Glutathione Peroxidase 1 Gene Polymorphism in Nephrolithiasis Patients From South of Iran

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Keywords. glutathione peroxidase 1, polymorphism, oxidative stress, nephrolithiasis

Introduction. Nephrolithiasis is a common multifactorial kidney disease with worldwide distribution. Compelling evidence, regarding the function of kidney in maintaining the body homeostasis, suggests the role of oxidative stress in the pathogenesis of nephrolithiasis. Glutathione peroxidase 1 is a major antioxidant enzyme, preventing oxidative damage to renal cells by detoxifying hydrogen and lipid peroxides, which may involve in its pathogenesis. The purpose of the present study was to determine the possible association of glutathione peroxidase 1 gene *(GPX1)* proline-to-leucine substitution at amino acid 198 (Pro198Leu polymorphism) with the risk of developing nephrolithiasis in south Iranian patients.

Materials and Methods. Association of Pro198Leu polymorphism in exon 2 of *GPX1* gene was investigated in 150 patients with nephrolithiasis and 184 healthy age-, sex-, and ethnically-matched control group using polymerase chain reaction-restriction fragment length polymorphism.

Results. Regression analysis demonstrated that the frequency of the genotypes carrying at least 1 Leu allele, in both dominant and codominant model for this allele, was significantly higher in patients compared with the controls. However, significant association was found neither with wild-type allele, nor with polymorphic allele with the risk of nephrolithiasis.

Conclusions. Findings of our study provide potential support in favor of the role of oxidative stress in the pathogenesis of nephrolithiasis in patients from south of Iran. The results indicate that *GPX1* may be a key player in nephrolithiasis development.

IJKD 2017;11:29-35
www.ijkd.org

Original Paper

INTRODUCTION

A common health problem, nephrolithiasis (stone disease) is influenced by a number of genes in combination with environmental factors. There is, however, no agreement as to which genes or pathways are involved.[1] Crystal formation depending on the supersaturated chemicals in urine (uric acid, calcium oxalate, calcium phosphate, and cystine), crystal aggregation, and crystal growth in tubular lumens are a sequence of events that result in stone formation.[2,3] Calcium oxalate, which is generally found mixed with calcium phosphate, is the major constituent of most calculi and found to exert a cytotoxic effect on renal epithelial cells through oxidative stress. This renal cell injury is considered a major risk factor for crystallization and crystal deposition in the kidney.[4] Furthermore, the role of kidney as a primary eliminator of toxins and drugs, and abundance of long-chain polyunsaturated fatty acids in the composition of renal lipids, make this organ vulnerable to damage caused by reactive oxygen species (ROS).[5] These pieces of evidence along with the results from in vivo and in vitro studies provide critical clue in

favor of the importance of oxidative stress in the pathogenesis of nephrolithiasis.[6]

Oxidative stress is characterized by an imbalance between the production of ROS and the activity of antioxidant enzymes, which contributes to susceptibility and pathology of many common and complex human disease.[7,8] Several studies suggest an association between stone formation and decreased antioxidant levels.[9] Glutathione peroxidase (GP), catalase, and superoxide dismutase (SOD) are the enzymatic contributor of ROS catabolism.[10]

Glutathione peroxidases are a family of selenium-dependent detoxifying enzymes promoting the reduction of hydrogen peroxide to water using glutathione as an obligate cosubstrate.[11] Glutathione peroxidase 1 (GP1) is the most abundant member of the GP family and is present in all cell types. Glutathione peroxidase 1 plays an essential role in ROS homeostasis and stress signaling by preventing the harmful accumulation of intracellular hydrogen peroxide and reduction of lipid hydroperoxides and other soluble hydroperoxides after their release from membrane lipids.[12,13] Several single nucleotide polymorphisms were found in the coding for glutathione peroxidase 1 gene (*GPX1*; 3p21.3) that mostly localized at 3′ and 5′ regions of the gene. However, the well-known polymorphism affecting the *GPX1* gene involves a C>T transition (*GPX1* C593T, dbSNP ID: rs1050450) in exon 2 of the gene, which leads to proline-to-leucine substitution at amino acid 198 (Pro198Leu). The *GPX1* C593T variant supposes to result in lower enzyme activity and mRNA expression in the presence of leu-allele compared with the pro-allele.[14]

The probable functional consequence of the *GPX1* Pro198Leu substitution draws attention to this polymorphism, which may contribute to nephrolithiasis. The aim of our study was to determine whether this polymorphism was associated with nephrolithiasis in south Iranian patients.

MATERIALS AND METHODS

Participants

A total of 150 patients who underwent percutaneous nephrolithotomy (mean age, 46.59 ± 13.94 years) and 184 controls (46.52 ± 13.92 years) were enrolled in this case-control study. All procedures including blood sampling, patients' clinical evaluation, molecular assessment testing, and data analysis were carried out from September 2014 to September 2015. All patients were recruited from the Department of Surgery, Shaheed Faghihi Hospital, Shiraz, Iran. The control group was selected randomly from the outpatient general clinic based on ultrasonography of the kidney and urine analysis. They were age-, sex-, and ethnically-matched to the patients group.

Written informed consent was obtained from all participants before collecting blood specimens. All participants were interviewed to collect detailed information on demographic characteristics such as age, weight, sex, and risk factors including family history of nephrolithiasis in first-degree relatives, alcohol consumption, smoking, coffee drink, and high-risk occupations.

To investigate the role of sunlight in the pathogenesis of nephrolithiasis, the participants were divided into 2 groups according to their job: high risk (farmers, drivers, policemen, etc) and low risk (housewives, teachers, etc). The study was approved by the Ethics Committee of Islamic Azad University, Arsanjan Branch.

Genotyping

Genomic DNA was extracted from peripheral blood cells using simple salting out procedure.[15] The target polymorphism of the *GPX1* (Pro198Leu) was detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism assay. The 317 bp region of the *GPX1* gene, encompassing the 593C>T polymorphic site was amplified by PCR using a set of primer pair: 5′-TGCCCCTACGCAGGTACAG-3′ (forward primer) and 5′-GTGTCAGCAGA ACTGTGTGTATGTC-3′ (reverse primer). Initially, the PCR was subjected to denaturation for 5 minutes at 95°C, followed by 35 cycles of amplification (60 seconds at 94°C, 45 seconds at 61°C and 40 seconds at 72°C). A final elongation step (7 minutes at 72°C) was applied at the end of the 35 cycles. Then, PCR was followed by an overnight digestion with the restriction enzyme ApaI (C allele, 79 and 238 bp; T allele, 317 bp) at 37°C. Digested PCR fragments were separated by an agarose gel electrophoresis and visualized by DNA safe dye staining.

Statistical Analysis

To determine whether the SNP was on the Hardy-Weinberg equilibrium, the observed

genotype frequency distributions were compared with the expected ones using the chi-square test. The Student *t* test and the chi-square test were used to compare selected characteristics between cases and controls. The association of Pro198Leu (rs1050450) genotypes and risk of nephrolithiasis were estimated by odds ratio (OR) and 95% confidence intervals (CI) calculated by logistic regression analysis. Logistic regression analysis was also used to assess the influence of some risk factors on the risk of nephrolithiasis. *P* values less than .05 were considered significant.

RESULTS

Baseline characteristics

Baseline characteristics of patients with nephrolithiasis and controls are presented in Table 1. Our data showed a higher frequency (more than 2 times) of disease among the men in comparison to the women. There was no significant difference in body mass index and body weight in the study group compared with controls (Table 1). Table 2 summarizes the estimation of the risk of kidney calculi considering some known risk factors through univariable analysis. Alcohol consumption, coffee drinking, family history of kidney calculus disease among first-degree relatives, and high-risk jobs were risk factors for nephrolithiasis, whereas no association was found between smoking or diabetes mellitus and nephrolithiasis.

Genotype Analysis

Agarose gel electrophoretography results for polymorphism analysis is shown in the Figure. It was confirmed that the genotype frequencies, both in the patients and the controls, fit the Hardy-Weinberg equilibrium for studied polymorphism. The genotypic and allelic distribution of *GPX1* (rs1050450, Pro198Leu) polymorphism is presented in Table 3. The results showed that *GPX1* Pro198Leu displayed significant differences in genotypic frequency between the patients and the controls. The frequency of Leu-allele carriage genotypes (Leu/Leu+ Leu/Pro) in nephrolithiasis cases was

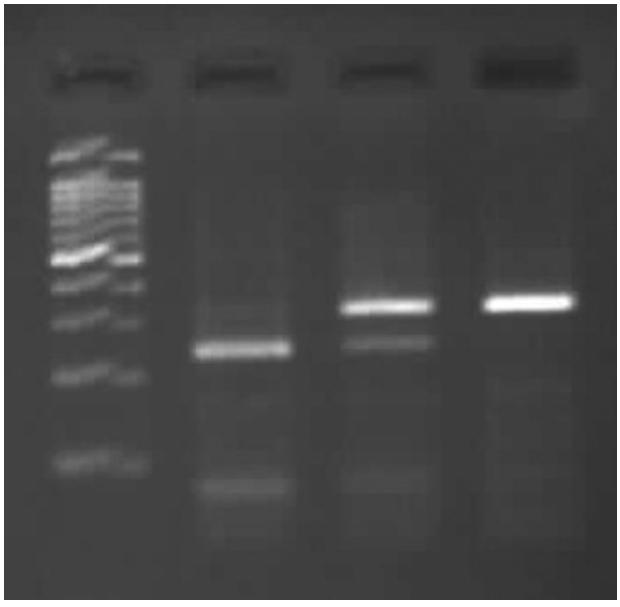
Table 1. Baseline Characteristics of Study Participants

Characteristics	Controls (n = 184)	Nephrolithiasis Patients (n = 150)	*P*
Sex			
Male	126 (68.5)	103 (68.7)	
Female	58 (31.5)	47 (31.3)	> .99
Age, y	46.52 ± 13.92	46.59 ± 13.94	.96
Age range, y	23 to 86	23 to 90	…
Disease duration, mo	…	120.61 ± 107.99	…
Body mass index, kg/m2	26.04 ± 3.91	25.67 ± 3.96	.39
Body weight, kg	75.14 ± 13.26	73.33 ± 13.40	.22
Stone component			
Calcium oxalate (may include < 50% calcium phosphate)	…	59 (17.7)	
Calcium phosphate (≥ 50%)	…	1 (0.3)	
Uric acid	…	18 (5.4)	
Cystine	…	2 (0.6)	
Mixed calcium oxalate and uric acid	…	34 (10.2)	
Unknown	…	36 (10.8)	…

Table 2. Risk Factors for Kidney Calculus Disease*

Risk Factor	Controls (n = 184)	Nephrolithiasis Patients (n = 150)	Crude Odds Ratio (95% Confidence Interval)	*P*
Alcohol consumption	21 (11.4)	8 (5.3)	2.30 (0.98 to 5.32)	.05
Smoking	54 (29.3)	48 (32.0)	0.88 (0.55 to 1.41)	.6
Coffee drinking	58 (46.2)	91 (60.7)	1.80 (1.16 to 2.80)	.009
Family history	49 (27.0)	107 (71.3)	7.30 (4.40 to 12.20)	< .001
High-risk job	33 (17.9)	67 (44.7)	8.50 (4.42 to 16.35)	< .001
Diabetes mellitus	11 (6.0)	16 (10.7)	1.88 (0.84 to 4.18)	.12

*Values for each group are frequencies of raisk factors (percentages).



Polymerase chain reaction-base restriction analysis of the P198L polymorphism was shown on 3% agarose gel electrophoresis. The region was amplified by polymerase chain reaction, resulting in a 317-bp fragment (Leu/Leu homozygote) in well 3 after size marker. Digestible fragments 79 bp and 238 bp represent the Pro/Pro homozygote (well 1). The presence of 3 bands 317, 79, and 238 belongs to heterozygote individuals (well 2). The 1st well from the left represents 100-bp DNA size marker.

95.3%, while in the control group, it was 83.20%, confirming a significant association between this polymorphism and nephrolithiasis (OR, 4.14; 95% CI, 1.77 to 9.70, P = .001). The Leu allele frequency among nephrolithiasis cases was higher than that in the control group (55% and 48%, respectively), but this difference was not significant (P = .09).

We further evaluated whether there was an association between *GPX1* genotypes and the risk factors of nephrolithiasis, including alcohol consumption, smoking, coffee drinking, family history of kidney calculi, and job in the case group. There were no significant differences in any characteristic analyses except for those stone formers with high-risk jobs (Table 4).

DISCUSSION

Epidemiological studies have suggested that nephrolithiasis might be multifactorial, with a possible genetic predisposition and involvement of environmental factors in its pathogenesis.[16] The complexity of the mode of inheritance, high incidence, as well as high degree of recurrence risk introduces nephrolithiasis as a challenging subject during decades.[17,18] Clinical and experimental data shed light on the importance of ROS and the development of oxidative stress as triggers of calcification and plaque formation in the process of the stone formation.[19] Cells are equipped with a number of scavenging

Table 3. Genotype and Allele Frequencies of Gluthatione Peroxidase 1 (*GPX1*) P198L Polymorphism for Study Participants*

GPX1 (rs1050450)	Controls (n = 184)	Nephrolithiasis Patients (n = 150)	*P*	Crude Odds Ratio (95% Confidence Interval)	Adjusted Odds Ratio (95% Confidence Interval)†	*P*
Genotypes						
Pro/Pro	31 (16.8)	7 (4.7)		1	1	
Pro/Leu	129 (70.1)	122 (81.3)	.001	4.18 (1.78 to 9.86)	4.27 (1.81 to 10.08)	.001
Leu/Leu	24 (13.0)	21 (14.0)	.008	3.87 (1.41 to 10.62)	3.89 (1.42 to 10.69)	.008
Leu/Leu + Pro/Leu	153 (83.2)	143 (95.3)	.001	4.14 (1.77 to 9.70)	4.21 (1.79 to 9.88)	.001
Alleles						
Pro	191 (52.0)	136 (45.0)		1	…	
Leu	177 (48.0)	164 (55.0)	.09	1.30 (0.96 to 1.77)	…	…

*Values for each group are frequencies of raisk factors (percentages).
†Data were adjusted for age, sex, and body mass index.

Table 4. Risk Factors for Kidney Calculus Disease Assessed by Glutathione Peroxidase Genotypes in Patients With Calculi*

Risk Factor	Pro/Pro (n = 7)	Pro/Pro + Pro/Leu (n = 143)	Odds Ratio (95% Confidence Interval)	*P*
Alcohol consumption	1 (14.3)	7 (4.9)	2.92 (0.41 to 20.58)	.28
Smoking	4 (57.1)	44 (30.8)	1.85 (0.93 to 3.69)	.16
Coffee drinking	6 (85.7)	85 (59.4)	1.44 (1.04 to 2.01)	.16
Family history	5 (71.4)	102 (71.3)	1.00 (0.62 to 1.62)	.99
High-risk job	6 (85.7)	61 (42.7)	2.00 (1.41 to 2.87)	.02
Diabetes mellitus	128 (89.5)	15 (10.5)	1.36 (0.21 to 8.89)	.75

*Values for each group are frequencies of risk factors (percentages).

enzymes to control ROS availability, such as catalase, superoxide dismutase, and GP.[20]

Glutathione peroxidase 1 is an important member of the natural enzymatic antioxidants protecting cells from oxidative damage and may be involved in cell signaling and reducing inflammatory processes as part of events involved in stone formation.[21] Several single nucleotide polymorphisms were mapped in *GPX1* gene sequence, one of which, Pro198Leu is supposed to be functional.[22] Several studies have been published concerning the association of the *GPX1* Pro198Leu polymorphism and various cancers.[23,24] However, there has not been a study of the association between the nephrolithiasis and *GPX1* gene polymorphisms. Erdem and coworkers evaluated the association of Pro198Leu polymorphism with prostate cancer.[23] They did not find any difference between genotype distributions in stone formers compared with normal individuals. However, they have been reported the lower *GPX1* activity in erythrocyte cells in cancer group than in healthy controls. They proposed that the Pro198Leu polymorphism might not be authenticated as a suitable marker for prognosis of prostate cancer. In similar studies, Ishimura and colleagues[24] and Paz-Y-Mino and colleagues[25] examined the association of *GPX1* Pro198Leu polymorphism with the susceptibility to bladder cancer. Both groups have been reported an increased risk of bladder cancer in *GPX1* Leu-allele carriers. Inevitably, further investigations require determining the influence of *GPX1* Pro198Leu polymorphism in human diseases.

This case-control study was conducted to clarify whether the functional *GPX1* Pro198Leu polymorphism was associated with nephrolithiasis. According to our results, the frequency of Leu-carriage genotypes was significantly different between nephrolithiasis cases and controls and were associated with an elevated risk of nephrolithiasis. Further analysis stratifying the data for age, sex, and body mass index did not modify the association. Examining the impact of some known factors related to environmental oxidative stress on nephrolithiasis showed a significant association between the stone formation and alcohol consumption, coffee drink, and high exposure to sunlight, which is mostly consistent with previous reports.[26,27] Smoking, alcohol, and coffee were found to increase oxidative stress and suppress antioxidant defense mechanism in the kidney.[26,27] A higher percentage of stone formation is seen in patients with positive family history, which may because of the existence of common susceptibility loci or exposure to the same environmental factors.[28] Inconsistent with the previous huge amount of studies considering the association of diabetes mellitus and kidney diseases, our results failed to find any association between diabetes mellitus and nephrolithiasis in cases compared with controls.[29] We also failed to find an association between Leu-carriage and Pro/Pro homozygote patients with a history of smoking, alcohol consumption, or coffee drinking. These findings support the independent influence of *GPX1* gene in the pathogenesis of nephrolithiasis regardless of the other risk factors.

Efforts to uncover the functional effect of Pro198Leu polymorphism provided inconsistent results. In this regard, Forsberg and coworkers and Jablonska and colelagues found no significant difference in *GPX1* activity by genotype.[14,30] Conversely, Hu and Diamond showed that the presence of Leu-allele resulted in a reduced response of *GPX1* enzyme followed by Selenium supplementation in vitro.[31] Also, in studies by the Ichimura and colleagues[24] and Ravn-Haren and colleagues,[33] a strong correlation was found between *GPX1* 198Leu allele and lower *GPX1* activity. This result was repeated by another group in 2010, which reported the lower *GPX1* activity in the presence of Leu-allele.[34] Our findings seem to support the oxidative stress-dependent pathology of nephrolithiasis: the effect of the *GPX1* genotype on nephrolithiasis risk would be expected if GP enzyme activity differs between the genotypes, such that a low-activity allele would be associated with a relatively high risk of nephrolithiasis due to less efficient prevention of ROS accumulation in cells and consequently cell injuries.

CONCLUSIONS

Results of our study demonstrated that Pro198Leu of *GPX1* might be a genetic risk factor in the development of nephrolithiasis. It is indicated that *GPX1* Leu-allele might be associated with higher nephrolithiasis risk among south Iranian population with lower GP enzyme activity.

ACKNOWLEDGMENTS

The data reported here were extracted from a

Master's of Science thesis by Raziyeh Aghakhani (Molecular Genetics, Islamic Azad University, Arsanjan Branch). We thank N Noruzi and N Jaafari (laboratory staffs) for their technical assistance. Finally, we would like to thank all the volunteers who took part in this study.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Rivers K, Shetty S, Menon M. When and how to evaluate a patient with nephrolithiasis. Urol Clin North Am. 2000;27:203-13.
2. Aggarwal S, Tandon CD, Forouzandeh M, Singla SK, Kiran R, Jethi RK. Role of biomolecules from human renal stone matrix on COM crystal growth. Mol Cell Biochem. 2000;210:109-19.
3. Ratkalkar VN, Kleinman JG. Mechanisms of Stone Formation. Clin Rev Bone Miner Metab. 2011;9:187-97.
4. Davalos M, Konno S, Eshghi M, Choudhury M. First Prize (Tie): oxidative renal cell injury induced by calcium oxalate crystal and renoprotection with antioxidants: a possible role of oxidative stress in nephrolithiasis. J Endourol. 2010;24:339-45.
5. Ozbek E. Induction of Oxidative Stress in Kidney. Int J Nephrol. 2012;2012:9p.
6. Gaspar S, Niculite C, Cucu D, et al. Effect of calcium oxalate on renal cells as revealed by real-time measurement of extracellular oxidative burst. Biosens Bioelectron. 2010;25:1729.
7. Betteridge DJ. What is oxidative stress? Metabolism. 2000;49:3-8.
8. Wei YH, Lu CY, Wei CY, Ma YS, Lee HC. Oxidative stress in human aging and mitochondrial disease-consequences of defective mitochondrial respiration and impaired antioxidant enzyme system. Chinese J Physiol. 2001;44:1-11.
9. Schwille PO, Manoharan M, Schmiedl A. Is idiopathic recurrent calcium urolithiasis in males a cellular disease? Laboratory findings in plasma, urine and erythrocytes, emphasizing the absence and presence of stones, oxidative and mineral metabolism: an observational study. Clin Chem Lab Med. 2005;43:590.
10. Yin J, Ren W, Wu X, et al. Oxidative stress-mediated signaling pathways: A review. J Food Agri Environ. 2013;11:132-9.
11. Lubos E, Loscalzo J, Handy DE. Glutathione Peroxidase-1 in Health and Disease: From Molecular Mechanisms to Therapeutic Opportunities. Antioxid Redox Signal. 2011;5:1958-69.
12. Arthur JR. The glutathione peroxidases. Cell Moll Life Sci. 2000;57:1825-35.
13. Miwa T, Adachi T, Ito Y, Hirano K, Sugiura M. Purification and properties of glutathione peroxidase from human liver. Chem Pharm Bull (Tokyo). 1983;31:179-85.
14. Jablonska E, Gromadzinska J, Reszka E, et al. Association between GPx1 activity and plasma selenium concentration in humans. Eur J Nutr. 2009;48:383-6.
15. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
16. Singh KB, Sailo S. Understanding epidemiology and etiologic factors of urolithiasis: an overview. Sci Vis. 2013;13:169-74.
17. Attanasio M. The genetic components of idiopathic nephrolithiasis. Pediatr Nephrol. 2011;26: 337-46.
18. Wilkilson H. Clinical investigation and management of patients with renal stones. Ann Clin Biochem. 2001;38:180-7.
19. Khan SR. Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol Res. 2005;33:349-57.
20. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv. 2015;5:27986-28006.
21. Brigelius-Flohe R. Selenium compounds and selenoproteins in cancer. Chem Biol. 2008;5:389-95.
22. Kuzuya M, Ando F, Iguchi A, Shimokata H. Glutathione peroxidase 1 Pro198Leu variant contributes to the metabolic syndrome in men in a large Japanese cohort. Am J Clin Nutr. 2008;87:1939-44.
23. Erdem O, Eken A, Akay C, et al. Association of GPX1 polymorphism, GPX activity and prostate cancer risk. Hum Exp Toxicol. 2012;31:24-31.
24. Ichimura Y, Habuchi T, Tsuchiya N, et al. Increased risk of bladder cancer associated with a glutathione peroxidase 1 codon 198 variant. J Urol. 2004;172:728-32.
25. Paz-y-Miño C, Muñoz MJ, López-Cortés A, et al. Frequency of polymorphisms pro198leu in GPX-1 gene and ile58thr in MnSOD gene in the altitude Ecuadorian population with bladder cancer. Oncol Res. 2010;18:395-400.
26. Raaschou-Nielsen O, Sorensen O, Hansen RD, et al. GPX1 Pro198Leu polymorphism, interactions with smoking and alcohol consumption, and risk for lung cancer. Cancer Lett. 2007;247:293-300.
27. Hansen RD, Krath BN, Frederiksen K, et al. GPX1 Pro198Leu polymorphism, erythrocyte GPX activity, interaction with alcohol consumption and smoking, and risk of colorectal cancer. Mut Res. 2009;664:13-9.
28. Polito C, La Manna A, Nappi B, Villani J, Di Toro R. Idiopathic hypercalciuria and hyperuricosuria: family prevalence of nephrolithiasis. Pediatr Nephrol. 2000;14:1102-4.
29. Robert C, Stanton T. Oxidative stress and diabetic kidney disease. Curr Diab Rep. 2011;11:330-6.
30. Forsberg L, de Faire U, Marklund SL, et al. Phenotype determination of a common Pro-Leu polymorphism in human glutathione peroxidase 1. Blood Cells Mol Dis. 2000;26:423-6.
31. 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;63:3347-51.

32. Ichimura Y, Habuchi T, Tsuchiya N, et al. Increased risk of bladder cancer associated with a glutathione peroxidase 1 codon 198 variant. J Urol. 2004;172:728-32.
33. Ravn-Haren G, Olsen A, Tjonneland A, et al. Associations between GPX1 Pro198Leu polymorphism, erythrocyte GPX activity, alcohol consumption and breast cancer risk in a prospective cohort study. Carcinogenesis. 2006;27:820-5.
34. Xiong YM, Mo XY, Zou XZ, et al. Association study between polymorphisms in selenoprotein genes and susceptibility to Kashin-Beck disease. Osteoarthritis Cartilage. 2010;18:817-24.

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Received April 2016
Revised July 2016
Accepted August 2016