesterone receptor membrane component 2). The *PLK4* gene, which contains two nonsynonymous SNPs, rs3811740 (S232T) and rs17012739 (E830D), within the coding sequence in this associated region, was singled out as a candidate causal gene based on its known role in the centrosome cycle, a process whose confusion can cause aneuploidy. The maternal genetic variant rs2305957 might contribute to mitotic-origin aneuploidy by disrupting the *PLK4* gene, thus causing abnormal chromosome segregation and cell division.

Therefore, the purpose of the present study was to evaluate whether common variant rs2305957 confers risk to embryo development in Northern Chinese Han (CHN) women. We first assessed the relevance of maternal variant rs2305957 and embryo development in infertile women undergoing IVF treatment. Given the important effect of embryonic aneuploidy in ERM, we further investigated whether maternal variant rs2305957 is related to ERM.

MATERIALS AND METHODS

Study Participants

A total of 2,015 infertile female patients who underwent IVF, 530 female patients with ERM, and 600 fertile female control subjects of the CHN population were recruited in the Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University. The study was approved by the Institutional Review Board of Reproductive Medicine, Shandong University, and all individuals provided written informed consents.

The first cohort of this study comprised 2,015 infertile female patients aged 20–40 years. The records of their first IVF treatment cycles were reviewed. Those infertile women who were characterized by the following issues were excluded: couples with chromosomal abnormalities; insufficient ovarian reserve (antral follicle count <5 follicles or FSH level >12 IU/L); poor ovarian response (<4 oocytes retrieved with a conventional stimulation protocol); known uterine or endometrial pathologies, such as uterine malformation, intrauterine adhesions, hydrosalpinx, endometriosis, or adenomyosis; and previous adverse pregnancy outcomes or complications.

In addition, 530 female patients who suffered from ERM and 600 age-matched fertile female control subjects were recruited in the second cohort of this study. All ERM patients had undergone a comprehensive examination to exclude known risk factors of ERM: couples with abnormal karyotypes, uterine pathologies, abnormal endocrinology functions, autoimmune dysfunction, infectious diseases, thrombophilia, and other systemic disorders. All of the enrolled control subjects had at least one live birth and no history of miscarriage or any other adverse pregnancy outcomes or complications.

SNP Genotyping

Genomic DNA was extracted from peripheral blood leukocytes with the use of a commercially available QIAamp DNA Blood Mini Kit (Qiagen). SNP rs2305957 genotyping was detected by means of polymerase chain reaction (PCR) amplification in the presence of a fluorescent double-stranded DNA binding dye combined with subsequent high-resolution melting (HRM) analysis on a Light Cycler 480 (Roche Diagnostics) (16). Briefly, PCR amplification was performed in a 10-μL reaction volume consisting of 20 ng genomic DNA, 1 μL 10× PCR buffer (20 mmol/L Mg^{2+} Plus; Takara), 0.8 μL dNTPs (2.5 mmol/L; Takara), 0.05 μL Hot-Start Taq DNA polymerase (5 U/μL; Takara), 0.5 μL forward and reverse primers (10 μmol/L), 0.5 μL LC-Green Plus (Idaho Technology), and H_2O. The amplification was achieved by a Hot-Start PCR protocol: first at 95°C for 5 minutes, then 35 cycles at 95°C for 10 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. After amplification, the PCR products