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The relationship between MMP-9 promoter polymorphism and IVF outcome

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Abstract

Angiogenesis, invasion and decidualization play an important role in implantation and embryo development. Matrix metalloproteinases (MMPs) are crucial for the degradation/remodeling of the extracellular matrix, and are involved in spiral artery formation and invasion of endometrium during implantation. A functional single-nucleotide polymorphism (SNP) in the *MMP9* promoter, -1562C/T, is known to influence gene expression in allele-specific manner. The present study evaluated the association between maternal genotype of SNP -1562C/T of *MMP9* and in vitro fertilization and embryo transfer (IVF-ET) outcome in infertile women. This case–control study was comprised of infertile patients (n= 123) and women having one healthy child as controls (n= 147). Genotyping for SNP-1562C/T was performed by PCR/RFLP. Allele and genotype distribution did not differ significantly between patients and controls ($P>0.05$). The *MMP9* genotype frequencies amongst the 123 cases were C/C=73.17%, C/T=24.40% and T/T=2.43%; the C and T allele frequencies were 85.36% and 14.63%, respectively. The *MMP9* genotype frequencies amongst the 147 controls were C/C=73.46%, C/T=20.40% and T/T=6.12%; the C and T allele frequencies were 83.67% and 16.32%, respectively. In conclusion, the results of this study indicate that SNP -1562C/T of *MMP9* may not be associated with IVF-ET outcome in this population.

Key words: Embryo Implantation, In Vitro Fertilization, Embryo Trnansfer, Matrix Metalloproteinase-9, Genetic Polymorphism.

Introduction

Global estimations suggest that nearly 72.4 million couples experience fertility problems (1). Infertility is a disorder of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Of all couples classified as infertile, female infertility accounts for about 40–50%. In 30–40% of infertile couples, male infertility is the cause, while the remaining 10–30% either is attributed to both male and female infertility or is unexplained. Moreover, the number of women experiencing infertility is expected to increase in the next 20 years due to women delaying childbearing. An increase in demand for infertility treatment is anticipated as a consequence (2).

The proportion of infertile couples seeking any infertility medical care ranged from 42% to 76.3% in more developed countries and from 27% to 74.1% in less developed countries (1). Fertility requires the production of a viable oocyte, transportation through the fallopian tube, and fertilization by a viable spermatozoon. The resulting zygote must then enter the uterus and get implanted into a suitably prepared endometrial lining (3). A defect in any of these steps will result in infertility. Indeed, the advances in basic research and the practical applications of the research that have been performed since the first successful in vitro fertilization (IVF) in 1978, have given rise to unthinkable strategies for achieving human reproduction (4). IVF is the most successful of the infertility treatments, and for many people is the last possibility for pregnancy (5). IVF is used in the treatment of various forms of infertility including endometriosis, ovulatory dysfunction, pelvic adhesions, cervical factor, tubal disease, luteal defects, immunological causes, male factor, and unexplained infertility. However, despite the progress achieved, people undertaking IVF often face a considerable risk of failure. There are many possible causes that may be responsible for this failure, including inappropriate stimulation protocol and its execution, adverse conditions in the embryology laboratory, a cycle-specific suboptimal response, a genetic abnormality in the gametes of either the male or female partner or a genetic abnormality in the embryo, maternal age and embryo implantation (6, 7).

Embryo implantation represents a critical step of the reproductive process and consists of a unique biological phenomenon. Successful implantation requires a receptive endometrium, a functional embryo at the blastocyst developmental stage and a synchronized dialogue between maternal and embryonic tissues (8). Implantation has three stages: apposition, adhesion and penetration. Apposition is an unstable adhesion of the blastocyst to the endometrial surface (9). This is followed by the adhesion stage in which the association of the trophoblast and the luminal epithelium is sufficiently intimate as to resist dislocation of the blastocyst by flushing the uterine lumen. Following adhesion, invasion or penetration of embryo occurs through the luminal epithelium into the stroma to establish a relationship with the maternal vasculature (10). With implantation, extensive remodeling of the uterus occurs throughout each reproductive cycle to support placentation. This remodeling of the extracellular environment depends on the cyclic hormonal changes associated with each estrous or menstrual cycle. In the uterus, there is extraordinary turnover of the endometrial connective tis-

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sue matrix during each menstrual cycle. In contrast, this turnover encompasses the complete breakdown and loss of this layer, followed by its subsequent regrowth (11). Several benign gynecological disorders including endometriosis, hydrosalpinx, leiomyoma and polycystic ovarian syndrome (PCOS) are associated with decreased cycle fecundity and impaired uterine receptivity (12). Assisted reproductive technology (ART) tools are now available that enable the selection of high-quality embryos, and ART protocols continue to evolve with the aim of achieving higher pregnancy rates. However, despite these advances, implantation rates are still relatively low and have not increased sufficiently in the last decade to allow widespread adoption of single-embryo transfer (13).

The dynamic changes in the uterine extracellular architecture are regulated, in part, by the matrix metalloproteinase (MMP) system. The MMP system acts to control connective tissue remodeling processes throughout the body and is comprised of proteolytic component, the MMPs, regulatory component and the associated tissue inhibitors of metalloproteinases. This multigene family of endopeptidases is capable of degrading components of the extracellular matrix and is important to many physiological and pathological processes, including embryo implantation and cyclic endometrial breakdown (14). Earlier studies showed that MMPs and their inhibitors (tissue inhibitors of metalloproteinase) are crucial during implantation and mediate in vitro trophoblast penetration. They are regulated by several cytokines, including IL-1 and TGF-β, which are expressed by decidual stromal cells and trophoblast cells (15). There are eight distinct structural classes of MMPs including secreted and membrane-type MMPs (MT-MMPs) (16). MMP-9 (92 kD) is an important secreted enzyme produced by a gene located in chromosome 20. Being involved in the degradation of the basement membrane (primarily collagen type IV), MMP9 found to be critical to trophoblast cells invasion. Moreover, in embryo implantation and placentation, the expression of MMP9 may play a central role (17). If the MMPs' activity or expression becomes interfered, it could give rise to inappropriate remodeling and as a result implantation failure. The aim of this project was to study the association between maternal genotype of SNP -1562C/T of *MMP9* and IVF-ET outcome in infertile women in northern Iran.

Materials and methods

Clinical samples

In this case–control study, 123 infertile patients enrolled who had unsuccessful IVF history and 147 control subjects who had at least one child were randomly selected among individuals visiting hospitals for regular health checks. Patients were recruited from Alzahra Hospital, IVF section, Rasht, Iran. All these subjects were women with median age of 34 years. A structured questionnaire was used during an in-person interview to elicit information on demographic features. Further, maternal pathology or genetic anomaly, maternal inflammatory disease, uterine malformation, diabetes, lupus erythematosus and embryonic aneuploidy were excluded from the study. The blood samples were collected from both groups. This study has been approved by the local ethical committee and consent has been obtained from patients. Genomic DNA was extracted from EDTA anticoagulated whole peripheral blood using DNG plus (Cinnagen, Iran).

Polymerase chain reaction-restriction fragment length polymorphism (PCR– RFLP) analysis

To analyze the -1562 C/T polymorphism, we amplified a region of the MMP-9 promoter with forward primer 5'-ACTTATTACGGTGCTTGACACA-3' and reverse primer 5'- TCACTCCTTTCTTCCTAGCCA -3' (BIONEER, Republic of Korea). The primers were designed by means of Oligo7 software (version 7.54, USA). PCR reactions were performed in 25µl reaction volume containing 1µl of each primer (100pmol/µl), 2.5 µl of 10x reaction buffer (100 mM Tris–HCl pH 8.3 at 25°C, 500 mM KCl, 15 mM $MgCl_2$), 0.5 µl of dNTPs (2.5 mM), 0.75 µl of $MgCl_2$, 0.3 µl of *Taq* DNA polymerase (Cinnagen, Iran), 4 µl of genomic DNA (80 ng/µl) and 14.95 µl H_2O. PCR cycle conditions consisted of an initial denaturation step of 95ºC for 5 minutes followed by 35 cycles of 30 seconds at 95ºC, 30 s at 57ºC, 1 min at 72ºC, and a final extension at 72ºC for 10 minutes. This results in an amplification product of 690 bp (Figure 1). The PCR product fragments were digested with SphI restriction enzyme (Fermentase, USA) for 4.5 hours at 37ºC. SphI cannot digest the C allele (690 bp) but generates 252 bp and 438 bp fragments for the T allele. Digested fragments were separated by electrophoresis on 2% agarose gels for 40 minutes at 70 V (Figure 2) so heterozygote samples had a combination of both alleles (690 bp, 252 bp and 438 bp bands).

Statistical analysis

In order to assess a possible distortion in allele frequencies between cases and controls, we performed a chi-square test with one degree of freedom for both allelic and genotypic distributions between the groups of cases and controls. Significant association was defined by $P \leq 0.05$.

Results

MMP9 1562C/T polymorphism was analysed in all the samples. Table 1 shows the allelic and genotypic distributions of the polymorphism in cases and controls. We compared the distribution of *MMP9* 1562C/T polymorphism, using χ^2 test.

There were no statistically significant differences in the allelic frequencies or genotype distributions

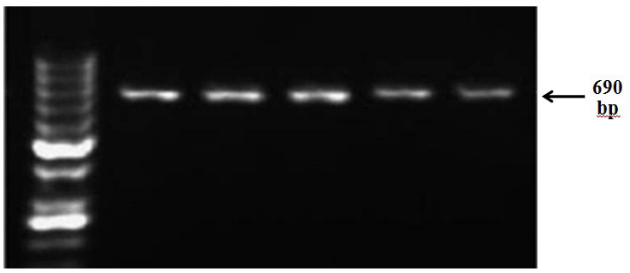

Figure 1. Agarose gel electrophoresis of PCR product. products (Lanes 1-5, 690 bp amplicon, Lane M, molecular size marker).

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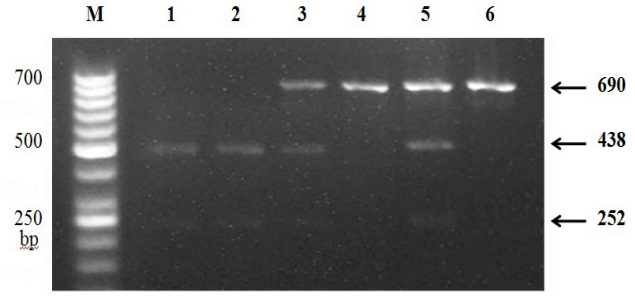

Figure 2. Detection of the MMP9 1562C/T by PCR-RFLP. C/C homozygote had a single band of 690 bp (lanes 4 and 6). C/T heterozygote had three bands of 690, 438 and 252 bp (lanes 3 and 5) and T/T homozygote had two fragment of 252 and 438 bp (lanes 1 and 2). M = 100 bp ladder.

Table 1. Genotype and allele frequencies of MMP9 single-nucleotide polymorphism -1562C>T in infertile and control groups. Values are n or n (%) unless otherwise stated.

	Patients n (%)	Controls n (%)
Genotype		
CC	90 (73.17%)	108 (73.46%)
CT	30(24.40%)	30 (20.40%)
TT	3(2.43%)	9 (6.12%)
Alleles		
C	210(85.36%)	246(83.67%)
T	36(14.63%)	48(16.32%)

between cases and controls (P>0.05). The *MMP9* genotype frequencies amongst the 123 cases were C/C=73.17%, C/T=24.40% and T/T=2.43%; the C and T allele frequencies were 85.36% and 14.63, respectively. The *MMP9* genotype frequencies amongst the 147 controls were C/C=73.46%, C/T=20.40% and T/T=6.12%; the C and T allele frequencies were 83.67% and 16.32%, respectively.

Discussion

30 years after the birth of the first 'test tube' baby, IVF has become a widely available treatment for most causes of sub-fertility. However, in humans and also in domestic animals the failure rate for this process is high. Despite ongoing advances in the associated assisted reproductive technologies (ART), pregnancy rates remain around 20–30% per started cycle (18). Being involved in a reciprocal dialogue between the blastocyst and uterus, implantation found to be a complex process essential for continued embryonic development within the uterus and achieve successful pregnancy (19, 20). So far, factors including production of a hatched blastocyst capable of implanting, development of endometrium (21), decidualization, vascular remodeling and invasion have been indicated as prominent processes for successful embryo implantation. Decidualization involves the reorganization of the extracellular matrix (ECM) (22). MMPs are implicated in ECM remodeling /degradation and allow trophoblast cells to invade the maternal endometrium and myometrium during embryo implantation (23). Inefficient or abnormal ECM remodeling /degradation of endometrium due to aberrant gene expression coordinating this process might lead to the failure of implantation. The *MMP9* SNP-1562C/T has been mostly studied in relation to cancer risk and both alleles have been differently associated with different cancers. The C allele has been associated with a decreased risk of prostate cancer (24) and T allele has been associated with the risk of breast cancer (25). In other studies, the C allele was found to be associated with increased risks of squamous cell carcinoma of the lung (26), endometrial carcinoma (27) and colorectal cancer (28). The-1562C/T polymorphism has also been studied in pregnancy-related disorders apart from cancer, such as pre-eclampsia (29, 30) and intrauterine growth restriction (31). The T allele was more commonly found in gestational hyper-tension subjects compared with the healthy pregnant group. However, no differences were found in the genotypes or allele distributions for the polymorphism when the pre-eclampsia and healthy pregnant groups were compared (30). In the present study, the C allele frequency (83.67%) in healthy controls was almost similar to the result obtained by Singh and colleagues which was 82% (32). There was no statistically significant difference in the genotype distributions between cases and controls (P>0.05). These results are in consistent with the results of Gremlich and colleagues who demonstrated that MMP9 gene polymorphism is not associated with IVF outcome (33).

The results of this study indicate that SNP -1562C/T of *MMP9* may not associate with IVF-ET outcome in this population. Further studies are needed to confirm the role of *MMP9* gene in IVF outcome.

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References

1. Boivin, J., Bunting, L., Collins, J.A. and Nygren, K.G., International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum. Reprod*. 2007, **22**: 1506-1512. doi: 10.1093/humrep/dem046

2. Stephen, E.H. and Chandra, A., Updated projections of infertility in the United States: 1995-2025. *Fertil Steril.* 1998, **70**: 30-34. doi: 10.1016/S0015-0282(98)00103-4

3. Makar, R.S. and Toth T.L., The evaluation of infertility. *Am. J. Clin. Pathol*. 2002, **117**: Suppl, S95-103. doi: 10.1309/W8LJ-K377-DHRA-CP0B

4. Fasouliotis, S.J. and Schenker, J.G., A historical perspective of the clinical evolution of the assisted reproductive technologies. *Gynecol. Endocrinol*. 1999, **13**: 420-440. doi: 10.3109/09513599909167589

5. Wright, V.C., Schieve, L.A., Reynolds M.A., Jeng, G. and Kissin, D., Assisted reproductive technology surveillance--United States, 2001. *MMWR. Surveill. Summ*. 2004, **53**: 1-20.

6. Tarin, J.J., Brines, J. and Cano, A., Long-term effects of delayed parenthood. *Hum. Reprod*. 1998, **13**: 2371-2376. doi: 10.1093/humrep/13.9.2371

7. Rubio, C., Buendía, P., Rodrigo, L., Mercader, A., Mateu, E., Peinado, V., Delgado, A., Milán, M., Mir, P., Simón, C., Remohí, J. and Pellicer, A., Prognostic factors for preimplantation genetic screening in repeated pregnancy loss. *Reprod. Biomed. Online* 2007, **18**: 687-93. doi: 10.1016/S1472-6483(10)60015-6

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8. Simon, C., Martin, J.C. and Pellicer, A., Paracrine regulators of implantation. *Baillieres Best Pract. Res. Clin. Obstet. Gynaecol*. 2000, **14**: 815-826. doi: 10.1053/beog.2000.0121

9. Tabibzadeh, S. and Babaknia, A., The signals and molecular pathways involved in implantation, a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue invasion. *Hum. Reprod*. 1995, **10**: 1579-1602. doi: 10.1093/HUMREP/10.6.1579

10. Sharkey, A.M. and Smith, S.K., The endometrium as a cause of implantation failure. *Best Pract. Res. Clin. Obstet*. *Gynaecol*. 2003, **17**: 289-307. doi: 10.1016/S1521-6934(02)00130-X

11. Uchida, H., Maruyama, T., Nishikawa-Uchida, S., Oda, H., Miyazaki, K., Yamasaki, A. and Yoshimura, Y., Studies using an in vitro model show evidence of involvement of epithelial-mesenchymal transition of human endometrial epithelial cells in human embryo implantation. *J. Biol. Chem*. 2012, **287**: 4441-50. doi: 10.1074/jbc.M111.286138

12. Donaghay, M. and Lessey, B.A., Uterine receptivity: alterations associated with benign gynecological disease. *Semin. Reprod. Med.* 2007, **25**: 461-475. doi: 10.1055/s-2007-991044

13. Andersen, A.N., Gianaroli, L., Felberbaum, R., de Mouzon, J. and Nygren, K.G., Assisted reproductive technology in Europe, 2001. Results generated from European registers by ESHRE. *Hum. Rep*. 2005, **20**: 1158-1176.

14. Curry TE, Jr. and Osteen, K.G., The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocr. Rev*. 2003, **24**: 428-465. doi: 10.1210/er.2002-0005

15. Huang, H.Y., Wen, Y., Irwin, J.C., Kruessel, J.S., Soong, Y.K. and Polan, M.L., Cytokine-mediated regulation of 92-kilodalton type IV collagenase, tissue inhibitor or metalloproteinase-1 (TIMP-1), and TIMP-3 messenger ribonucleic acid expression in human endometrial stromal cells. *J. Clin. Endocrinol. Metab*. 1998, **83**: 1721-1729. doi: 10.1210/jc.83.5.1721

16. Egeblad, M. and Werb, Z., New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer* 2002, **2**: 161-174. doi: 10.1038/nrc745

17. Lockwood, C.J., Oner, C., Uz, Y.H., Kayisli, U.A., Huang, S.J., Buchwalder, L.F., Murk, W., Funai, E.F. and Schatz F., Matrix metalloproteinase 9 (MMP9) expression in preeclamptic decidua and MMP9 induction by tumor necrosis factor alpha and interleukin 1 beta in human first trimester decidual cells. *Biol. Reprod*. 2008, **78**: 1064-72. doi: 10.1095/biolreprod.107.063743

18. Macklon, N.S., Stouffer, R.L., Giudice, L.C. and Fauser, B.C., The science behind 25 years of ovarian stimulation for in vitro fertilization. *Endocr. Rev*. 2006, **27**: 170-207.

19. Psychoyos, A., Hormonal control of ovoimplantation. *Vitam. Horm*. 1973, **31**: 201-256. doi: 10.1016/S0083-6729(08)60999-1

20. Dey, S.K., Lim, H., Das, S.K., Reese, J., Paria, B.C., Daikoku, T. and Wang, H., Molecular cues to implantation. *Endocr. Rev*. 2004, **25**: 341-373. doi: 10.1210/er.2003-0020

21. Sharkey, A., Cytokines and implantation. *Rev. Reprod*. 1998, **3**: 52-61. doi: 10.1530/revreprod/3.1.52

22. Reynolds, L.P. and Redmer D.A., Utero-placental vascular development and placental function. *J. Anim. Sci*. 1995, **73**: 1839-1851.

23. Campbell, S., Rowe, J. and Jackson, C.J., Gallery ED. In vitro migration of cytotrophoblasts through a decidual endothelial cell monolayer: the role of matrix metalloproteinases. *Placenta* 2003, **24**: 306-315. doi: 10.1053/plac.2002.0911

24. Sfar, S., Saad, H., Mosbah, F., Gabbouj, S. and Chouchane, L., TSP1 and MMP9 genetic variants in sporadic prostate cancer. *Cancer Genet. Cytogenet*. 2007, **172**: 38-44. doi: 10.1016/j.cancergencyto.2006.07.014

25. Sadeghi, M., Motovali-bashi, M. and Hojati, Z., MMP-9 promoter polymorphism associated with tumor progression of breast cancer in Iranian population. *Int. J. Integ. Biol.* 2009, **6**: 1-37.

26. Rollin, J., Regina, S., Vourc'h, P., Iochmann, S., Blechet, C., Reverdiau, P. and Gruel, Y., Influence of MMP-2 and MMP-9 promoter polymorphisms on gene expression and clinical outcome of non-small cell lung cancer. *Lung cancer* 2007, **56**: 273-280. doi: 10.1016/j.lungcan.2006.11.021

27. Sugimoto, M., Yoshida, S., Kennedy, S., Deguchi, M., Ohara, N. and Maruo, T., Matrix metalloproteinase-1 and -9 promoter polymorphisms and endometrial carcinoma risk in a Japanese population. *J. Soc. Gynecol. Investig.* 2006, **13**: 523-529. doi: 10.1016/j.jsgi.2006.07.001

28. Park, K.S., Kim, S.J., Kim, K.H. and Kim, J.C., Clinical characteristics of TIMP2, MMP2, and MMP9 gene polymorphisms in colorectal cancer. *J. Gastroenterol. Hepatol*. 2011, **26**: 391-7. doi: 10.1111/j.1440-1746.2010.06504.x

29. Coolman, M., de Maat, M., Van Heerde, W.L., Felida, L., Schoormans, S., Steegers, E.A.P., Bertinad, R.M. and de Groot, C.J.M., Matrix metalloproteinase-9 gene -1562C/T polymorphism mitigates preeclampsia. *Placenta* 2007, **28**: 709-713. doi: 10.1016/j.placenta.2006.06.017

30. Palei, A.C., Sandrim, V.C., Duarte, G., Cavalli, R.C., Gerlach, R.F. and Tanus-Santos, J.E., Matrix metalloproteinase (MMP)-9 genotypes and haplotypes in preeclampsia and gestational hypertension. *Clinica. Chimica. acta; international journal of clinical chemistry* 2010, **411**: 874-877. doi: 10.1016/j.cca.2010.03.002

31. Gremlich, S., Nguyen, D., Reymondin, D., Hohlfeld, P., Vial, Y., Witkin, S.S. and Gerber, S., Fetal MMP2/MMP9 polymorphisms and intrauterine growth restriction risk. *Journal of reproductive immunology.* 2007, **74**: 143-151. doi: 10.1016/j.jri.2007.02.001

32. Singh, K., Nair, R.R. and Khanna, A., Functional SNP -1562C/T in the promoter region of MMP9 and recurrent early pregnancy loss. *Reproductive biomedicine online* 2012, **24**: 61-65. doi: 10.1016/j.rbmo.2011.09.007

33. Gremlich S, Fratta S, Rebellato E, Uras R, Reymondin D, Damnon F, Germond M, Gerber S. Interleukin-1 receptor antagonist gene (IL-1RN) polymorphism is a predictive factor of clinical pregnancy after IVF. Hum Reprod. 2008, **23**: 1200-6. doi: 10.1093/humrep/den034

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