J Assist Reprod Genet (2014) 31:379–382
DOI 10.1007/s10815-013-0163-2

GENETICS

Glutathione S-transferase A1 polymorphism and the risk of recurrent spontaneous abortion in Chinese Han population

Chen Zong · Yanwei Sha · Huifen Xiang · Jing Wang · Dawei Chen · Juan Liu · Binbin Wang · Yunxia Cao

Received: 6 October 2013 / Accepted: 17 December 2013 / Published online: 5 January 2014

Abstract
Objective Recurrent spontaneous abortion (RSA) is a multifactor and distressing disease. There are still approximately half of the RSA patients with cause not being identified to date. Accumulating studies have confirmed that genetic polymorphisms in glutathione S-transferases (GSTs) were associated with the risk of recurrent spontaneous abortion. In this study, we aimed to investigate the relationship between the polymorphism of GSTA1, which is GSTA1 -69C/T (rs3957357), and the development of recurrent spontaneous abortion.

Methods A case–control study of 127 cases with RSA and 112 ethnic and age matched women as controls was conducted. And measurement of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was performed to genotype all of samples in order to analyze the association between GSTA1 -69C/T (rs3957357) and the risk of RSA.
Results We found that the frequencies of genotypes between cases and controls have no significant difference (P=0.908) and GSTA1 mutant allele GSTA1 −69 T was present at a frequency of 0.122 in case group, while in controls the frequency was 0.125 (P=0.922).
Conclusion The polymorphism of GSTA1 (rs3957357) may not be associated with the risk of recurrent spontaneous abortion in Chinese Han population.

Keywords GSTA1 · Recurrent spontaneous abortion · Variant · Genetics

Capsule The *GSTA1* polymorphism may not be associated with the development of RSA.

Chen Zong and Yanwei Sha contributed equally to the work.

C. Zong · H. Xiang · D. Chen · Y. Cao (✉)
Reproductive Medicine Center, The First Affiliated Hospital, Anhui Medical University, 218 Jixi Road, Hefei, Anhui 230022, China
e-mail: caoyunxia7@126.com

C. Zong · H. Xiang · D. Chen · Y. Cao
Institute of Reproductive Genetics, The First Affiliated Hospital, Anhui Medical University, Hefei 230022, China

Y. Sha
Maternal and Child Health Hospital of Xiamen city, Reproductive Medicine Center, 361005 Xiamen city, Fujian Province, China

J. Wang
Department of Medical Genetics, School of Basic Medical Sciences, Capital Medical University, Beijing, China

J. Liu · B. Wang (✉)
Center for Genetics, National Research Institute for Family Planning, 12 Dahuisi Road, Haidian, Beijing 10081, China
e-mail: wbbahu@163.com

B. Wang
Graduated School, Peking Union Medical College, Beijing 100080, China

Introduction

Recurrent spontaneous abortion (RSA) is defined by two or more consecutive failed clinical pregnancy [1]. As a common and frustrating pregnancy complication, it has been reported that approximately 15 % clinical pregnancies ended up with miscarriage, and RSA occurs in 0.4–2 % of diagnosed pregnancies [2]. Since RSA is a multifactor disease, chromosomal abnormalities, thrombophilic disorders, anatomical anomaly, endocrine abnormalities, immune dysfunction, antiphospholipid syndrome and infections have been identified as risk factors for RSA [3]. However, there are only half of the RSA patients with cause identified, the other half still need to be elucidated, which are called unexplained recurrent spontaneous abortion (URSA). What's worse, the safety and efficacy of current therapies to prevent RSA are inadequate, and this is due, in part, to our poor understanding of the

complex mechanisms underlie the RSA. In recent years, accumulating studies have suggested that oxidative stress (OS) plays an important role in the development of RSA and the sensitive to oxidative damage could be modified by the genetic variants in individual biotransformation enzymes.

Oxidative stress (OS) [4] is an imbalanced state that the ability to degrade dangerous molecules, such as reactive oxygen species (ROS), is weaker than the generation of them. Since pregnancy is a high metabolic state and accompanied by elevated requirement for tissue oxygen, women with pregnancies are more subject to the oxidative stress compared to the non-pregnant women [5]. A deficient detoxification system in pregnant women may result in the RSA due to the increased exposure of embryo to exogenous and endogenous compounds. These compounds may be degraded by the phase II enzymes, especially glutathione S-transferase (GST). GST gene encodes the glutathione S-transferases (GSTs) which play a key role in detoxification of a wide range of exogenous and endogenous compounds, such as carcinogens, therapeutic drugs, environmental toxins and reactive oxygen species [6]. GSTs are divided into three families: cytosolic, mitochondrial, and the membrane-associated proteins in eicosanoid and glutathione metabolism proteins, among them, the cytosolic subfamily is by far the most abundant one [7]. Numbers of studies have suggested that the genetic polymorphism of cytosolic GST genes was associated with the risk of RSA [8–12].

At present, cytosolic glutathione S-transferases have been identified into eight distinct classes: alpha, kappa, mu, omega, pi, sigma, theta and zeta [13]. GSTA1 gene encodes a glutathione S-transferase belonging to the alpha class. The GSTA1 enzymes have the ability to protect cells from reactive oxygen species, products of peroxidation and also play a role in the metabolism of polycyclic aromatic hydrocarbons, which are dangerous air pollutants [14]. There was study suggest that the genetic variant of GSTA1 gene, which is GSTA1 −69C/T polymorphism (rs3957357) has significant association with the risk of RSA in Italian women with RSA [15].

The aim of our study was to explore the role of GSTA1 −69C/T polymorphism (rs3957357) in URSA pathogenesis in Chinese Han population.

Materials and methods

Selection of patients

This case–control study was performed in 127 women with RSA and 112 control women who were referred to The First Affiliated Hospital of Anhui Medical University *during the years 2010–2012*. RSA was defined as *at least two spontaneous consecutive miscarriages* before 12 weeks of gestation after conceiving from the same partner. All the female with RSA included are primary aborters with no previous history of live births. Furthermore, RSA patients enrolled in the study underwent a routine investigation to exclude the known risk factors for RSA, such as chromosomal aberrations of partners and the embryos, anatomical abnormalities, thrombophilia, metabolic disorders and positive lupus anticoagulant, anticardiolipin antibodies. As a control group, 121 healthy women with at least one previous live birth and no spontaneous abortion were included. The women enrolled in control group had no pregnancy-associated complications either. Informed written consent was obtained from all individuals. The study was approved by the Ethics Review Committee on Family Planning of Anhui Medical University.

DNA extraction

Genomic DNAs from Peripheral blood leukocytes of RSA patients and controls were extracted by a QIAGEN blood kit (Qiagen, Hilden, Germany) according to the manufacturer's methods.

Genotyping

Genotype analysis of GSTA1 −69C/T polymorphism was detected by Polymerase Chain Reaction Fragment Length Polymorphism (PCR-RFLP).

The primers used for PCR procedure were selected by the Genetool, then we inputted the primer sequences to the BLAST database to compare the homology with other GSTs and to make sure that the primers were unique to GSTA1. Primers used were for GSTA1 forward primer CCAACATA ACCCCCTACATGGTAT and reverse primer CAATTCTT GAACTGTCACCCAAGC. The PCR procedure was as follows: an pre-incubation at 95 centigrade for 3 min, the PCR mixture was subject to 35 cycles of 95 °C for 30 s, 60.9 °C for 30s and 72 °C for 40s. Followed, the PCR reaction mixture products were analyzed by gel electrophoresis.

To determine the polymorphism of the GSTA1 −69C/T, the resulting 899 bp fragment was digested with the restriction enzyme EarI(Fermentas, EU) [16]. The genotypes were defined by the different electrophoretic bands. The wild type allele (C) still presented a 899 bp band as a result of having no EarIsite. The heterozygous (CT) and rare homozygous (TT) resulted in 3 bands and 2 bands, respectively.

Statistical analysis

Demographic and clinical characteristics of patients and control samples were described in terms of median±minimum/maximum. The standard chi-test was used to assess the differences in the genotype distributions and the Hardy-Weinberg equilibrium among RSA patients and healthy females. The statistically significance was accepted when P value <0.05.

Table 1 Characteristics of women with URSA and healthy individuals

	Patients	Controls
No.of cohorts	127	112
Age	27.81±4.19	29.71±4.20
No.of abortion	2.54±0.68	0
No.of live births	0	1.11±0.36
menstrual cycle(d)	29.64±3.30	30.23±1.39

Results

Genotype and allele frequencies of 127 women with RSA and 112 healthy women were analyzed for the variant single nucleotide polymorphism (SNP) in GSTA1. Three different genotypes (CC, CT and TT) of GSTA1 −69C/T polymorphism reported previously were observed after digesting the amplified DNA fragment of 899 bp with the restriction enzyme EarI [17]. The enzymatic restriction products of the rs3957357 were of the expected size. Three different banding patterns were obtained: the wild type (CC) produced only a band at 899 bp, homozygous mutant genotype (TT) is characterized by 639 bp and 260 bp products, while heterozygous genotype (CT) resulted in three bands (899, 639 and 260 bp). All genotype frequencies in our controls were within the ranges reported previously in Chinese population [18].

The characteristics of patients and control group recruited for this study were summarized in Table 1. The genotype frequencies of GSTA1 −69C/T polymorphism in both case and control groups were in Hardy-Weinberg equilibrium (χ^2= 0.842, P=0.359; χ^2=0.047, P=0.829).

Genotypes and allelic frequencies of GSTA1 −69C/T polymorphism were showed in Table 2. Between both study samples, there was no significant difference in the frequencies of the GSTA1 alleles. We found that GSTA1 variant allele GSTA1 −69 T was present at a frequency of 0.122 in case group, while in controls the frequency was 0.125 (χ^2= 0.009592, P=0.922). The chi-test for GSTA1 showed that the genotypes distribution of case groups did not differ significantly from control groups, either (χ^2=0.193, P=0.908). In our analysis of GSTA1 −69C/T, the proportion of the wild genotype, heterozygous and homozygous genotype were 0.780, 0.197 and 0.024 respectively in women with RSA, whereas in controls the proportions of these three genotypes were 0.768, 0.214 and 0.018, respectively.

Discussion

Pregnancy is a dramatic complicated process, accompanied with increased metabolic demand and elevated oxygen requirement. Thus, compared to non-pregnancy women, pregnancy women is in a state of high oxidative load. The genetic variants in metabolic detoxification activities could provoke the imbalance between the interactions of phase Iand II biotransformation enzymes, as a result, increased exposure of conceptus to endo- and exogenous toxins might contribute to the development of RSA [12].

GSTA is a subfamily of GSTs, which is most expressed in the liver. There was report suggesting that glutathione S-transferase alpha (GSTA) may have potential defense mechanisms against reactive oxygen species in mouse embryonic cells [19]. Recently, Polimanti,R have confirmed the genetic association between GSTA1 −69C/T polymorphism and the risk of RSA in Italian population. However, the exact mechanisms of GSTA1 in the development of RSA has not been clarified, thus, in order to provide a theoretical basis for subsequent functional exploration, the aim of this study was to investigate the possible association between rs3957357 polymorphism at the GSTA1 gene and the risk of RSA in Chinese Han population.

Our study reported that the GSTA1 −69C/T polymorphism have no significant association with the development of RSA in Chinese Han population. Therefore, the results suggested that the GSTA1 −69C/T polymorphism may be excluded as a genetic cause of RSA in Chinese Han population or perhaps it has a week function in the risk of RSA.

Our study failed to reveal the relationship between the GSTA1 polymorphism and risk of RSA in Chinese Han patients, while this result may not be considered contradictory

Table 2 Frequency distribution of GSTA1 genotype and relative alleles in case and control group

	Allele		Genotype		
	C (freq)	T (freq)	C/C (freq)	C/T (freq)	T/T (freq)
Case n=127	223 (0.878)	31 (0.122)	99 (0.780)	25 (0.197)	3 (0.024)
Control n=112	196 (0.875)	28 (0.125)	86 (0.768)	24 (0.214)	2 (0.018)
χ^2		0.009592			0.193
P value[a]		0.922			0.908

[a] Calculated by χ^2-test (Pearson)

to the report in Italian individuals. This discrepancy might possibly due to factors as follows:

On the one hand, the frequency distribution of GSTA1 −69C/T genotype was not identical in different ethnics. According to the study of Ping [18], Chinese population has lower mutant allele frequency (12.9 %) of GSTA1 gene as compared to the Caucasian (26 %), African-American (38 %), and Spanish (39 %). This diversity may lead to the contrary observation between the previous study and ours.

On the other hand, RSA is a kind of multifactor disease, both genetic, environment factors and life-style can have an effect on the development of the RSA. Among them, caffeine and cigarette smoking are considered to be fetotoxic and may have an adverse effect on the pregnancy outcome. There was research suggested that GSTA1 gene may play an important role in detoxification in the fetus exposure to the toxicants of maternal smoking [20]. Bech,B.H. and colleagues also hypothesized that GSTA1 may be indispensable in the metabolism of caffeine [21]. Unfortunately, our study failed to collect relative data. Therefore, the relationship between life-style and RSA may be analyzed in our further research.

In conclusion, our study reported that the GSTA1 −69C/T polymorphism has no significant association with the development of RSA in Chinese Han population for the first time. However, this result could not be considered as a contradiction to the previous study in the Italian population. In the further study, the assessment of cigarette smoking, caffeine intake and alcohol beverage consumption, larger sample size and the included variety of population had better take into consideration in order to better elucidate the relationship and function of GSTA1 in the development of RSA.

Acknowledgments This work was supported by the National Basic Research Program of China (2012CB944704), the National Natural Science Foundation of 150 China (30973197), and Anhui Provincial Natural Science Foundation (11010402166).

The authors thank all the women for their cooperation, and thank the clinicians who provided these patients.

Declaration of interest The authors report no declaration of interest.

References

1. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril. 2013;99(1):63. doi:10.1016/j.fertnstert.2012.09.023.
2. McNamee K, Dawood F, Farquharson R. Recurrent miscarriage and thrombophilia: an update. Curr Opin Obstet Gynecol. 2012;24(4):229–34. doi:10.1097/GCO.0b013e32835585dc.
3. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368(9535):601–11. doi:10.1016/S0140-6736(06)69204-0.
4. Peter Stein T, Scholl TO, Schluter MD, Leskiw MJ, Chen X, Spur BW, et al. Oxidative stress early in pregnancy and pregnancy outcome. Free Radic Res. 2008;42(10):841–8. doi:10.1080/10715760802510069.
5. Gitto E, Reiter RJ, Karbownik M, Tan DX, Gitto P, Barberi S, et al. Causes of oxidative stress in the pre- and perinatal period. Biol Neonate. 2002;81(3):146–57.
6. Polimanti R, Piacentini S, Fuciarelli M. HapMap-based study of human soluble glutathione S-transferase enzymes: the role of natural selection in shaping the single nucleotide polymorphism diversity of xenobiotic-metabolizing genes. Pharmacogenet Genomics. 2011;21(10):665–72. doi:10.1097/FPC.0b013e328349da4d.
7. Frova C. Glutathione transferases in the genomics era: new insights and perspectives. Biomol Eng. 2006;23(4):149–69. doi:10.1016/j.bioeng.2006.05.020.
8. Parveen F, Faridi RM, Das V, Tripathi G, Agrawal S. Genetic association of phase I and phase II detoxification genes with recurrent miscarriages among North Indian women. Mol Human Reprod. 2010;16(3):207–14. doi:10.1093/molehr/gap096.
9. Nonaka T, Takakuwa K, Tanaka K. Analysis of the polymorphisms of genes coding biotransformation enzymes in recurrent miscarriage in the Japanese population. J Obstet Gynaecol Res. 2011;37(10):1352–8. doi:10.1111/j.1447-0756.2011.01529.x.
10. Sata F, Yamada H, Kondo T, Gong Y, Tozaki S, Kobashi G, et al. Glutathione S-transferase M1 and T1 polymorphisms and the risk of recurrent pregnancy loss. Mol Human Reprod. 2003;9(3):165–9.
11. Suryanarayana V, Deenadayal M, Singh L. Association of CYP1A1 gene polymorphism with recurrent pregnancy loss in the South Indian population. Hum Reprod. 2004;19(11):2648–52. doi:10.1093/humrep/deh463.
12. Zusterzeel PL, Nelen WL, Roelofs HM, Peters WH, Blom HJ, Steegers EA. Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. Mol Human Reprod. 2000;6(5):474–8.
13. Dourado DF, Fernandes PA, Ramos MJ. Mammalian cytosolic glutathione transferases. Curr Protein Pept Sci. 2008;9(4):325–37.
14. Coles BF, Kadlubar FF. Human alpha class glutathione S-transferases: genetic polymorphism, expression, and susceptibility to disease. Methods Enzymol. 2005;401:9–42. doi:10.1016/S0076-6879(05)01002-5.
15. Polimanti R, Piacentini S, Lazzarin N, Vaquero E, Re MA, Manfellotto D, et al. Glutathione S-transferase genes and the risk of recurrent miscarriage in Italian women. Fertil Steril. 2012;98(2):396–400. doi:10.1016/j.fertnstert.2012.05.003.
16. Polimanti R, Piacentini S, Lazzarin N, Re MA, Manfellotto D, Fuciarelli M. Glutathione S-transferase variants as risk factor for essential hypertension in Italian patients. Mol Cell Biochem. 2011;357(1–2):227–33. doi:10.1007/s11010-011-0893-3.
17. Coles BF, Morel F, Rauch C, Huber WW, Yang M, Teitel CH, et al. Effect of polymorphism in the human glutathione S-transferase A1 promoter on hepatic GSTA1 and GSTA2 expression. Pharmacogenetics. 2001;11(8):663–9.
18. Ping J, Wang H, Huang M, Liu ZS. Genetic analysis of glutathione S-transferase A1 polymorphism in the Chinese population and the influence of genotype on enzymatic properties. Toxicol Sci Off J Soc Toxicol. 2006;89(2):438–43. doi:10.1093/toxsci/kfj037.
19. Osman AM, van Dartel DA, Zwart E, Blokland M, Pennings JL, Piersma AH. Proteome profiling of mouse embryonic stem cells to define markers for cell differentiation and embryotoxicity. Reprod Toxicol. 2010;30(2):322–32. doi:10.1016/j.reprotox.2010.05.084.
20. O'Shaughnessy PJ, Monteiro A, Bhattacharya S, Fowler PA. Maternal smoking and fetal sex significantly affect metabolic enzyme expression in the human fetal liver. J Clin Endocrinol Metab. 2011;96(9):2851–60. doi:10.1210/jc.2011-1437.
21. Bech BH, Autrup H, Nohr EA, Henriksen TB, Olsen J. Stillbirth and slow metabolizers of caffeine: comparison by genotypes. Int J Epidemiol. 2006;35(4):948–53. doi:10.1093/ije/dyl116.