Serum paraoxonase-1 gene polymorphism and enzyme activity in patients with urolithiasis

Arda Atar, Asuman Gedikbas, Erkan Sonmezey, Zeynep Kusku Kiraz, Semra Abbasoglu, Ali Ihsan Tasci and Volkan Tugcu

Department of Urology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey; Department of Biochemistry, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey

ABSTRACT

Objectives Paraoxonase-1 (PON1) is a high-density lipoprotein-associated enzyme implicated in the pathogenesis of atherosclerosis by protecting lipoproteins against peroxidation. PON1 has two genetic polymorphisms both due to amino acid substitution, one involving glutamine and arginine at position 192 and the other leucine and methionine at position 55. Recent reports suggest that nephrolithiasis and atherosclerosis share a number of risk factors. Our study aimed to compare the effects of PON1 192, PON1 55 polymorphisms, and PON1 activity in patients with urolithiasis and controls.

Materials and methods PON1’s arylesterase/paraoxonase activities and phenotype were determined in 158 stone forming cases (Group 1) and 138 non-stone forming controls (Group 2). The PON1 192 and PON1 55 polymorphisms were studied by polymerase chain reaction/restriction fragment length polymorphism.

Results Paraoxonase activity was significantly lower in Group 1 than Group 2 (112 ± 31.8 vs. 208 ± 53.1 IU/L) (p < 0.001). The PON1 L55M polymorphism was significantly higher in Group 1. The “M” allele coding for PON1 was higher in Group 1 (p < 0.001). PON1 192 RR homozygotes had significantly higher PON1 activity than QR and QQ genotypes among all the patients (p < 0.001).

Conclusion The results of our study demonstrate that the PON1 55 gene “M” allele is associated with renal stone disease. Individuals possessing the “M” allele have a higher incidence of urolithiasis. The results of this study provide genetic evidence that the PON1 gene may play a role in stone formation. PON1 genotype determination may provide a tool to identify individuals who are at risk of urolithiasis.

Introduction

Paraoxonase-1 (PON1) is a serum high-density lipoprotein (HDL)-bound enzyme with an antioxidant function. It plays an important role in lipid metabolism as preventing low-density lipoproteins from oxidative modifications. The PON gene clusters maps to human chromosome 7q21-22, and several polymorphisms in the promoter and coding regions have been identified. It contains 2 coding region polymorphisms leading 2 different PON1 isoforms: 1 at position 192 (glutamine [Q] to arginine [R] substitution) and the second at position 55 (leucine [L] to methionine [M] substitution). PON1 activity is reduced in high oxidative stress (OS) diseases such as coronary heart disease, dyslipidemia, inflammatory processes, diabetes, and certain neuropathies. Decreased PON1 activity is associated with the increased lipid peroxidation; this might be a factor for determining predisposition to stone formation.

The prevalence of nephrolithiasis has increased dramatically over the last two decades, along with comorbidities, such as hypertension, diabetes mellitus, atherosclerosis, and myocardial infarction. Despite extensive study into the pathogenesis of nephrolithiasis, the recurrence rates remain high, indicating that substantial progress in the understanding of stone pathogenesis remains elusive.

The role of OS in the pathogenesis of nephrolithiasis remains to be determined; however, multiple animal model and tissue culture studies have revealed that high calcium oxalate (CaOx), calcium phosphate, and oxalate (Ox) crystals produced excessive reactive oxygen species (ROS) followed by localized inflammation, extracellular matrix mineralization, and fibrosis. Results of a recent study of 17,695 participants in NHANES III (National Health and Nutrition Examination Survey) showed significantly lower antioxidants, carotene, and...
β-cryptoxanthin in those with a kidney stone history. ROS overproduction or decreased antioxidants lead to OS, inflammation and injury, and are related to stone comorbidity. There is little and controversial data regarding PON1 polymorphisms as well as PON1/arylesterase activities in stone formation. Hence, the present study was aimed to investigate the possible association between L55M and Q192R polymorphisms as well as PON1 activities and urolithiasis.

Materials and methods
This case–control study was performed at the Department of Urology and Clinical Biochemistry of BEAH between June 2012 and November 2013. The study groups consisted of 158 patients with urolithiasis (Group 1) and a control group of 138 healthy individuals (Group 2). The study project was approved by the ethics committee of BEAH and informed consent was obtained from all participants.

Exclusion criteria of the urolithiasis patients included usage of supplementary vitamins, the presence of hyperlipidemia, acute-chronic liver diseases, or renal dysfunction. The control group consisted of 138 healthy subjects (without a history of chronic or recurrent disease). The subjects in the control group were asymptomatic with an unremarkable medical history and a normal physical examination. None of the control subjects were receiving the treatment of antibiotics or antioxidant vitamin supplements including vitamins C and E.

Venous blood samples were collected in tubes from the antecubital vein, followed by an overnight fasting. The tubes were centrifuged at 2000 × g (10 min) to remove the plasma and serum. The plasma and serum samples were kept at −80 °C until the analysis of PON 1 activity. Paraoxonase activities were determined using a novel automated measurement method developed by Erel (Rel Assay Diagnostics, Gaziantep, Turkey). Briefly, the rate of paraoxon hydrolysis was measured by the increased absorbance at 412 nm at 25 °C. The PON activity is expressed as U/L serum. The coefficient of variation for individual samples was 1.8%. Other biochemical parameters were determined by Abbott Architect C16200 chemistry auto-analyzer and using commercial kits (Abbott Laboratories, Abbott Park, IL).

Genomic DNA was isolated from peripheral blood leukocytes by using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). Detection of polymorphisms was done by rapid capillary PCR with melting curve analysis using fluorescence-labeled hybridization probes in a LightCycler (Roche Diagnostics GmbH, Mannheim, Germany).

Table 1. Demographical data and PON1 activity among all patients.

|                         | Control | Urolithiasis |
|-------------------------|---------|--------------|
| Age (y)                 | 55.93 ± 17.14 | 53.42 ± 17.55 |
| Sex (M/F)               | 88/50   | 110/48       |
| Serum Ca (mg/dL)        | 9.21 ± 0.45 | 9.57 ± 0.42  |
| Serum uric acid (mg/dL) | 4.88 ± 1.29 | 5.46 ± 1.58  |
| Creatinine (mg/dL)      | 0.82 ± 0.15 | 0.95 ± 0.28  |
| PON1 activity U/L*      | 208 ± 53.1 | 112 ± 31.8   |

*p < 0.05.

Table 2. Distribution of PON1 genotypes defined by polymorphism at position 55 and 192 of the mature enzyme protein.

| Genotype Control Urolithiasis | OR (95% CI) |
|-------------------------------|-------------|
| PON1 192 genotype frequency   |             |
| QQ                            | 20          | 28          | 0.084 | 0.51 |
| QR                            | 58          | 85          | 0.959 | 0.98 |
| RR                            | 60          | 45          |       |     |
| PON1 192 allele frequency (Q/R)|             |
| Q                             | 0.35        | 0.45        | 0.053 | 1.19 |
| R                             | 0.65        | 0.55        |       |     |
| PON1 55 genotype frequency   |             |
| LL                            | 62          | 23          |       |     |
| LM                            | 58          | 68          | 0.002 | 3.21 |
| MM                            | 18          | 67          | 0.001 | 9.88 |
| PON1 55 allele frequency (L/M)|             |
| L                             | 0.66        | 0.36        | 0.001 | 3.41 |
| M                             | 0.34        | 0.63        |       |     |

All statistical analyses were performed with SPSS 11.0 for Windows (Chicago, IL). Differences in genotype distributions and allele frequencies in the cases and the controls were compared for statistical significance using the chi-square (χ²) test. The statistical significance for deviations from the Hardy–Weinberg Equilibrium (HWE) was determined using the Pearson χ²-test. The associations between genotypes of PON1 gene and urolithiasis were estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. The wild-type genotype/allele served as a reference category. Haplotype frequencies were estimated using the Haploview software and compared between cases and controls using a contingency χ²-test.

Results
Demographic and clinical characteristics of the healthy controls and the urolithiasis patients are presented in Table 1. The groups were similar in terms of age, sex, and the presence of risk factors for stone formation (p > 0.05). The activity of PON1 was significantly lower in urolithiasis group than in control individuals (p = 0.001).

The frequency of PON1 Q192R and PON1 L55M polymorphism in control and urolithiasis subjects is shown in Table 2. The results showed that there were no significant differences regarding Q192R genotypes or
allele frequencies among case and control groups ($p > 0.05$). The PON1 L55M polymorphism was significantly higher in urolithiasis group ($p = 0.002$). The “M” allele coding for PON1 was higher in urolithiasis group ($p = 0.0001$). The patients with a MM genotype showed more possibilities of urolithiasis than those with a LM genotype (OR = 9.88, 95% CI). The results showed that the “R” allele was not significantly associated with risk of urolithiasis. The Q192R genotype polymorphism in case and control groups was consistent with HWE.

PON1 192 RR homozygotes had significantly higher PON1 activity than QR and QQ genotypes. PON1 activity (U/L) for RR, RQ, and QQ genotypes are 164.44 ± 41.25, 98.3 ± 26.97, 76.98 ± 14.98, respectively ($p < 0.05$; Table 3).

### Discussion

Although little is known about its etiology, several lines of evidence point out that ROS production and OS development may be the primary starting point in the pathogenesis of urolithiasis. Under normal conditions, ROS such as superoxide anions, nitric oxide radicals, hydroxyl-free radicals, and hydrogen peroxides normally occur at steady state levels and have many significant regulatory roles. They are generated as needed and are then cleared by the activity of various antioxidants and scavengers. ROS can also produce chemical modifications of and damage to proteins, lipids, carbohydrates, and nucleotides, and they modulate renal and cardiovascular systems through redox-dependent signaling pathways. Uncontrolled generation of reactive oxygen and/or a decrease in endogenous antioxidant capacity creates OS which may lead to inflammation and injury.15

### Table 3: Distribution of stone parameters and PON1 activity among 192 Q > R genotypes.

| PON1 192, Q > R | RR, n = 35 | RQ, n = 68 | QQ, n = 22 | p |
|-----------------|------------|------------|------------|---|
| Serum Ca (pg/mL) | 9.56 ± 0.38 | 9.56 ± 0.41 | 9.6 ± 0.54 | 0.926 |
| Serum uric acid (mg/dL) | 5.94 ± 1.61 | 5.37 ± 1.61 | 4.96 ± 1.29 | 0.058 |
| Serum creatinine (mg/day) | 1.27 ± 1.53 | 0.87 ± 0.16 | 0.9 ± 0.23 | 0.067 |
| Cr/24-h urine (mg/day) | 1585.11 ± 367.51 | 1542.81 ± 351.2 | 1377.25 ± 340.47 | 0.184 |
| Uric acid/24-h urine (mg/day) | 537.47 ± 188.22 | 568.77 ± 219.21 | 550.44 ± 156.01 | 0.839 |
| Ca/24-h urine (mg/day) | 139.15 ± 88.84 | 143.59 ± 85.94 | 135.1 ± 102.03 | 0.944 |
| K/24-h urine (mmol/day) | 34.32 ± 16.7 | 31.96 ± 10.86 | 48.39 ± 60.19 | 0.151 |
| Citrate/24-h urine (mmol/day) | 550.79 ± 577.79 | 440.02 ± 367.37 | 259.4 ± 262.89 | 0.127 |
| Oxalate/24-h urine (mg/day) | 1.75 ± 2.14 | 2.95 ± 12.67 | 1.49 ± 1.06 | 0.836 |
| PON1 activity (U/L) | 164.44 ± 41.25 | 98.3 ± 26.97 | 76.98 ± 14.98 | 0.0001 |

ROS also have a role in the prevention of stone formation via production of crystallization inhibitors. Decrease in antioxidant capacity would hamper the crystallization inhibitors and coupled with persistent oversaturation of urine would result in an increase in crystallization and stone formation.9

Despite promising preliminary findings, prospective data evaluating the role of antioxidants in humans for prevention of stone formation are lacking. Taylor et al. demonstrated that adherence to the high-antioxidant dietary approaches to stop hypertension (DASH) diet which reduces the risk of stroke and cardiovascular disease (CVD), also reduces the risk of stone formation up to 45%. Tungsanga et al. demonstrated that a cohort of patients who were scheduled for surgical stone removal had higher levels of blood lipid peroxidation products and a decreased antioxidant status compared with healthy non-stone formers. In addition, Boonla et al. showed elevated urinary 8-hydroxy deoxyguanosine excretion in nephrolithiasis patients indicating increased oxidative DNA damage. Mushtaq et al. also reported urinary excretion of the anti-inflammatory proteins calgranulin, α-defensin, and myeloperoxidase-8 by stone patients and the presence of these proteins in the inner core of CaOx stones, indicating a putative role in nephrolithiasis. Sur et al. recently investigated the impact of statin medications on urinary stone formation and found 50% decreased risk of stone formation in patients who used statins compared to those who did not.11,16–19

Animal models also suggest that renal responses to Ox and crystals are mediated by ROS. An increase in the renal tissue and urine levels of lipid peroxides is reported in rats with hyperoxaluria and CaOx stones.20 With vitamin E therapy, tissue levels of antioxidant enzymes increased, peroxidase tissue injury subsided and CaOx crystal deposition came to a halt. In the kidneys with CaOx crystal deposition, a decrease in total renal cellular glutathione and an increase in lipid peroxides were reported. Losartan, an angiotensin-converting enzyme...
inhibitor that is known to decrease the OS, increased the glutathione, and decreased the thiobarbituric acid levels in the kidneys when administered to rats. In hyperoxaluric rats, elevations in kidney catalase and Mn superoxide dismutase activities were noted along with increased levels of α and μ glutathione-S-transferase in the urine.21

PON1, a hydrolytic antioxidant enzyme, is associated with a wide range of substrates including HDL, to which it is exclusively bound in the plasma.22,23 By hydrolyzing hydrogen peroxide, HDL-associated PON takes part in the neutralization of the lipid peroxidation byproducts, which decreases the oxidation of LDL and eventually the risk of CVD.24 Due to its anti-inflammatory and antioxidative properties, PON1 is frequently implicated in immune mediated, inflammatory and oxidative and nitrosative stress (O&NS)-related disorders, including CVD, chronic obstructive pulmonary disorder, lung cancer, and inflammatory bowel disease.25–28

Enzymatic activity of PON1 is determined by the PON1 gene and reports indicate an important role for the Q-R polymorphism at the 192 position (Q192R).29 While some reports associate RR genotype with an increased risk of ischemic stroke,30 others suggest it to be more protective against OS. QQ genotype, on the other hand, is related to weaker paraoxon hydrolyzing activity, lower protection against HDL and LDL oxidation, and higher susceptibility to genotoxicity.30–32 We found a positive association between the PON1 55 “M” allele and the possibility of stone formation, whereas no significant difference in PON1 Q192R polymorphism was observed between study and control groups. In addition, serum PON1 activity was significantly lower in urolithiasis group than in the control group.

Serum paraoxonase (PON1) is an antioxidant enzyme that hydrolyzes lipid peroxidation products and H2O2 and contributes to the prevention of LDL oxidation. On the other hand, it has been reported that the PON1 192 “R” allele is less effective at hydrolyzing lipid peroxides than the “Q” allele.33 Therefore, the findings of our research can be explained by the lower ability of PON1 192 “R” allele in hydrolyses of lipid peroxides in patients with urolithiasis.

To our knowledge, our study is the first investigation evaluating association between PON1 polymorphism and nephrolithiasis. Tracy et al. evaluated the association between levels of antioxidant PON1 activity and CaOx stone formation. They examined differences between recurrent stone formers and non-stone-formers regarding OS and the effect of pomegranate administration on risk factors for CaOx stone formation. Baseline serum PON1 activity was similar between the two groups, but after pomegranate supplementation there was a significant 10% increase in PON1 activity and a tendency toward significantly lower values for CaOx supersaturation.34

There are some major limitations in the current study. Some of these are sample size, different ethnic groups, and different environmental conditions, all affecting oxidase–antioxidase balance. Larger sample sizes with different ethnic groups are required to validate our findings. In conclusion, we found that there is an association between L55M polymorphism of PON1 and urolithiasis and “M” allele is a risk factor for urolithiasis. No association was observed between PON1 Q192R polymorphism and urolithiasis. Serum PON1 and ARE activities were found to be lower in urolithiasis group than in control groups. More studies in large samples with different ethnicities are necessary to find out the exact mechanisms affecting high PON1 and ARE activities in urolithiasis.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**References**

1. La Du BN, Adkins S, Kuo CL, et al. Studies on human serum paraoxonase/arylesterase. *Chem Biol Interact*. 1993;87:25–34.
2. Humbert R, Adler DA, Disteche CM, et al. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat Genet*. 1993;3:73–76.
3. Garin MC, James RW, Dussoix P, et al. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J Clin Invest*. 1997;99:62–66.
4. Aviram M, Hardak E, Vaya J, et al. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation*. 2000;101:2510–2517.
5. Mackness B, Mackness MI, Arrol S, et al. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *Febs Lett*. 1998;423:57–60.
6. Khan SR. Is oxidative stress, a link between nephrolithiasis and obesity, hypertension, diabetes, chronic kidney disease, metabolic syndrome? *Urol Res*. 2012;40:95–112
7. Lotan Y, Buendia Jimenez I, Lenoir-Wijnkoop I, et al. Primary prevention of nephrolithiasis is cost-effective for a national healthcare system. *BJU Int*. 2012;110:E1060–E1067
8. Tracy CR, Pearle MS. Update on the medical management of stone disease. *Curr Opin Urol*. 2009;19:200–204.
9. Khan SR. Reactive oxygen species as the molecular modulators of calcium oxalate kidney stone formation:
Evidence from clinical and experimental investigations. J Urol. 2013;189:803–811.

10. Holoch PA, Tracy CR. Antioxidants and self-reported history of kidney stones: The National Health and Nutrition Examination Survey. J Endourol. 2011;25:1903–1908

11. Boonla C, Wunsuwan R, Tungsanga K, et al. Urinary 8-hydroxydeoxyguanosine is elevated in patients with nephrolithiasis. Urol Res. 2007;35:185–191

12. 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;25:1729–1734.

13. Tugcu V, Kemahli E, Ozbek E, et al. Protective effect of a potent antioxidant, pomegranate juice, in the kidney of rats with nephrolithiasis induced by ethylene glycol. J Endourol. 2008;22:2723–2731.

14. Ilbey YO, Ozbek E, Simsek A, et al. Effects of pomegranate juice on hyperoxaluria-induced oxidative stress in the rat kidneys. Ren Fail. 2009;31:522–531

15. Manea A. NADPH oxidase-derived reactive oxygen species: Involvement in vascular physiology and pathology. Cell Tissue Res. 2010;342:325–339.

16. Taylor EN, Fung TT, Curhan GC, et al. DASH-style diet associates with reduced risk for kidney stones. J Am Soc Nephrol. 2009;20:2253–2259.

17. Tungsanga K, Sriroonblue P, Futrakul P, et al. Renal tubular cell damage and oxidative stress in renal stone patients and the effect of potassium citrate treatment. Urol Res. 2005;33:65–69.

18. Mustaq S, Siddiqui AA, Naqvi ZA, et al. Identification of myeloperoxidase, alpha-defensin and calgranulin in calcium oxalate renal stones. Clin Chim Acta. 2007;384:41–47.

19. Sur RL, Masterson JH, Palazzi KL, et al. Impact of statins on nephrolithiasis in hyperlipidemic patients: A 10-year review of an equal access health care system. Clin Chim Acta. 2013;399:351–355.

20. Thamilselvan S, Menon M. Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. BJU Int. 2005;96:117–126.

21. Huang HS, Ma MC, Chen J, et al. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. J Urol. 2002;167:2584–2593.

22. Litvinov D, Mahini H, Garelnabi M, et al. Antioxidant and anti-inflammatory role of paraoxonase 1: Implication in arteriosclerosis diseases. N Am J Med Sci. 2012;4:523–532.

23. Razavi AE, Ani M, Pourfarzam M, et al. Associations between high density lipoprotein mean particle size and serum paraoxonase-1 activity. J Res Med Sci. 2012;17:1020–1026.

24. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest. 1998;101:1581–1590.

25. Furlong CE, Suzuki SM, Stevens RC, et al. Human PON1, a biomarker of risk of disease and exposure. Chem Biol Interact. 2010;187:355–361.

26. Isik B, Isik RS, Ceylan A, Calik O. Trace elements and oxidative stress in chronic obstructive pulmonary disease. Saudi Med J. 2005;26:1882–1885.

27. Rothem L, Hartman C, Dahan A, Lachter J, Eliakim R, Shamir R. Paraoxonases are associated with intestinal inflammatory diseases and intracellularly localized to the endoplasmic reticulum. Free Radic Biol Med. 2007;43:730–739.

28. Goswami B, Tayal D, Gupta N, Mallika V. Paraoxonase: A multifaceted biomolecule. Clin Chim Acta. 2009;410:1–12.

29. Liu ME, Liao YC, Lin RT, et al. A functional polymorphism of PON1 interferes with microRNA binding to increase the risk of ischemic stroke and carotid atherosclerosis. Atherosclerosis. 2013;228:161–167.

30. Mackness M, Mackness B. Targeting paraoxonase-1 in atherosclerosis. Expert Opin Ther Targets. 2013;17:829–837.

31. Singh S, Kumar V, Thakur S, et al. Paraoxonase-1 genetic polymorphisms and susceptibility to DNA damage in workers occupationally exposed to organophosphate pesticides. Toxicol Appl Pharmacol. 2011;252:130–137.

32. Kotani K, Tsuzaki K, Sakane N. Paraoxonase Q192R polymorphism and reactive oxygen metabolites. J Int Med Res. 2012;40:1513–1518.

33. Gamboa R, Zamora J, Rodriguez-Perez JM, et al. Distribution of paraoxonase PON1 gene polymorphisms in Mexican populations. Its role in the lipid profile. Exp Mol Pathol. 2006;80:85–90.

34. Tracy CR, Henning JR, Newton MR, et al. Oxidative stress and nephrolithiasis: A comparative pilot study evaluating the effect of pomegranate extract on stone risk factors and elevated oxidative stress levels of recurrent stone formers and controls. Urolithiasis. 2014;42:401–408.