ORIGINAL RESEARCH
General Urology

Doi: 10.4274/jus.galenos.2023.2023-10-2
J Urol Surg 2023;10(4):290-294

The Association Between *Sodium Citrate Cotransporter (NaDC-1)* Gene Polymorphism and Urinary Citrate Excretion in Patients with Calcium-containing Kidney Stones

Ahmet Çalışkan[1], Ömür Memik[2], Selma Düzenli[3], Ali Tekin[4]

[1]Akçakoca State Hospital, Clinic of Urology, Düzce, Turkiye
[2]University of Health Sciences Turkiye, Kocaeli Derince Training and Research Hospital, Clinic of Urology, Kocaeli, Turkiye
[3]Bolu Abant İzzet Baysal University Faculty of Medicine, Department of Medical Genetics, Bolu, Turkiye
[4]Acıbadem University Atakent Hospital, Department of Urology, İstanbul, Turkiye

What's known on the subject? and What does the study add?

The I550V single-nucleotide polymorphism (SNP-rs11567842) has been associated with hypocitraturia and calcium kidney stones. Previous studies have reported that the rs11567842 mutation may be protective against hypocitraturia and kidney stones. However, in these studies, urine levels of oxalate, uric acid, and calcium in stone formers were higher than those in healthy individuals in the control group. This indicates that hypocitraturia cannot be the main factor in those who form stones. The current study investigated the relationship between patients with calcium-containing kidney stones and those with normal and low citrate excretion.

Abstract

Objective: To evaluate the relationship between *sodium citrate cotransporter (NaDC-1)* gene polymorphism and urinary citrate excretion in patients with kidney stones containing calcium.

Materials and Methods: Between June 2009 and August 2011, stone materials obtained from patients treated for nephrolithiasis at the Urology Clinic were examined using X-ray diffraction, and patients with calcium-containing stones (calcium oxalate and calcium phosphate) were identified. Patients were divided into two groups based on their 24-hour urine citrate levels: (1) those with normal urine citrate levels and (2) hypocitraturia. To analyze the rs11567842 mutation in the *NaDC-1* gene, their blood was collected in a Na-EDTA hemogram tube and stored at -40 °C. The genotypes of the cases were determined by analyzing the obtained genomic DNAs in real-time polymerase chain reaction.

Results: Ninety-six patients with calcium-containing nephrolithiasis were eligible for this study, 40 with normal urine citrate levels and 56 with hypocitraturia. The mean 24-hour urine citrate levels in the normal- and hypo-citraturia groups were 773 mg/1.73 m^2/24 hours and 152 mg/1.73 m^2/24 hours, respectively. Citrate measurements revealed a statistically significant difference between the two groups ($p<0.001$). Twenty-four-hour urine oxalate, magnesium, calcium, and uric acid levels did not differ significantly between the groups (all $p>0.05$). *NaDC-1* gene rs11567842 homozygous mutation (GG genotypes) was detected in 4 (10%) of normocitraturia and 4 (7%) of hypocitraturia. The normocitraturia group had a higher mutation rate than the hypocitraturia group, but this difference was insignificant ($p=0.618$).

Conclusion: This study suggests that the *NaDC-1* gene polymorphism does not cause hypocitraturia in calcium-containing kidney stones. Larger studies are needed to understand genetic disorders' impact on low urinary citrate excretion, with patient groups and healthy controls, and a standard diet.

Keywords: Citrate, hypocitraturia, NaDC-1, polymorphism, kidney stone, urolithiasis

Correspondence: Ömür Memik MD, University of Health Sciences Turkiye, Kocaeli Derince Training and Research Hospital, Clinic of Urology, Kocaeli, Turkiye
Phone: +90 505 385 71 99 **E-mail:** memikomur@yahoo.com.tr **ORCID-ID:** orcid.org/0000-0003-0328-8444
Received: 08.10.2023 **Accepted:** 17.10.2023

Cite this article as: Çalışkan A, Memik Ö, Düzenli S, Tekin A. The Association Between *Sodium Citrate Cotransporter (NaDC-1)* Gene Polymorphism and Urinary Citrate Excretion in Patients with Calcium-containing Kidney Stones. J Urol Surg 2023;10(4):290-294.

Introduction

Urinary citrate prevents the formation of calcium-containing kidney stones by inhibiting the crystallization and precipitation of calcium, so hypocitraturia, or low urinary citrate excretion, is an essential metabolic risk factor for the formation and recurrence of urinary stones (1). Hypocitraturia ranges from 20% to 60% among patients with calcium-containing kidney stones (2). The mechanisms underlying hypocitraturia in calcium-containing kidney stones still need to be fully clarified. Citrate's intestinal transportation, serum concentration, and filtered load do not differ between patients with kidney stones and healthy volunteers (3-5). But, metabolic abnormalities or a diet high in acid-producing foods affect the renal handling of citrate, resulting in alterations in citrate excretion levels in the urine (6-9). However, hypocitraturia can be observed in patients with calcium-containing kidney stones without any metabolic abnormalities, and despite urine collection being performed with fixed diets to minimize dietary factors in the studies, kidney stone formers still have lower citrate excretion compared to controls (4,5,10). These reports suggest that genetic predispositions play a role in the formation of kidney stones in patients with hypocitraturia (2).

Citrate is reabsorbed in the proximal tubule apical membrane by the Na^+/$citrate^{2-}$ cotransporter, also known as Na^+/dicarboxylate cotransporter (NaDC-1). Therefore, NaDC-1 is a significant determinant of citrate excretion in the urine. The cDNA of the human *NaDC-1* gene contains 12 exons with 1.953 base pairs and encodes 593 amino acids (11-13). The genetic polymorphism of NaDC-1 (I550V or rs11567842) has been reported to be associated with urinary citrate excretion in Japanese patients with calcium stone formation (14). An *in vitro* experiment revealed that the single nucleotide polymorphisms (SNP) - rs11567842 in *NaDC-1* gene affects the function of NaDC-1, as it causes a decrease in protein expression and transport activity (15). Although the effect of the SNP - rs11567842 on citrate excretion has been demonstrated, its association with calcium stone formation remains unclear. In the current study, the relationship between *NaDC-1* gene SNP (I550V/rs11567842) and stone formation was evaluated by analyzing *NaDC-1* gene polymorphism in two separate patient groups with calcium-containing kidney stones and normal or low citrate excretion.

Materials and Methods

Between June 2009 and August 2011, stone materials obtained from patients treated for kidney stones at the Düzce University Faculty of Medicine Hospital, Clinic of Urology were examined using X-ray diffraction, and patients with calcium-containing kidney stones (calcium oxalate and calcium phosphate) were identified. The patients' weight and height were measured, a detailed medical history was obtained, and they were questioned about familial stone formation, recurrent stone formation, receiving treatment for the urolithiasis (surgical or medical), and systemic or metabolic disease. Excluded from the study were patients with any systemic disease (except hypertension), non-calcium component kidney stones, any treatment that could affect acid-base balance, diuretic treatment, and calcium or vitamin C supplements. For metabolic evaluation, serum creatinine, sodium, potassium, calcium, and uric acid levels were measured in each patient, as well as 24-hour urine citrate, oxalate, magnesium, calcium, and uric acid levels. In addition, a urinalysis and culture of the urine were conducted. Patients were instructed to avoid excessive consumption of red meat, salty foods, chocolate, leafy greens, tea, and coffee prior to the 24-hour urine test. Solute excretion in 24-hour urine samples were measured using the photometric method with Ben Biochemical Enterprise™ (CI8820, Milan, Italy) kits in the biochemistry laboratory of Düzce University Faculty of Medicine Hospital. Patients were divided into two groups based on their 24-hour urine citrate levels: normal and low. To analyze the rs11567842 mutation in the *NaDC-1* gene, their blood was collected in a Na-EDTA hemogram tube and stored at -40 °C.

The study was conducted in line with the principles of the Declaration of Helsinki and was approved by the local ethics committee of Düzce University, Turkiye (approval no: 2010/102, date: 30.12.2010).

Real-time Polymerase Chain Reaction (PCR)

DNA isolation and PCR studies were conducted in the Molecular Genetics Laboratory of the Medical Genetics Department at the Bolu Abant İzzet Baysal University Faculty of Medicine Hospital. Genomic DNA was isolated and obtained using the High Pure PCR Template Preparation Kit-Roche™ kit. The genotypes of the cases were determined by analyzing the obtained genomic DNAs in real-time PCR (Light Cycler480 II™) using primers and probes covering the relevant polymorphism. Each sample was classified as wild type (AA), heterozygous type (AG), or homozygous type (GG) based on the results of the analysis

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 15 (IBM, Chicago, IL, USA). A $p<0.05$ indicated statistical significance. The Kolmogorov-Smirnov test was used to determine the normality of the numeric data. The two groups were compared using the Student's t-test for numerical variables and the Pearson chi-square test for nominal and ordinal variables, including genetic analysis results, gender, family history, and positive urine culture.

Results

Ninety-six patients with calcium-containing kidney stones were eligible for this study. Within the scope of the study, patients

were evaluated: 40 patients with normal 24-hour urine citrate levels and 56 patients with low levels. Twenty-four men and 16 women comprised the normocitraturia group, whereas 33 men and 23 women comprised the hypocitraturia group. The mean ages of the normocitraturia and hypocitraturia groups were 44.0 (9-72) and 43.3 (3-70) years, respectively (p=0.84). There were no statistically significant differences between the groups concerning age, gender, body mass index, or positive family history (all p>0.05, Table 1). The normocitraturia group had mean serum creatinine levels of 0.84 mg/dL, while the hypocitraturia group had mean serum creatinine levels of 0.88 mg/dL (p=0.417). Serum calcium and urine pH and density exhibited no statistically significant differences between groups (all p>0.05).

The mean 24-hour urine citrate level in the normocitraturia group was 773 mg/1.73 m^2/24 hours; in the hypocitraturia group, it was 152 mg/1.73 m^2/24 hours. Citrate measurements revealed a statistically significant difference between the two groups (p<0.001). The normocitraturia group had an average 24-hour urine oxalate concentration of 39.2 mg/1.73 m^2/24 hours, while the hypocitraturia group had an average 24-hour urine oxalate concentration of 33.3 mg/1.73 m^2/24 hours. Regarding oxalate measurement, there was no statistically significant difference between the groups (p=0.130). In addition, 24-hour urine magnesium, calcium and uric acid levels did not differ significantly between the groups (all p>0.05). The results of a 24-hour urine analysis (urine volume, oxalate, magnesium, calcium, and uric acid) are shown in Table 2.

Comparing the urine culture results of the normocitraturia and hypocitraturia groups, urine culture positivity was detected in 2 (5%) normocitraturia and 4 (7.2%) hypocitraturia patients. In terms of urine culture positivity, there was no statistically significant difference between the two groups (p=0.665). In 4 (10%) normocitraturia patients and 4 (7%) hypocitraturia

Table 1. Patient characteristics and familial stone formation rates

		Nephrolithiasis with normal citraturia (n=40)	Nephrolithiasis with hypocitraturia (n=56)	p-value*
Age (years)		44.0±15.0	43.3±15.0	0.839
Height (cm)		168.8±7.7	166.9±13.2	0.425
Weight (kg)		75.1±11.1	74.7±13.4	0.903
BMI (kg/m^2)		26.3±3.9	26.9±4.0	0.820
Gender	Male	24 (60)	33 (59)	0.916
	Female	16 (40)	23 (41)	
Familial stone formation	Yes	22 (55)	21 (38)	0.089
	No	18 (45)	35 (63)	

*: To compare mean values, the Student's t-test was used, and Pearson's chi-squared test was used to compare proportional values

Table 2. The outcomes of serum, spot urine, and 24-hour urine collection tests

	Nephrolithiasis with normal citraturia (n=40)	Nephrolithiasis with hypocitraturia (n=56)	p-value
Serum (blood)			
Creatinine (mg/dL)	0.84±0.1	0.88±0.2	0.417
Calcium (mg/dL)	10.1±3.8	9.5±0.5	0.224
Urinalysis			
pH	5.2±0.7	5.1±0.8	0.308
Density	1017±6.0	1016±6.0	0.377
24-hour urine collection			
Volume (mL)	2276±758	2075±925	0.264
Citrate (mg/1.73 m^2/24 hours)	773±301	152±87	**<0.001**
Oxalate (mg/1.73 m^2/24 hours)	39.2±19.5	33.3±18.0	0.130
Magnesium (mg/dL)	4.7±2.2	5.0±2.2	0.527
Calcium (mg/dL)	9.6±5.2	9.9±6.0	0.810
Uric acid (mg/dL)	23.4±9.6	23.8±12.5	0.859

Bold values denote statistical significance at the p<0.05

Table 3. Genotype frequencies of *NaDC-1 (SLC13A2/I550V)* gene polymorphism			
Genotypes, n, (%)	**Nephrolithiasis with normal citraturia n=40**	**Nephrolithiasis with hypocitraturia n=56**	**p-value**
AA genotype	20 (50)	29 (51.8)	0.618
AG genotype	16 (40)	23 (41.1)	
GG genotype (rs11567842 mutation)	4 (10)	4 (7.1)	

patients, the *NaDC-1* gene rs11567842 homozygous mutation (AA) was found. The hypocitraturia group had a higher mutation rate than the normocitraturia group, but this difference was not statistically significant (p=0.618, Table 3).

Discussion

The approximately 23.8 kb human *NaDC-1* gene is located on chromosome 17 p11.1-q11.1 and comprises 12 exons (16). The *NaDC-1* gene encodes the 592-residue NaDC-1 protein, which shares 54% and 43% sequence identity with the human NaCT and NaDC-3 proteins (17). The kidney and small intestine express NaDC-1 predominately. More specifically, NaDC-1 is localized on the apical membrane of renal proximal tubular and small intestine cells where it reabsorbs tricarboxylic acid cycle intermediates from urine and diet, respectively (18). According to *in vivo* and *in vitro* studies, acidosis stimulates both NaDC-1 function (citrate transport activity) and expression (mRNA and protein levels), whereas alkalosis only affects its citrate transport function (9,19). The primary physiological function attributed to NaDC-1 is citrate elimination in the kidneys. Urinary citrate is essential for preventing the formation of kidney stones by complexing Ca^{2+} ions, thereby preventing urine supersaturation and precipitation of Ca^{2+} salts-based calculi. This suggests that NaDC-1 is involved in the pathophysiology of kidney stones (18). Furthermore, the human *NaDC-1* gene I550V-SNP has been genetically related to hypocitraturia and kidney stones (14). Since citrate reabsorption by NaDC-1 determines urinary citrate concentration, inhibition of NaDC-1 is expected to increase urinary citrate excretion. However, potent specific NaDC-1 inhibitors are not yet available. A specific inhibitor could clarify the connection between NaDC-1-mediated urinary citrate excretion and calcium nephrolithiasis, and it could have been used as a treatment agent today. Therefore, additional research and evidence are required to conclude that NaDC-1-mediated hypocitraturia is a fundamental mechanism underlying calcium-containing kidney stones.

A SNP (pI550V/rs11567842) in the *NaDC-1* gene causes a change from isoleucine (I) to valine (V) at amino acid 550th. Three genotypes have been identified in SNP. These are AA (wild type), AG (heterozygous mutant), and GG (homozygous mutant) genotypes. In the present study, 51.8%, 41.1%, and 7.1% of individuals with hypocitraturia had the AA, AG, and GG genotypes, respectively. Stone formers with normal citrate levels comprised 50% AA, 40% AG, and 10% GG genotypes. There was no statistical difference in the frequency of genotypes between groups.

Okamoto et al. (14) evaluated the effect of I550V-SNP on hypocitraturia and calcium stone formation and found that those with AA genotype had lower urinary citrate levels than other genotypes. They also detected AA genotypes at a higher rate in stone-forming patients than in healthy individuals. They demonstrated that the I550V polymorphism is associated with hypocitraturia and kidney stones and that having the AA genotype may be a risk factor for hypocitraturia and kidney stones. However, according to this study's 24-hour urine examination results, stone-forming groups had lower pH and citrate levels and higher calcium, oxalate, and uric acid levels than stone-free groups. The presence of these values, which may be a risk factor for the formation of kidney stones, makes it difficult to interpret the findings correctly, and it may not be accurate to state that stone formation is only due to low citrate levels.

Udomsilp et al. (20) evaluated the impact of the I550V polymorphism on hypocitraturia and recurrent calcium stone formation. They discovered that individuals with the AA genotype had lower urinary citrate levels than those with other genotypes. However, there was no noticeable distinction in the frequency of genotypes between stone-forming individuals and healthy individuals. They concluded that having the AA genotype is associated with hypocitraturia and may be a risk factor for kidney stone formation. This study did not report urinary levels of calcium, oxalate, and uric acid. In the present study, all I550V polymorphism-examined patients had calcium-containing kidney stones. In addition, pH, calcium, oxalate, and uric acid levels in urine were similar between groups. The current study design minimizes the influence of confounding variables and lacks the limitations of previous research.

Pajor and Sun (15) examined the effect of SNPs on NaDC-1 expression and function using the COS-7 cell heterologous expression system. They showed that the I550V variant had an increased sensitivity to lithium inhibition, although there was no significant effect on protein expression. They also concluded that all SNP mutations reduced the transport activity or expression of NaDC-1, leading to reduced intestinal and renal absorption

of citric acid cycle intermediates. In the current study, although those with *NaDC-1* gene rs11567842 mutation (GG genotype) were detected less frequently in the hypocitraturia group than those with normal urine citrate levels, this difference was not statistically significant. However, when evaluating the findings of our study, it must be kept in mind that no strict diet was adhered to during the research. As is well known, environmental factors, particularly foods that make the urine more acidic, also influence the urinary citrate excretion of individuals with normal renal function. Consequently, environmental factors cannot be ruled out as a cause of hypocitraturia in these patients.

Study Limitations

This study's most significant limitations are its small sample size and lack of a healthy control group. Comprehensive studies with larger patient groups, healthy controls, and a strict diet are required to clarify this relationship.

Conclusion

These results do not support a role for *NaDC-1* gene polymorphism in the etiopathogenesis of hypocitraturia in calcium-containing idiopathic kidney stones. Important limitations of this study include the absence of healthy control subjects and a standard diet. To further elucidate the role of genetic disorders in low urinary citrate excretion, comparative studies with larger patient groups and healthy controls, excluding environmental effects (with a standard diet), should be conducted.

Ethics

Ethics Committee Approval: The study was conducted in line with the principles of the Declaration of Helsinki and was approved by the Local Ethics Committee of Düzce University, Turkiye (approval no: 2010/102, date: 30.12.2010).

Informed Consent: The authors declare that they have no relevant financial.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.Ç., Ö.M., A.T., S.D., Concept: A.Ç., Ö.M., A.T., S.D., Design: A.Ç., A.T., Data Collection or Processing: A.Ç., Ö.M., A.T., Analysis or Interpretation: A.Ç., Ö.M., AT., S.D., Literature Search: A.Ç., Ö.M., A.T., Writing: A.Ç., Ö.M., A.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that they have no relevant financial.

References

1. Leslie SW, Bashir K. Hypocitraturia and Renal Calculi. 2023 May 30. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.
2. Parks J, Ruml L, Pak C. Hypocitraturia: Kidney Stones-Medical and Surgical Management. Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM. 1996.
3. Fegan J, Khan R, Poindexter J, Pak CY. Gastrointestinal citrate absorption in nephrolithiasis. J Urol 1992;147:1212-1214.
4. Minisola S, Rossi W, Pacitti MT, Scarnecchia L, Bigi F, Carnevale V, Mazzuoli G. Studies on citrate metabolism in normal subjects and kidney stone patients. Miner Electrolyte Metab 1989;15:303-308.
5. Rudman D, Kutner MH, Redd SC, Waters WC 4th, Gerron GG, Bleier J. Hypocitraturia in calcium nephrolithiasis. J Clin Endocrinol Metab 1982;55:1052-1057.
6. Breslau NA, Brinkley L, Hill KD, Pak CY. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. J Clin Endocrinol Metab 1988;66:140-146.
7. Windus DW, Cohn DE, Heifets M. Effects of fasting on citrate transport by the brush-border membrane of rat kidney. Am J Physiol 1986;251:F678-F682.
8. Levi M, McDonald LA, Preisig PA, Alpern RJ. Chronic K depletion stimulates rat renal brush-border membrane Na-citrate cotransporter. American Journal of Physiology-Renal Physiology 1991;261:F767-F773.
9. Aruga S, Wehrli S, Kaissling B, Moe OW, Preisig PA, Pajor AM, Alpern RJ. Chronic metabolic acidosis increases NaDC-1 mRNA and protein abundance in rat kidney. Kidney Int 2000;58:206-215.
10. Nicar MJ, Skurla C, Sakhaee K, Pak CY. Low urinary citrate excretion in nephrolithiasis. Urology 1983;21:8-14.
11. Pajor AM. Molecular cloning and functional expression of a sodium-dicarboxylate cotransporter from human kidney. Am J Physiol 1996;270:F642-F648.
12. Pajor AM. Sequence and functional characterization of a renal sodium/dicarboxylate cotransporter. J Biol Chem 1995;270:5779-5785.
13. Hamm LL. Renal handling of citrate. Kidney Int 1990;38:728-735.
14. Okamoto N, Aruga S, Matsuzaki S, Takahashi S, Matsushita K, Kitamura T. Associations between renal sodium-citrate cotransporter (hNaDC-1) gene polymorphism and urinary citrate excretion in recurrent renal calcium stone formers and normal controls. Int J Urol 2007;14:344-349.
15. Pajor AM, Sun NN. Single nucleotide polymorphisms in the human Na+-dicarboxylate cotransporter affect transport activity and protein expression. Am J Physiol Renal Physiol 2010;299:F704-F711.
16. Pajor AM. Molecular properties of sodium/dicarboxylate cotransporters. J Membr Biol 2000;175:1-8.
17. Markovich D, Murer H. The SLC13 gene family of sodium sulphate/carboxylate cotransporters. Pflugers Arch 2004;447:594-602.
18. Bergeron MJ, Clémençon B, Hediger MA, Markovich D. SLC13 family of Na^+-coupled di- and tri-carboxylate/sulfate transporters. Mol Aspects Med 2013;34:299-312.
19. Aruga S, Pajor AM, Nakamura K, Liu L, Moe OW, Preisig PA, Alpern RJ. OKP cells express the Na-dicarboxylate cotransporter NaDC-1. Am J Physiol Cell Physiol 2004;287:C64-C72.
20. Udomsilp P, Saepoo S, Ittiwut R, Shotelersuk V, Dissayabutra T, Boonla C, Tosukhowong P. rs11567842 SNP in SLC13A2 gene associates with hypocitraturia in Thai patients with nephrolithiasis. Genes Genomics 2018;40:965-972.