Systems Biology in Reproductive Medicine

http://informahealthcare.com/aan
ISSN: 1939-6368 (print), 1939-6376 (electronic)

Syst Biol Reprod Med, 2014; 60(5): 304–307
 DOI: 10.3109/19396368.2014.892651

informa healthcare

CLINICAL CORNER: COMMUNICATION

Polymorphisms of glutathione peroxidase 1 (GPX1 Pro198Leu) and catalase (CAT C-262T) in women with spontaneous abortion

Eliza Eskafi Sabet[1], Zivar Salehi[1]*, Siamak Khodayari[2], Samin Sabouhi Zarafshan[1], and Ziba Zahiri[3]

[1]*Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran,* [2]*Department of Biology, International Pardis, University of Guilan, Rasht, Iran, and* [3]*Reproductive Health Research Center, Obstetrics & Gynecology Department, Alzahra Hospital, Guilan University of Medical Sciences, Rasht, Iran*

Abstract

About 10%–15% of conceptions are lost spontaneously prior to 20 weeks. Apart from the clinical problems, genetic variations have also been proposed as a susceptibility factor to miscarriage. Glutathione peroxidase 1 (GPX1) and catalase (CAT) encode two antioxidant enzymes that detoxify H_2O_2 and protect the cells from oxidative damage. A functional polymorphism at codon 198 of the GPX1 gene causes a C/T substitution in exon 2, which encodes for either proline or leucine (Pro198Leu). The CAT gene has a polymorphic site in the promoter region at position −262 (C-262T) which alters the expression and enzyme blood levels, leading to some pathological clinical conditions. In this study, we evaluated the association of these two polymorphisms with the risk of spontaneous abortion. Genomic DNA from 105 cases with spontaneous abortion and 90 healthy women were genotyped using allele-specific PCR (AS-PCR) and polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP). The genetic distributions for GPX1 did not differ significantly between cases and controls ($p = 0.680$). However, C-262T polymorphism was significantly associated with the risk of the disease (OR, 5.50; 95% CI, 1.43–21.09; $p = 0.012$). In conclusion, this study indicates that CAT −262T/T genotype confers less susceptibility to spontaneous abortion, while GPX1 Pro198Leu polymorphism may not be correlated with the disease.

Abbreviations: CAT: catalase; GPX1: glutathione peroxidase 1; AS-PCR: allele specific-polymerase chain reaction; RFLP: restriction fragment length polymorphism; ROS: reactive oxygen species; SNP: single nucleotide polymorphism; BMI: body mass index; ETS: exposure to environmental tobacco smoke

Keywords

Catalase, gene polymorphism, glutathione peroxidase 1, reactive oxygen species, spontaneous abortion

History

Received 3 November 2013
Revised 21 December 2013
Accepted 8 January 2014
Published online 17 July 2014

Introduction

The balance between oxidant and antioxidant forces has a pivotal role in the maintenance of physiological conditions as in normal pregnancy. However, damage from reactive oxygen species (ROS) including hydroxyl radicals, superoxide anion, hydrogen peroxide, and nitric oxide in the absence of efficient antioxidant defenses leads to a variety of pathological and clinical conditions such as pregnancy complications [Wang et al. 1991].

Spontaneous abortion is defined as the pregnancy termination before 20 weeks of gestation, with an incidence up to 10%–15%. The true incidence is not well known because many abortions occur before pregnancy is clinically recognized [Tummers et al. 2003]. Fetal chromosomal abnormalities, increased maternal age, and some maternal diseases such as uterine structural abnormalities, diabetes, infections, endocrine and immune disorders, as well as environmental factors like cigarette smoking and radiation exposure are associated with pregnancy loss [Clifford et al. 1994; Fretts et al. 1995]. Apart from these factors, it has been proposed that the imbalance between ROS production and antioxidant defense is also involved in spontaneous abortion [Lagod et al. 2001].

There are two antioxidant enzymes, namely glutathione peroxidase 1 (GPX1) and catalase (CAT), which protect the cells against ROS by controlling hydrogen peroxide concentrations through its conversion to water and oxygen [Rohrdanz and Kahl 1998]. Glutathione peroxidase 1 is a cytosolic selenium-dependent enzyme, whose gene is located on chromosome 3p21 and is comprised of two exons [Flohe 1988; Kiss et al. 1997]. Among several single nucleotide polymorphisms (SNPs) that have been accounted for this gene, a functional SNP at codon 198 in exon 2 alters the protein coding region due to substitution of proline with

*Address correspondence to Dr. Zivar Salehi, Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran. Tel: 0098-9113337003. E-mail: geneticzs@yahoo.co.uk

leucine (Pro198Leu, rs1050450) [Foster et al. 2006; Moscow et al. 1994]. Studies have shown that the Leu variant is less responsive to increased selenium levels than the Pro allele, making it responsible for some diseases [Hu and Diamond 2003]. Todorova et al. [2005] found that diabetic patients with spontaneous abortion have increased GPX expression but reduced selenium levels, suggesting that weak antioxidant defenses may lead to spontaneous abortion [Todorova et al. 2005].

Catalase is an ubiquitous antioxidant enzyme which is most abundantly found in the liver, kidney, and erythrocytes [Deisseroth and Dounce 1970]. The CAT gene is located on chromosome 11p13 and consists of 13 exons. Its transcription start site is preceded by a GC-rich, TATA-less promoter containing several Sp1 binding sites [Quan et al. 1986]. A functional SNP in the promoter region of the CAT gene results from C/T substitution at position −262 (C-262T, rs1001179). It has been demonstrated that the T allele has a significantly higher transcriptional activity than the C variant, leading to higher catalase levels in the T/T genotype [Forsberg et al. 2001]. Since there is more protection against H_2O_2 accumulation in cells with high catalase expression, it is possible that the variability associated with this polymorphism plays a role in response to oxidative stress [Chang et al. 1999]. Thus, in this study we investigated the role of GPX1 Pro198Leu and CAT C-262T polymorphisms on the risk of spontaneous abortion in northern Iran.

Results and Discussion

The characteristics of the subject population are summarized in Table 1. The range of each characteristic are presented and means are presented in parenthesis. There was no significant difference in body mass index (BMI), exposure to environmental tobacco smoke (ETS), and age distributions between cases and controls. Table 2 shows the effect of GPX1 Pro198Leu and CAT C-262T polymorphisms on the risk of spontaneous abortion. The size of PCR products for both CAT alleles was 340 bp (Figure 1A). Similarly, as shown in Figure 1(B), samples homozygote for the GPX1 Leu/Leu genotype revealed a 314 bp fragment. Individuals carrying the Pro/Leu genotype produced 314, 237, and 77 bp fragments upon restriction digestion with A*paI*. The Pro/Pro homozygotes yielded 237 and 77 bp fragments (Figure 1B). For GPX1, genotype frequencies were 11.4% for Pro/Pro, 82.9% for Pro/Leu, and 5.7% for Leu/Leu among women with abortion, and 10.0% for Pro/Pro, 86.7% for Pro/Leu, and 3.3% for Leu/Leu in the control group. We found no significant difference in genotype distributions of GPX1 between cases and controls ($p = 0.680$). The allele frequencies also revealed no significant difference between the two groups ($p = 0.993$).

For CAT, genotype frequencies were 4.8% for T/T, 74.3% for C/T, and 20.9% for C/C among cases, and 11.1% for T/T,

Table 1. Characteristics of the samples.

Characteristics	Women with abortion ($n = 105$)	Controls ($n = 90$)
Maternal Age (mean)	18–44 (28.8)	20–40 (27.4)
BMI[a] (mean)	18.4–29.3 (23.5)	19.0–27.6 (24.0)
Daily ETS[b] (%)	17 (16.1)	13 (14.4)
Number of abortion (%)		
1	51 (48.6)	–
2	24 (22.8)	–
≥3	30 (28.6)	–
Number of live births (%)		
0	58 (55.2)	–
1	38 (36.1)	29 (32.2)
2	9 (8.5)	45 (50.0)
≥3	–	16 (17.7)

[a]BMI: Body Mass Index (kg/m^2), [b]ETS: Exposure to Environmental Tobacco Smoke

Table 2. Genotype and allele distributions of GPX1Pro198Leu and CAT C-262T.

	Controls (%)	Cases (%)	OR (95% CI)	*p* Value
GPX1 Pro198Leu				
Pro/Pro	9 (10.0)	12 (11.4)	1.00 (Ref)	
Pro/Leu	78 (86.7)	87 (82.9)	0.83 (0.33–2.09)	0.702
Leu/Leu	3 (3.3)	6 (5.7)	1.50 (0.29–7.68)	0.626
Pro	96 (53.3)	111 (52.9)	1.00 (Ref)	
Leu	84 (46.7)	99 (47.1)	1.01 (0.68–1.51)	0.925
CAT C-262T				
T/T	10 (11.1)	5 (4.8)	1.00 (Ref)	
C/T	72 (80.0)	78 (74.3)	2.16 (0.70–6.64)	0.176
C/C	8 (8.9)	22 (20.9)	5.50 (1.43–21.09)	0.012
T	92 (51.1)	88 (41.9)	1.00 (Ref)	
C	88 (48.9)	122 (58.1)	1.44 (0.97–2.16)	0.069

GPX1: glutathione peroxidase 1; Pro/Leu: proline/leucine; CAT: catalase.

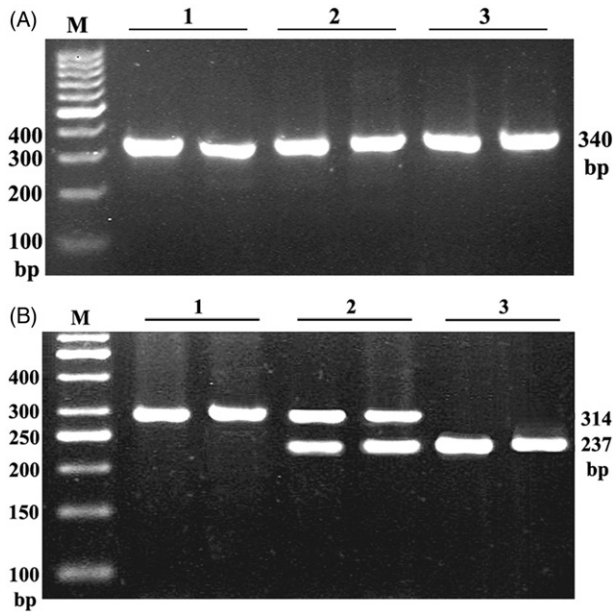

Figure 1. Agarose gel electrophoresis of CAT and GPX1 PCR products. The PCR-products were visualized following ethidium bromide staining. (A) Allele-specific polymerase chain reaction amplification of CAT using one reveres and two forward allele-specific primers. Lanes: (M), 100 bp DNA marker; (1), (2), and (3) show the CAT C/C, C/T, and T/T genotypes, respectively. (B) GPX1 fragments generated by PCR after digestion with *Apa*I. Lanse: (M), 50 bp DNA marker; (1), fragments presenting homozygote Leu/Leu genotype; (2), fragments indicating heterozygote Pro/Leu genotype; (3), fragments showing homozygote Pro/Pro genotype. CAT: catalase; GPX1: glutathione peroxidase 1; PCR: polymerase chain reaction.

80.0% for C/T, and 8.9% for C/C among controls. There was a significant association between C/C genotype and the increased risk of spontaneous abortion (OR, 5.50; 95% CI, 1.43–21.09; $p = 0.012$,). The allele frequencies showed no significant difference between cases and controls, but there was an increased risk of spontaneous abortion among women carrying the C allele (OR, 1.44; 95% CI, 0.97–2.16; $p = 0.069$, Table 2).

Placental structure has an important role in maintaining the embryo during pregnancy, and its high metabolic rate leads to an increased generation of ROS [Safronova et al. 2003]. The elevated oxidative stress along with inefficient antioxidant status in placenta impairs its function and may result in fetal abnormalities or miscarriage [Jauniaux et al. 2000]. In spite of considerable progress in identifying the causes of spontaneous abortion, the etiology remains unclear in about 40% of the cases [Hempstock et al. 2003]. In recent years, there has been an increasing interest in gene polymorphisms as a susceptibility factor. It is possible that the balance of oxidants and antioxidants is affected by numerous genetic variants, as well as endogenous and exogenous factors [Sata et al. 2003; Shin et al. 2010]. Allele substitutions in antioxidant enzymes may cause functional changes and play an important role in host response to oxidative stress. In this study, we evaluated functional polymorphisms of GPX1 and CAT to determine their role in spontaneous abortion. This is the first study, to our knowledge, which attempts to detect such an association.

Glutathione peroxidase 1 is the main antioxidant enzyme to reduce hydrogen peroxide in normal conditions while CAT protects against severe oxidative stress [Kinnula et al. 1992]. Qanungo and Mukherjea [2000] observed enhanced CAT activities in placental and fetal tissues against ROS toxicity in the feto-placenal system. The best known polymorphism of GPX1 is Pro198Leu, with the Leu allele being less responsive to the stimulation of enzyme activity during selenium supplementation [Moscow et al. 1994]. The most widely studied polymorphism of CAT results from C/T substitution at position −262. Forsberg et al. [2001] reported that the T variant causes an increased basal expression in different cell types and has a higher enzyme activity in red blood cells.

As the above-cited function of CAT, it is supposed that in severe oxidative stress conditions present in placenta, the role of this enzyme is more significant. Thus, any change in gene expression that enhances CAT activity, as the case in T/T genotype, may lead to efficient detoxification and prevention of spontaneous abortion [Forsberg et al. 2001]. Consistent with this hypothesis, our results demonstrated that the risk of spontaneous abortion had increased in women with C/C genotype. Reduced risk of the T allele has also been reported in patients with type 1 diabetes [Chistiakov et al. 2004], hypertension [Jiang et al. 2001], and breast cancer [Ambrosone et al. 2005]. Overdominant selection favoring heterozygotes and associative overdominance can lead to excess of heterozygote observed in the results.

In conclusion, the current study indicates that CAT −262C/C genotype significantly increases the risk of spontaneous abortion but the GPX1 Pro198Leu polymorphism may not be associated with this susceptibility. However, larger population-based studies are needed to clarify the results.

Materials and Methods

Sampling

In this study, 105 women with spontaneous abortion and 90 women with normal pregnancy history were investigated. All the participants resided in Guilan province and attended the study between September 2011 and March 2012. Clinical data including age at abortion, number of abortions, history of medications, and disease backgrounds as well as other factors were recorded for all the samples. Excluded criteria were: chromosomal abnormalities, reproductive diseases, maternal endocrine and immune disorders, maternal coagulation defects, hormonal treatment, irregular menstrual cycle, thrombophilia, antiphospholipid syndrome, rhesus incompatibility, and anti-inflammatory drugs. This research was approved by the local institutional review board. Informed consent was obtained from all participants and ethical principles of the Helsinki Declaration were followed.

DNA extraction

For each sample, 2 ml blood was collected by venipuncture and drawn into EDTA-K3 coated tubes (Venoject, Belgium). Genomic DNAs were extracted from peripheral leukocytes using DNG-plus kit (CinnaGen, Iran) following standard procedures. Extracted DNAs were stored at −20°C until further analysis.

Genotyping

The GPX1 Pro198Leu and CAT C-262T genotypes were determined by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) and allele-specific PCR (AS-PCR), respectively. PCR amplifications were carried out in a total volume of 25 µl containing 30 ng genomic DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM each primer, and 1.5 U of super Taq DNA polymerase (Gen Fanavaran, Iran). Primer sequences were as follows: for GPX1, 5′-GTGTGCCCCTACGCAGGTA-3′ (forward) and 5′-CACACAGTTCTGCTGACACC-3′ (reverse); for CAT, 5′-GCCCTGGGTTCGGCTATC-3′ (forward C allele), 5′-GCCCTGGGTTCGGCTATT-3′ (forward T allele) and 5′-GGTTTGCTGTGCAGAACACT-3′ (common reverse). The thermal profile started with initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 54°C for GPX1 and 56°C for CAT for 45 s, extension at 72°C for 45 s, and completed with a final extension at 72°C for 5 min. The size of PCR products for both CAT alleles was 340 bp.

For genotyping GPX1, 314 bp PCR products were digested in a total volume of 32 µl containing 0.1 µg DNA, 1x Buffer, and 10 U of *Apa*I (Fermentas, USA) at 37°C overnight. The digested products were separated on 3% agarose gel stained with ethidium bromide and visualized under UV light. Samples homozygote for Leu/Leu genotype revealed a 314 bp fragment. Individuals carrying the Pro/Leu genotype produced 314, 237, and 77 bp fragments. The Pro/Pro homozygotes yielded 237 and 77 bp fragments.

Statistical analysis

Genotype and allele frequencies were calculated in each group. The differences in genetic distributions between patients and controls were estimated by chi-square (χ^2) test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for each genotype. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were conducted by MedCalc statistical software (Version 12.1, Belgium).

Acknowledgments

This study was partly supported by the University of Guilan. The authors thank all people in the Genetics and Developmental Biology laboratories, Department of Biology, Faculty of Sciences, University of Guilan, for their technical assistance and all the sample donors for their collaboration.

Declaration of interest

All the research experiments related to this research were performed in the laboratories of the University of Guilan, and the authors are not otherwise employed in any respect by the Government of Iran. The authors report no declarations of interest.

Author contributions

Designed the study and directed its implementation: ZS; Performed the study: EES, SSZ; Analyzed and interpreted the data: EES; Designed the primers: SK; Wrote the manuscript: EES; Revised the draft: SK, ZS, ZZ.

References

Ambrosone, C.B., Ahn, J., Singh, K.K., Rezaishiraz, H., Furberg, H., Sweeney, C., et al. (2005) Polymorphisms in genes related to oxidative stress (MPO, MnSOD, CAT) and survival after treatment for breast cancer. Cancer Res **65**:1105–11.

Chang, M.S., Lee, S.G., and Rho, H.M. (1999) Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenosides extracted from Panax ginseng. Phytother Res **13**:641–4.

Chistiakov, D.A., Savost'anov, K.V., Turakulov, R.I., Titovich, E.V., Zilberman, L.I., Kuraeva, T.L., et al. (2004) A new type 1 diabetes susceptibility locus containing the catalase gene (chromosome 11p13) in a Russian population. Diabetes Metab Res Rev **20**:219–24.

Clifford, K., Rai, R., Watson, H., and Regan, L. (1994) An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. Hum Reprod **9**:1328–32.

Deisseroth, A., and Dounce, A.L. (1970) Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. Physiol Rev **50**:319–75.

Flohe, L. (1988) Glutathione peroxidase. Basic Life Sci **49**:663–8.

Forsberg, L., Lyrenas, L., de Faire, U., and Morgenstern, R. (2001) A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. Free Radic Biol Med **30**:500–5.

Foster, C.B., Aswath, K., Chanock, S.J., McKay, H.F., and Peters, U. (2006) Polymorphism analysis of six selenoprotein genes: support for a selective sweep at the glutathione peroxidase 1 locus (3p21) in Asian populations. BMC Genet **7**:56.

Fretts, R.C., Schmittdiel, J., McLean, F.H., Usher, R.H., and Goldman, M.B. (1995) Increased maternal age and the risk of fetal death. N Engl J Med **333**:953–7.

Hempstock, J., Jauniaux, E., Greenwold, N., and Burton, G.J. (2003) The contribution of placental oxidative stress to early pregnancy failure. Hum Pathol **34**:1265–75.

Hu, Y.J., and Diamond, A.M. (2003) Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. Cancer Res **63**:3347–51.

Jauniaux, E., Watson, A.L., Hempstock, J., Bao, Y.P., Skepper, J.N., and Burton, G.J. (2000) Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. Am J Pathol **157**:2111–22.

Jiang, Z., Akey, J.M., Shi, J., Xiong, M., Wang, Y., Shen, Y., et al. (2001) A polymorphism in the promoter region of catalase is associated with blood pressure levels. Hum Genet **109**:95–8.

Kinnula, V.L., Everitt, J.I., Mangum, J.B., Chang, L.Y., and Crapo, J.D. (1992) Antioxidant defense mechanisms in cultured pleural mesothelial cells. Am J Respir Cell Mol Biol **7**:95–103.

Kiss, C., Li, J., Szeles, A., Gizatullin, R.Z., Kashuba, V.I., Lushnikova, T., et al. (1997) Assignment of the ARHA and GPX1 genes to human chromosome bands 3p21.3 by in situ hybridization and with somatic cell hybrids. Cytogenet Cell Genet **79**:228–30.

Lagod, L., Paszkowski, T., Sikorski, R., and Rola, R. (2001) The antioxidant-prooxidant balance in pregnancy complicated by spontaneous abortion. Ginekol Pol **72**:1073–8.

Moscow, J.A., Schmidt, L., Ingram, D.T., Gnarra, J., Johnson, B., and Cowan, K.H. (1994) Loss of heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer. Carcinogenesis **15**: 2769–73.

Qanungo, S., and Mukherjea, M. (2000) Ontogenic profile of some antioxidants and lipid peroxidation in human placental and fetal tissues. Mol Cell Biochem **215**:11–19.

Quan, F., Korneluk, R.G., Tropak, M.B., and Gravel, R.A. (1986) Isolation and characterization of the human catalase gene. Nucleic Acids Res **14**:5321–35.

Rohrdanz, E., and Kahl, R. (1998) Alterations of antioxidant enzyme expression in response to hydrogen peroxide. Free Radic Biol Med **24**: 27–38.

Safronova, V.G., Matveeva, N.K., Avkhacheva, N.V., Sidel'nikova, V.M., Van'ko, L.V., and Sukhikh, G.T. (2003) Changes in regulation of oxidase activity of peripheral blood granulocytes in women with habitual abortions. Bull Exp Biol Med **136**:257–60.

Sata, F., Yamada, H., Kondo, T., Gong, Y., Tozaki, S., Kobashi, G., et al. (2003) Glutathione S-transferase M1 and T1 polymorphisms and the risk of recurrent pregnancy loss. Mol Hum Reprod **3**: 165–9.

Shin, S.J., Lee, H.H., Cha, S.H., Kim, J.H., Shim, S.H., Choi, D.H., et al. (2010) Endothelial nitric oxide synthase gene polymorphisms (786T > C, 4a4b, 894G > T) and haplotypes in Korean patients with recurrent spontaneous abortion. Eur J Obstet Gynecol Reprod Biol **152**:64–7.

Todorova, K., Ivanov, S., Mazneikova, V., and Genova, M. (2005) Glucooxidative stress and spontaneous abortion in pregnant women with diabetes mellitus type 1. Akush Ginekol (Sofiia) **44**:3–10.

Tummers, P., De Sutter, P., and Dhont, M. (2003) Risk of spontaneous abortion in singleton and twin pregnancies after IVF/ICSI. Hum Reprod **18**:1720–3.

Wang, Y.P., Walsh, S.W., Guo, J.D., and Zhang, J.Y. (1991) The imbalance between thromboxane and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood. Am J Obstet Gynecol **165**:1695–700.