Effects of GPX3 and GSTA1 Polymorphisms on the Risk of Schizophrenia in Chinese Han Population

CURRENT STATUS: POSTED

Chao Liu  
Jining Medical University

Sijia Song  
Rizhao mental health center

Junkai Zhang  
Jining Medical University

Xiao Li  
Jining Medical University

huijie gao  
Jining medical university

Corresponding Author  
miayigao@163.com

ORCiD: https://orcid.org/0000-0001-5029-6573

DOI:  
10.21203/rs.2.13319/v1

SUBJECT AREAS  
Medical Genetics

KEYWORDS  
Oxidative stress, Schizophrenia, Glutathione peroxidase, Glutathione S-transferase, Gene polymorphism
Abstract
Background: Several lines of evidence support the fact that the presence of oxidative stress plays an important role in the pathophysiological mechanisms of schizophrenia (SCZ). The glutathione peroxidases (GPXs) and glutathione S-transferases (GSTs) are the major antioxidant enzymes. Polymorphic variants of GPX and GST can affect the antioxidant activities of their encoded enzymes. This study explored the possible associations of the GPX3 and GSTA1 gene polymorphisms and schizophrenia in the Chinese Han population. Methods: DNA from 316 healthy controls and 303 schizophrenic patients was genotyped for single nucleotide polymorphisms (SNPs) rs736775 in GPX3 and rs3957357 in GSTA1 using a PCR-LDR genotyping assay. The χ² test compared differences in genetic distributions between the two groups in a case-control study. Results: No significant differences in allelic or genotypic frequencies of GPX3 rs736775 or GSTA1 rs3957357 were detected between cases and controls (GPX3 rs736775: χ² = 0.036, P = 0.982 by genotype, χ² = 0.20, P = 0.888, odds ratio = 1.017, 95% confidence interval = 0.801-1.292 by allele; GSTA1 rs3957357: χ² = 1.100, P = 0.577 by genotype, χ² = 0.924, P = 0.336, odds ratio = 1.176, 95% confidence interval = 0.845-1.637 by allele). Conclusions: Our results suggest that GPX3 rs736775 and GSTA1 rs3957357 SNPs are unlikely to be a candidate gene for susceptibility to SCZ in at least Chinese Han population. However, these results should be validated by replication in different populations.

Background
Schizophrenia (SCZ) is a complex and severe mental disorder and is the most common among psychotic illness, with a prevalence of around 1% in the worldwide population[1-3]. Although the detailed mechanisms underlying the pathophysiology of SCZ is unknown for certain, multiple pieces of evidence illustrated that a complex interaction between genetic background and environmental factors was likely to be involved in the development of SCZ[4, 5].

Dopamine (DA) is the principal neurotransmitter in the brain and play a significant role in the functions of neurons. However, the oxidative metabolites of dopamine are hydrogen peroxide (H₂O₂) and DA quinones which is a principal source of reactive oxygen species (ROS) in the brain[6]. Oxidative stress arises when the balance between antioxidant activity and the formation of ROS is disrupted, and ROS can cause neuronal inflammation as a consequence of oxidative stress. Interestingly, several studies suggested that neuronal inflammation induced by oxidative stress play an important role in SCZ pathophysiological mechanisms [7-9].
Recently, glutathione (GSH) levels were reported to be decreased in the cerebrospinal fluid and in the prefrontal cortex of SCZ patients[10-12]. Furthermore, some studies showed changes in antioxidant enzyme activity in the plasma and postmortem brain of schizophrenia patients[7, 13]. Taking all these factors into consideration, we had to focus our attention on the role of oxidative stress in the pathophysiology of SCZ through investigating two important antioxidant enzymes: glutathione peroxidases (GPXs) and glutathione S-transferases (GSTs).

GPX family belongs to selenium-dependent peroxidases and plays important role in protecting cells from oxidative damage by reducing free hydrogen peroxide to water. The GPX3, a member of GPX family, was found to be highly expressed in the prefrontal cortex suggests that GPX3 may be involved in antioxidant activity in brain[14]. Human GPX3 is located on chromosome 5q33.1 and has a common single nucleotide polymorphism (SNP), GPX3 rs736775. Several studies have suggested the effect of this variant on GPX3 activity and many disorders[15-18].

GSTs consist of phase II detoxication enzyme and can catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification[19]. The GST alpha, a member of GSTs family, are located in chromosome 6 and shows an important detoxifying activity that protects the cell from ROS. GST alpha 1 (GSTA1) represents one of the most abundant alpha-class GST isoenzymes. Besides its through conjugation of reduced form glutathione GSH, GSTA1 can also inactivates quinones[20]. SNP rs3957357 in GSTA1 is just located in the promoter region of GSTA1 genes, and several studies suggested the pathogenic effects of this variant in many disorders[21, 22].

Although polymorphic variants of oxidative stress-related candidate genes including GSTP1, GSTT1, GSTM1, GPX1 and GSTA1 have been shown to be risk factors for SCZ[23-25], genetic polymorphism vary by race considerably and we therefore estimated the possible associations of the GPX3 rs736775 and GSTA1 rs3957357 gene polymorphisms and schizophrenia in the Chinese Han population for the purpose of identifying potential prognostic or predictive tools for the individuals at risk of SCZ.

Methods
Population
The study was approved by the Ethical Committees of Jining Medical University in accordance with the Code of Ethics of the Declaration of Helsinki. The participants were recruited from the Rizhao Mental Health Center and Affiliated Hospital of Jining Medical University and they were original northern Han Chinese individuals. The sample consisted of 303 patients with SCZ (140 men and 163 women, mean age 47.2 ± 4.3 years) and 316 healthy controls (145 men and 171 women, mean age: 46.9 ± 4.4 years) living in the same geographic area. The patients with SCZ were interviewed by two board-certified psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) criteria. The normal controls were confirmed to be free from any mental illness by two
board-certified psychiatrists. All participants gave written informed consent to participate in the study.

Genetic studies

Total Genomic DNA was extracted from whole blood using TIANamp Genomic DNA Kit (TIANGEN, China), according to the manufacturer’s instructions. Genotyping for SNPs GPX3 rs736775 and GSTA1 rs3957357 was performed using the polymerase chain reaction-ligase detection reaction (PCR-LDR) method. The sequences of primers are listed in Table 1. PCR was performed in a volume of 15 μl reaction system, containing 7.5 μl 2×PCR Master Mix, 2 μl Primer mix, and 2 μl genomic DNA and DNase-free water. Multiplex PCR amplifications were performed under the following conditions: an initial denaturation at 94°C for 3min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 7 °C for 30 s, and a terminal extension 72 °C for 3min. After multiplex PCR amplification, the LDR was performed in a volume of 10 μl reaction system, including 3 μl PCR product, 1 μl 10× Taq DNA ligase buffer, 0.125 μl Taq DNA ligase(40 U/μl), 2 μl Probe mix, and ddH₂O, followed by 30 cycles at 94 °C for 30 s, 56 °C for 3min. The sequences of probes are listed in Table 2. Hence, the final reaction system containing 1 μl LDR product and 9 μl highly deionized formamide were performed under denaturation at 95°C for 3min and the genotypes were analyzed by ABI 3730XL sequencer and Genemapper software.

Table 1 The information of primer of GPX3 rs736775 and GSTA1 rs3957357 polymorphism

| Primer name | Sequence (5'-3') | PCR length |
|-------------|-----------------|------------|
| rs736775    | TAAACCCAAGTCCCCCTGAGT | 96bp       |
|             | RCTCTTGAGTAATGGTGACGTATCA |           |
| rs3957357   | FACAACTGAAATCCCCAGGTCTCTAATG | 114bp     |
|             | GCCCATGAAATGTTGGGAGT |           |

Table 2 The information of probe of GPX3 rs736775 and GSTA1 rs3957357 polymorphism

| Probe name      | Sequence (5'-3') | LDR length |
|-----------------|-----------------|------------|
| Rs736775_modify | TGCCCTACCCCTCAAGGAGGCTCATGAC |      |
| rs736775_CY     | CCTAGGCTCTTTCCACTGCCTCCGGG | 50        |
| rs736775_T      | CTGCGTACGGCTCTTCCACTGCCCCG | 53        |
| Rs3957357_modify| GTCAAGTGGGAAAGCCTGGACTGAC |      |
| rs3957357_A     | CTGAAATAGTTCTCTCCCCTGAAAAGAAGA | 57        |
| rs3957357_G     | CTGACCTGATAGTTCTCTCCCCTGAAAGAAGG | 60        |

Statistical analysis

The χ² test was used to test the Hardy–Weinberg equilibrium (HWE) of the genotype distribution and to compare the differences in genotypic and allelic frequencies of GPX3 rs736775 and GSTA1 rs3957357 between cases and controls. P<0.05 was considered to denote statistical significance. The
degree of relative risk was estimated by Odds ratios (ORs) and 95% confidence intervals (CIs). Statistical analysis in this study was carried out using the Statistical Package (version 21.0 for Windows; SPSS Inc., Chicago, Illinois, USA).

Results

The genotype distributions of the two tested alleles were in accordance with Hardy-Weinberg equilibrium (for rs736775, $P=0.071$; rs3957357, $P=0.157$). The allelic and genotypic frequencies of GPX3 rs736775 and GSTA1 rs3957357 in SCZ and control groups are shown in Table 3. No significant differences in allelic or genotypic frequencies of GPX3 rs736775 and GSTA1 rs3957357 were observed between schizophrenic and control groups. (GPX3 rs736775: $\chi^2 =0.036, P=0.982$ by genotype, $\chi^2=0.020, P=0.888$, odds ratio=1.017, 95% confidence interval=0.801-1.292 by allele; GSTA1 rs3957357: $\chi^2 =1.100, P=0.577$ by genotype, $\chi^2=0.924, P=0.336$, odds ratio=1.176, 95% confidence interval=0.845-1.637 by allele).

Table 3  The comparison of genotypic and allelic frequencies between SCZ and control groups

| SNP   | Allele | SCZ/control | $p$  | $\chi^2$ | OR(95% CI) | Genotype | SCZ/control | $p$  | $\chi^2$ |
|-------|--------|-------------|------|----------|------------|----------|-------------|------|----------|
| rs3957357 | G      | 521/55      | 0.336| 0.924    | 1.176 (0.845-1.637) | GG       | 220/241     | 0.577| 1.100    |
|        | A      | 85/77       |      |          |            | AG       | 81/73       |      |          |
|        |        |             |      |          |            | AA       | 2/2         |      |          |
| rs736775  | T      | 411/43      | 0.888| 0.020    | 1.017 (0.801-1.292) | TT       | 132/140     | 0.982| 0.036    |
|        | C      | 195/20      |      |          |            | CT       | 147/151     |      |          |
|        |        |             |      |          |            | CC       | 24/25       |      |          |

Furthermore, in order to ensure sufficient statistical power for the detection of disease susceptibility loci, additive, dominant and recessive genetic models were used to analyze genotype frequencies of GPX3 rs736775 and GSTA1 rs3957357. Table 4 shows that rs736775 and rs3957357 were not the risk factors for SCZ on the basis of these models (all $P > 0.05$).

Table 4  Analysis of the two SNPs based on three genetic models
| SNP      | Genotype | SCZ/control | Additive model | Dominant model | Recessive model |
|----------|----------|-------------|----------------|----------------|----------------|
|          |          |             | p      | OR(95% CI) | p      | OR(95% CI) | p      | OR(95% CI) |
| rs3957357| GG       | 220/241     | 0.93   | 1.10 (0.15-7.84) | 0.29 | 1.21 (0.84-1.74) | 0.97 | (          |
|          | AG       | 81/73       |         |               |         |               |         |            |
|          | AA       | 2/2         |         |               |         |               |         |            |
| rs736775 | TT       | 132/140     | 0.95   | 1.02 (0.55-1.87) | 0.85 | 1.03 (0.75-1.42) | 0.99 | (          |
|          | CT       | 147/151     |         |               |         |               |         |            |
|          | CC       | 24/25       |         |               |         |               |         |            |

**Discussion**

It is well known that the oxygen consumption of brain is the highest in our body and hence generation of ROS increase. Moreover, the oxidative metabolites of dopamine are hydrogen peroxide (H$_2$O$_2$) and DA quinones, which is a principal source of ROS in the brain[6]. Therefore, the brain is considered particularly vulnerable to ROS. Normally, ROS can be eliminated by the antioxidant system. When the balance between the formation of ROS and intrinsic antioxidant capacity is upset, oxidative metabolite damage to neurons arises. Increasing evidence indicates that oxidative injury to neurons can play important roles in the pathophysiology of neuropsychiatric disorders including schizophrenia[7-9].

The antioxidant system comprises of enzymatic and nonenzymatic antioxidants, and enzymes involved in the antioxidant systems comprise of glutathione peroxidase (GPx), glutathione S transferase (GST), and so on. Polymorphic variants of GPX and GST may affect their antioxidant activities, contributing to the imbalance of ROS production and antioxidant capacity in SCZ patients[23, 24, 26].

GPXs are encoded by the GPX gene family and consists of eight groups, GPx1-8. The GPX3, a member of GPX family, was found to be highly expressed in the prefrontal cortex suggests that GPX may be involved in antioxidant activity in brain. The human GPX3 gene is located on chromosome 5q33.1 and multiple GPX3 SNPs have been reported, with rs736775 being one of the most common. For instance, Noci et al. demonstrated that GPX3 rs736775 was associated with overall survival in colorectal cancer patients[16]. Zhang et al. also suggested GPX3 rs736775 was a prognostic markers in patients with gastric cancer[17]. Another study showed that the increased risk for cardiovascular toxicity among patients was associated with GPX3 rs736775[27]. Taking these results into consideration, GPX3 rs736775 might contribute to the altered antioxidant capacity in SCZ patients. In
In this study, we compared allelic and genotypic frequencies of rs736775 between 303 SCZ patients and 316 controls. However, we observed a TT genotype frequency of 43.56%, a CT genotype frequency of 48.51%, and a CC genotype frequency of 7.92% for patients with SCZ, compared with 44.30%, 47.79%, and 7.91%, respectively, in controls. No significant differences in allelic or genotypic frequencies of GPX3 rs736775 were observed between SCZ patients and controls in our study. This finding suggest that the GPX3 rs736775 polymorphism may not be the genetic risk factor for SCZ patients.

GSTs are encoded by the GST family of genes located on different chromosomes and the cytosolic GST can be classified into four major groups: Alpha, Mu, Pi, and Theta. The GST alpha (GSTA) family are located in chromosome 6 and shows an important detoxifying activity that protects the cell from ROS. The GSTA1 rs3957357 is one of five polymorphisms just located in the promoter region of GSTA1 genes, resulting in decreased enzyme activity. In recent years, GSTA1 rs3957357 has been reported to be associated with many disorders including bladder cancer, leukemia and gestational hypertension, as well as SCZ. For instance, Rossi et al. reported that GSTA1 rs3957357 may associate with event-free survival in patients with diffuse large B-cell lymphoma[21]. Iorio et al. suggested that the GSTA1 rs3957357 was significantly associated with gestational hypertension risk[28]. However, we are intrigued by the effects of GSTA1 polymorphisms on the risk of SCZ. Gravina et al. found a higher frequency of the combined genotypes including GSTA1 polymorphisms in Italy patients with SCZ[23]. Spalletta et al. suggested GSTA1 polymorphisms was associated with the altered microstructure of the thalami in Italy patients with SCZ[24]. Because of several factors, such as population and sample, is necessary to test the gene polymorphisms on the risk of some disorders, we sought to analyze the Chinese Han population in the present study. We found GG, AG and AA genotype frequencies of 72.61%, 26.73%, and 0.66% in patients with SCZ, compared with 76.27, 23.10, and 0.63%, respectively, in controls. Consequently, no significant differences in allelic or genotypic frequencies of GSTA1 rs3957357 between SCZ patients and controls in our study, and GSTA1 rs3957357 does not appear to be a potential predictive tool for Chinese population at risk of SCZ.

Conclusions
In conclusion, this is the first report to investigate the effects of GPX3 and GSTA1 SNPs in SCZ in a Chinese population. Our results suggest that GPX3 rs736775 and GSTA1 rs3957357 SNPs are unlikely to be a candidate gene for susceptibility to SCZ in at least Chinese Han population. However, most subjects were Han nationality in Shandong region in our study and the sample size is relatively small, larger-scale studies are necessary to validate this findings.

Abbreviations
SCZ: Schizophrenia; DA: Dopamine; ROS: Reactive oxygen species; GSH: Glutathione; GPX:
Glutathione peroxidase; GST: Glutathione S-transferase; SNP: Single-nucleotide polymorphism; PCR-LDR: Polymerase chain reaction-ligase detection reaction; ORs: Odds ratios; CIs: Confidence intervals.

Declarations

Acknowledgements
Not applicable.

Authors' contributions
LC and GHJ designed the experiments. LC, SSJ, ZJK and LX collected the blood samples and extracted DNA from the blood samples. LC, ZJK and LX analyzed the raw data. LC and SSJ wrote the manuscript. GHJ participate in revising the manuscript. All authors reviewed and approved the final manuscript.

Funding
This work was supported by the Shandong Provincial Natural Science Foundation of China (Grant no. ZR2016HL22), the NSFC cultivation project of Jining Medical University (2016), and the National Students' innovation training program(201810443020), the Projects of Medical and Health Technology Development Program of Shandong Province (2015WS0420 and 2017WS338).

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study was approved by the Ethical Committees of Jining Medical University in accordance with the Code of Ethics of the Declaration of Helsinki. All participants gave written informed consent to participate in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 College of Pharmacy, Jining Medical University, Rizhao, Shandong, China. 2Rizhao Mental Health Center, Rizhao, Shandong, China

References
1. Gysin R, Kraftsik R, Sandell J, Bovet P, Chappuis C, Conus P, Deppen P, Preisig M,
Ruiz V, Steullet P et al: Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence. Proc Natl Acad Sci U S A 2007, 104(42):16621-16626.

2. Tamminga CA, Holcomb HH: Phenotype of schizophrenia: a review and formulation. Mol Psychiatry 2005, 10(1):27-39.

3. Freedman R: Schizophrenia. N Engl J Med 2003, 349(18):1738-1749.

4. Thaker GK, Carpenter WT, Jr.: Advances in schizophrenia. Nat Med 2001, 7(6):667-671.

5. Mowry BJ, Gratten J: The emerging spectrum of allelic variation in schizophrenia: current evidence and strategies for the identification and functional characterization of common and rare variants. Mol Psychiatry 2013, 18(1):38-52.

6. Shinkai T, Muller DJ, De Luca V, Shaikh S, Matsumoto C, Hwang R, King N, Trakalo J, Potapova N, Zai G et al: Genetic association analysis of the glutathione peroxidase (GPX1) gene polymorphism (Pro197Leu) with tardive dyskinesia. Psychiatry Res 2006, 141(2):123-128.

7. Reddy R, Sahebarao MP, Mukherjee S, Murthy JN: Enzymes of the antioxidant defense system in chronic schizophrenic patients. Biol Psychiatry 1991, 30(4):409-412.

8. Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT: Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. Int J Neuropsychopharmacol 2011, 14(1):123-130.

9. Yao JK, Keshavan MS: Antioxidants, redox signaling, and pathophysiology in schizophrenia: an integrative view. Antioxid Redox Signal 2011, 15(7):2011-
10. Behrens MM, Sejnowski TJ: Does schizophrenia arise from oxidative dysregulation of parvalbumin-interneurons in the developing cortex? *Neuropharmacology* 2009, **57**(3):193-200.

11. Tosic M, Ott J, Barral S, Bovet P, Deppen P, Gheorghita F, Matthey ML, Parnas J, Preisig M, Saraga M et al: Schizophrenia and oxidative stress: glutamate cysteine ligase modifier as a susceptibility gene. *Am J Hum Genet* 2006, **79**(3):586-592.

12. Do KQ, Trabesinger AH, Kirsten-Kruger M, Lauer CJ, Dydak U, Hell D, Holsboer F, Boesiger P, Cuenod M: Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *Eur J Neurosci* 2000, **12**(10):3721-3728.

13. Gawryluk JW, Wang JF, Andreazza AC, Shao L, Yatham LN, Young LT: Prefrontal cortex glutathione S-transferase levels in patients with bipolar disorder, major depression and schizophrenia. *Int J Neuropsychopharmacol* 2011, **14**(8):1069-1074.

14. Kim WS, Wong J, Weickert CS, Webster MJ, Bahn S, Garner B: Apolipoprotein-D expression is increased during development and maturation of the human prefrontal cortex. *J Neurochem* 2009, **109**(4):1053-1066.

15. Wang JY, Yang IP, Wu DC, Huang SW, Wu JY, Juo SH: Functional glutathione peroxidase 3 polymorphisms associated with increased risk of Taiwanese patients with gastric cancer. *Clin Chim Acta* 2010, **411**(19-20):1432-1436.

16. Noci S, Dugo M, Bertola F, Melotti F, Vannelli A, Dragani TA, Galvan A: A subset of genetic susceptibility variants for colorectal cancer also has prognostic value. *Pharmacogenomics J* 2016, **16**(2):173-179.

17. Zhang H, Zhao W, Gu D, Du M, Gong W, Tan Y, Wang M, Wen J, Zhai Y, Xu Z:
18. Fullerton JM, Tiwari Y, Agahi G, Heath A, Berk M, Mitchell PB, Schofield PR: Assessing oxidative pathway genes as risk factors for bipolar disorder. *Bipolar Disord* 2010, **12**(5):550-556.

19. Bjork K, Saarikoski ST, Arlinde C, Kovanen L, Osei-Hyiaman D, Ubaldi M, Reimers M, Hyytia P, Heilig M, Sommer WH: Glutathione-S-transferase expression in the brain: possible role in ethanol preference and longevity. *FASEB J* 2006, **20**(11):1826-1835.

20. Hayes JD, Flanagan JU, Jowsey IR: Glutathione transferases. *Annu Rev Pharmacol Toxicol* 2005, **45**:51-88.

21. Rossi D, Rasi S, Franceschetti S, Capello D, Castelli A, De Paoli L, Ramponi A, Chiappella A, Pogliani EM, Vitolo U et al: Analysis of the host pharmacogenetic background for prediction of outcome and toxicity in diffuse large B-cell lymphoma treated with R-CHOP21. *Leukemia* 2009, **23**(6):1118-1126.

22. Akhdar H, El Shamieh S, Musso O, Desert R, Joumaa W, Guyader D, Aninat C, Corlu A, Morel F: The rs3957357C>T SNP in GSTA1 Is Associated with a Higher Risk of Occurrence of Hepatocellular Carcinoma in European Individuals. *PLoS One* 2016, **11**(12):e0167543.

23. Gravina P, Spoletini I, Masini S, Valentini A, Vanni D, Paladini E, Bossu P, Caltagirone C, Federici G, Spalletta G et al: Genetic polymorphisms of glutathione S-transferases GSTM1, GSTT1, GSTP1 and GSTA1 as risk factors for schizophrenia. *Psychiatry Res* 2011, **187**(3):454-456.

24. Spalletta G, Piras F, Gravina P, Bello ML, Bernardini S, Caltagirone C: Glutathione S-
transferase alpha 1 risk polymorphism and increased bilateral thalamus mean diffusivity in schizophrenia. Psychiatry Res 2012, 203(2-3):180-183.

25. Gao H, Liu C, Song S, Zhang C, Ma Q, Li X, Xu L: GPX1 Pro198Leu polymorphism and GSTP1 Ile105Val polymorphisms are not associated with the risk of schizophrenia in the Chinese Han population. Neuroreport 2017, 28(15):969-972.

26. Hu YJ, Diamond AM: Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. Cancer Res 2003, 63(12):3347-3351.

27. Kraus S, Hummler S, Toriola AT, Poole EM, Scherer D, Kotzmann J, Makar KW, Kazanov D, Galazan L, Naumov I et al: Impact of genetic polymorphisms on adenoma recurrence and toxicity in a COX2 inhibitor (celecoxib) trial: results from a pilot study. Pharmacogenet Genomics 2013, 23(8):428-437.

28. Iorio A, Spinelli M, Polimanti R, Lorenzi F, Valensise H, Manfellotto D, Fuciarelli M: GSTA1 gene variation associated with gestational hypertension and its involvement in pregnancy-related pathogenic conditions. Eur J Obstet Gynecol Reprod Biol 2015, 194:34-37.