

Meta-analysis of the urokinase gene 3'-UTR T/C polymorphism and susceptibility to urolithiasis

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Received December 2, 2012; Accepted January 18, 2013

DOI: 10.3892/br.2013.73

Abstract. Urokinase is involved in the processes of initiating urinary stones. Several published case-control studies have examined the relationship of urokinase gene 3'-untranslated region (3'-UTR) T/C polymorphism and urolithiasis, but yielded inconsistent findings. In this study, a comprehensive meta-analysis was conducted by pooling relevant studies to obtain reliable conclusions. Studies focusing on the association between urokinase gene 3'-UTR T/C polymorphism and urolithiasis were retrieved through PubMed, Medline, Web of Science and the China National Knowledge Infrastructure platform without any limit on language, until October 2012. Four independent articles were eventually identified as eligible for the final meta-analysis, involving 1,195 subjects. Crude odds ratios (ORs), as well as 95% confidence intervals (CIs), were assessed for the association by either fixed- or random-effects models using RevMan 5.0 software. Significant associations were noted in the 'TC vs. CC' codominant model for total population (OR=2.53; 95% CI, 1.43-4.46; P=0.001), Asian population (OR=2.46; 95% CI, 1.38-4.40; P=0.002), male (OR=2.98; 95% CI, 1.43-6.21; P=0.004), Hardy-Weinberg equilibrium (HWE) (OR=2.46; 95% CI, 1.38-4.40; P=0.002) and recurrence (OR=2.66; 95% CI, 1.51-4.67; P=0.00). Statistically significant associations were also observed in the 'TT+TC vs. CC' dominant model for the Asian, male, HWE and recurrence population (P<0.05). Additionally, a significant difference was detected in the 'T vs. C' allele model for HWE. However, there were no associations in either the 'TT vs. CC' codominant model or 'TT vs. TC+CC' recessive model. In

conclusion, the present meta-analysis suggests that urokinase gene T allele may increase the susceptibility of urolithiasis.

Introduction

Urolithiasis is a global health problem and is the third most common urologic disease. Approximately 5% of females and 12% of males are likely to develop nephrolithiasis during their lifetime (1). It is also notorious for its high rate of recurrence, which is >40% in five years (2). Various causes contribute to the formation of urinary calculi, including genetic factors (3,4). Efforts have been made to investigate the causes of the disease; however, the detailed pathogenic mechanism for the occurrence and recurrence of urolithiasis remains intangible.

Urolithiasis formation is thought to be involved in the progression of nucleation, growth, aggregation and retention of mineral crystals. A number of proteins and molecules, such as osteocalcin, osteopontin, fetuin-A, heparan sulfate and urokinase, are likely to inhibit calcium oxalate supersaturation and crystalization in various stages of stone formation. Urokinase was first discovered in urine in 1947 and is a multifunctional protein synthesized by a variety of human organs and cells, besides kidney cells (5,6). The activity and concentration of urokinase was verified to be lower in stone patients compared with normal subjects (7). Urokinase, originally known as a plasminogen activator, degrades the organic matrix of urinary stones to prevent their complete formation and growth (8). Thus, it is important in averting the development of urinary stones.

Single nucleotide polymorphisms (SNPs) are considered responsible for interindividual diversity in mediating genetic predisposition to the complex disease (9), including cancer (10,11) and urinary stones (12). The urokinase gene is located on chromosome 10q24 (13). Three polymorphic sites have been commonly reported; a T/C substitution in exon 6, a C/T change in intron 7 (14) and a T/C polymorphism at the +4065 nucleotide in the 3'-untranslated region (3'-UTR). Of the three sites, the 3'-UTR T/C polymorphic site is the most widely studied, and its relationship to complex diseases, such as non-small cell lung, prostate and bladder cancer, has been examined (15-17). Tsai *et al* (18) reported that the 'T' allele in 3'-UTR increased the risk of calcium stone disease. Ozturk *et al* (19) demonstrated that 3'-UTR T/C polymorphism played a role in childhood recurrence urolithiasis. By

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Key words: urokinase, polymorphism, meta-analysis, urolithiasis

Table I. Detailed characteristics of eligible studies.

Variables	Tsai <i>et al</i> (18)	Mittal <i>et al</i> (16)	Ozturk <i>et al</i> (19)	Kim <i>et al</i> (21)
Sample sizes, n				
Patients	153	130	80	370
Controls	105	150	40	167
Age, mean (SD) years				
Controls	54.7	40 (11.5)	10.5 (0.67)	-
Patients	44.2 (12.0)	40.5 (10.5)	10.5 (4.33)	-
			11.2 (3.8)	
Gender, male/female				
Patients	118/35	106/24	55/25	-
Controls	65/40	120/30	25/15	-
P-value for HWE				
Patients	0.44	<0.05	<0.05	<0.05
Controls	0.84	0.75	<0.05	<0.05
Ethnicity	Chinese	Indian	Turkish	Korean
Genotypes, (patients/controls), n				
TT	-/-	32/48	14/12	158/63
CT	18/4	82/72	4/-	212/104
CC	135/101	16/30	62/28	-/-
Alleles, (patients/controls), n				
T	18/4	146/168	32/24	528/230
C	288/206	114/132	128/56	212/104

HWE, Hardy-Weinberg equilibrium.

contrast, two studies did not find any association between 3'-UTR T/C polymorphism and nephrolithiasis (20,21). It is assumed that the inconsistent findings may be secondary to ethnic background or small sample size. In this study, a search was conducted for all studies regarding the association of 3'-UTR T/C polymorphism and urolithiasis. Results were pooled together to obtain reliable conclusions.

Materials and methods

Identification and eligibility of relevant studies. A search of the electronic literature was performed via PubMed, Medline, Web of Science and the China National Knowledge Infrastructure platform without any limit on language, until October 2012. Search terms used included 'urokinase/uPA/urokinase-type plasminogen activator/rs4065' and 'urolithiasis/nephrolithiasis/stone'.

Studies were included in the meta-analysis if they i) focused on the relationship of urokinase gene 3'-UTR T/C polymorphism and the risk of urolithiasis; ii) were case-control designed; iii) provided concrete data for various genotypes for calculating odds ratio (OR) and its corresponding 95% confidence interval (CI). Studies not meeting the inclusion criteria, as well as abstracts without sufficient information were excluded.

Data management. Searches and data extraction for the study were carried out independently by two authors (D.W. Li and

J.K. Liu). The extraction information included: name of the first author, year of publication, ratio of male/female, mean age of the subjects, ethnicity of the population studied, number of cases and controls with the various genotypes of urokinase gene rs4065 polymorphism, and P-value for Hardy-Weinberg equilibrium (HWE) of the genotypes in the patient and control groups.

Statistical analysis. The HWE was determined using the Chi-square test for the urolithiasis and control groups, and was considered statistically significant when $P < 0.05$. In this meta-analysis, RevMan 5.0 software, developed by the Cochrane Collaboration, was used to analyze the data. Publication bias was tested by inverted funnel plots, and subsequently assessed by the Egger's or Begg's test with Stata software version 12.1. Heterogeneity between these studies was tested using the Chi-square-based Q statistics test and was considered statistically significant when $P < 0.10$. Random-effects models were employed when $P_{\text{heterogeneity}} < 0.10$, while the fixed-effects models was used when $P_{\text{heterogeneity}} > 0.10$ (22). The allele, recessive, codominant and dominant models of the associations between urokinase gene 3'-UTR T/C polymorphism and urolithiasis susceptibility were simultaneously calculated by crude ORs and 95% CIs. Sensitivity analysis was carried out by deleting the study with the most subjects if the meta-analysis included three or four studies. In this study, P-value was two-tailed and was considered significant at 0.05.

Table II. Detailed results of meta-analysis.

Category	Patients, n	P _{heterogeneity}	I ² (%)	Statistical method	OR	95% CI	P-value
T vs. C							
Total	733	0.05	62	R	1.05	0.72-1.53	0.82
Asian	653	0.14	49	F	1.13	0.92-1.40	0.24
Male	224	0.03	78	R	2.14	0.25-18.51	0.49
Female	59	0.82	0	F	1.54	0.77-3.08	0.22
HWE	283	0.97	0	F	3.29	2.45-4.41	0.000
Recurrence	323	0.12	54	F	1.09	0.82-1.45	0.54
TT+TC vs. CC							
Total	363	0.06	64	R	1.53	0.66-3.51	0.32
Asian	283	0.33	0	F	2.51	1.23-3.77	0.007
Male	224	0.14	55	F	2.46	1.21-4.99	0.01
Female	59	0.73	0	F	1.67	0.62-4.50	0.31
HWE	283	0.33	0	F	2.51	1.23-3.77	0.007
Recurrence	323	0.34	8	F	1.83	1.14-2.95	0.01
TT vs. TC+CC							
Total	580	0.07	62	R	0.83	0.49-1.40	0.48
Asian	500	0.08	67	R	0.95	0.55-1.67	0.87
Recurrence	170	0.97	0	F	0.69	0.43-1.10	0.12
TC vs. CC							
Total	317	0.74	0	F	2.53	1.43-4.46	0.001
Asian	251	0.49	0	F	2.46	1.38-4.40	0.002
Male	201	0.21	37	F	2.98	1.43-6.21	0.004
Female	50	0.83	0	F	1.52	0.53-4.34	0.44
HWE	251	0.49	0	F	2.46	1.38-4.40	0.002
Recurrence	282	0.53	0	F	2.66	1.51-4.67	0.000
TT vs. CC							
Total	124	0.15	53	F	0.88	0.50-1.56	0.66
Recurrence	84	0.46	0	F	1.06	0.58-1.93	0.86

OR, odds ratio; CI, confidence interval; R, random-effects model; F, fixed-effects model; HWE, Hardy-Weinberg equilibrium. Bold, statistically significant.

Results

Characteristics of eligible studies. The initial study search by electronic engines supplemented with a manual search yielded 54 potential articles, of which 49 were excluded after scanning the titles and abstracts. Of the remaining five, two were published by the same author group and had overlapping data, thus the article (19) with detailed information was included following consensus. Four case-control publications (18-21) concerning the relationship of urokinase gene 3'-UTR T/C polymorphism and urolithiasis susceptibility were identified (Fig. 1). In total, 1,195 subjects, including 462 healthy controls and 733 urolithiasis patients, were included in the meta-analysis. Of the four case-control studies, one publication (19) involved Caucasian populations, while the remaining three (18,20,21) pertained to Asian populations. One (19) of the four studies meeting the inclusion criteria focused on child patients. Control groups in two eligible studies (19,21) deviated from the HWE ($P < 0.05$). Additionally, three studies (18-20)

were related to urokinase gene 3'-UTR T/C polymorphism and urolithiasis recurrence. The detailed information of the studies included are shown in Tables I and II, respectively.

Main meta-analysis results. Table II and Fig. 2 show the results of the association between urokinase gene 3'-UTR T/C polymorphism and risk of urolithiasis in the total population. A significant association was noted in the codominant model (TC vs. CC: OR=2.53; 95% CI, 1.43-4.46; $P=0.001$). However, no significant associations between urokinase gene 3'-UTR T/C polymorphism and risk of urolithiasis were observed in the allele model (T vs. C: OR=1.05; 95% CI, 0.72-1.53; $P=0.82$), the dominant model (TT+TC vs. CC: OR=1.53; 95% CI, 0.66-3.51; $P=0.32$), the recessive model (TT vs. TC+CC: OR=0.83; 95% CI, 0.49-1.40; $P=0.48$) and the codominant model (TT vs. CC: OR=0.88; 95% CI, 0.50-1.56; $P=0.66$) (Table II and Fig. 2).

As for the subgroup meta-analysis, we stratified the analysis in terms of gender (male/female), ethnicity (Asian),

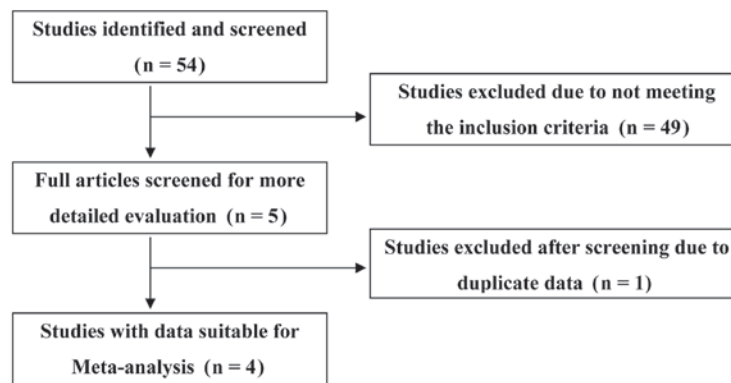


Figure 1. Flow chart of the systematic article search progress.

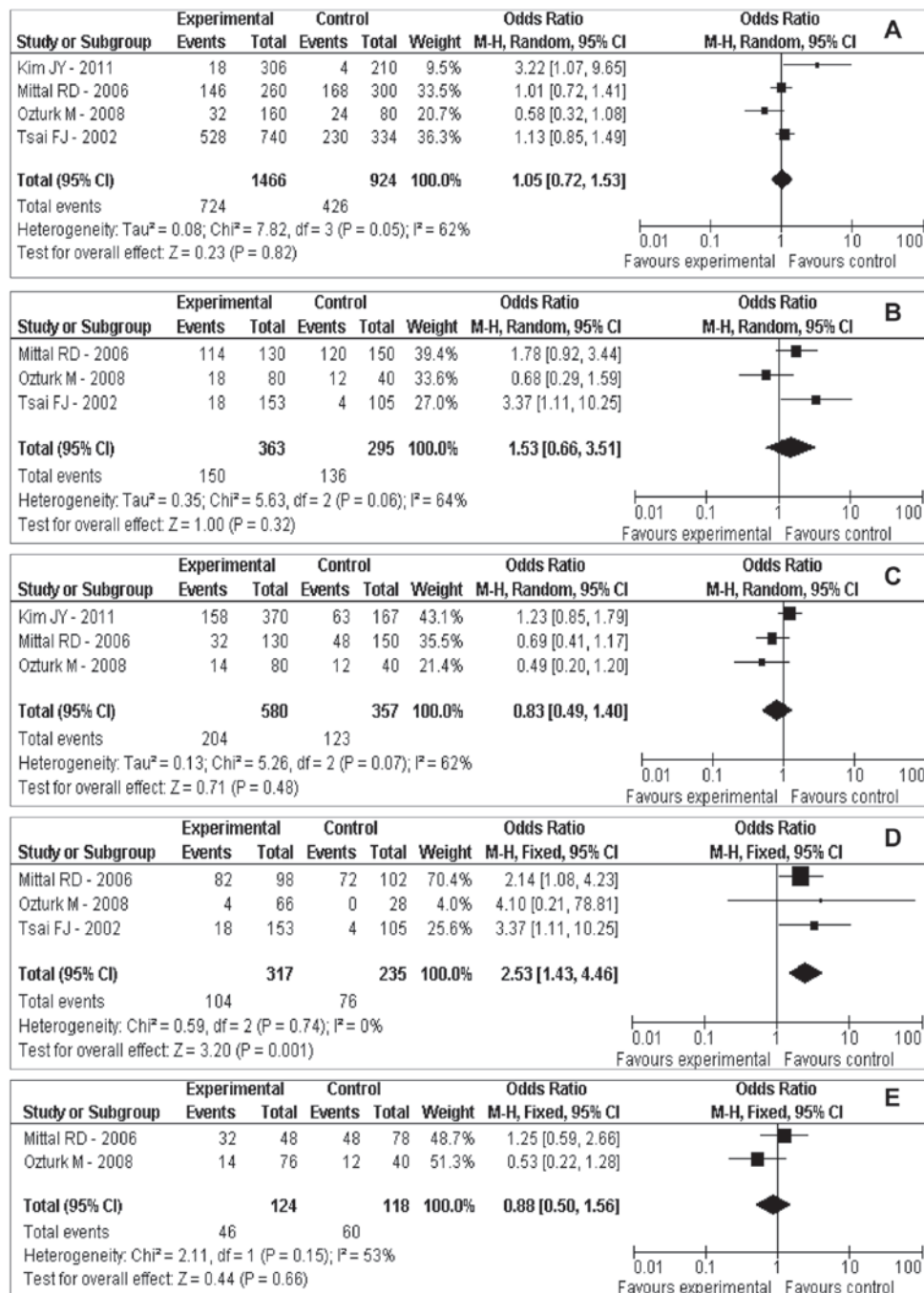


Figure 2. Meta-results for total population in (A) allele, (B) dominant, (C) recessive and (D) and (E) codominant models.

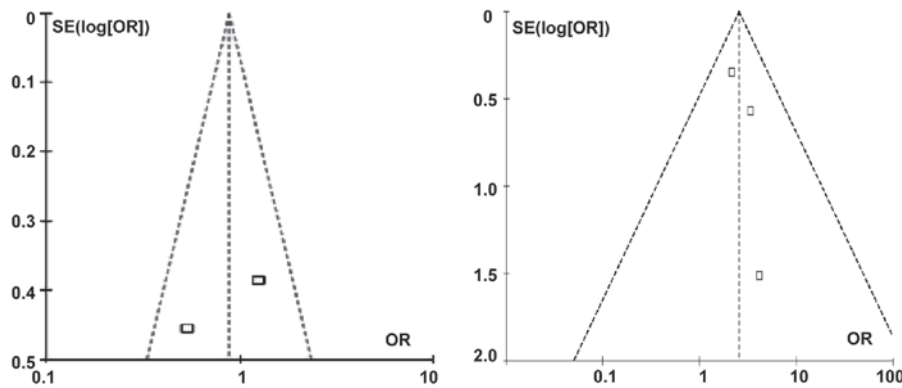


Figure 3. Inverted funnel plots for the total population in codominant models.

HWE and urolithiasis status (recurrence) (Table II). The association of urokinase gene 3'-UTR T/C polymorphism and urolithiasis risk was evident in some genetic models, such as: allele model (T vs. C: HWE OR=3.29; 95% CI, 2.45-4.41; $P=0.000$), the dominant model (TT+TC vs. CC: Asian OR=2.51; 95% CI, 1.23-3.77; $P=0.007$; male OR=2.46; 95% CI, 1.21-4.99; $P=0.01$; HWE OR=2.51; 95% CI, 1.23-3.77; $P=0.007$; recurrence OR=1.83; 95% CI, 1.14-2.95; $P=0.01$) and the codominant model (TC vs. CC: Asian OR=2.46; 95% CI, 1.38-4.40; $P=0.002$; male OR=2.98; 95% CI, 1.43-6.21; $P=0.004$; HWE OR=2.46; 95% CI, 1.38-4.40; $P=0.002$; recurrence OR=2.66; 95% CI, 1.51-4.67; $P=0.000$) (Table II).

The publication bias was assessed by the inverted funnel plot. Funnel plots of the codominant models (TC vs. CC and TT vs. CC) for the total population are shown in Fig. 3. No existing publication bias was evident and neither the Egger's nor Begg's tests identified publication bias. A significant difference was observed in the subgroup analysis of recurrence urolithiasis for the dominant model when the study by Mittal *et al* (20), with the largest number of subjects, was deleted.

Discussion

Urolithiasis is a global problem and affects almost all populations with a prevalence rate of 4-20% (23) and a recurrence rate of >40% in five years (2). Due to such distinguishing features, extensive efforts have mainly focused on three aspects: i) screening useful markers to prevent the occurrence of urolithiasis; ii) perfecting the means of clearing stones; and iii) finding effective methods, to avoid the recurrence of nephrolithiasis.

Stone formation in the kidney is a multifactor and complex process. According to the matrix theory, proteins, such as uromucoid, promote precipitation of calcium crystals to initiate the crystallization process. Therefore, factors that affect this procedure may be important in precluding stone development. Urokinase, originally isolated from human urine, is a plasminogen activator synthesized by the kidney, which can cleave plasminogen to plasmin and then stimulate fibrinolysis. Urokinase then becomes a natural inhibitor of abnormal initiation and growth of stone. The quantity and activity of urokinase were evidently downregulated in *in vivo* (8) and *in vitro* (24) studies. These findings suggest that a

higher urinary excretion of urokinase may play a protective role in preventing calcium urolithiasis formation.

Single nucleotide polymorphisms (SNP) have been identified as a powerful tool for predicting complex diseases, including cancers (10,11) and urolithiasis (18-21,25). Determination of SNPs in these genes is useful in the identification of available markers and in the clarification of the role of urokinase in the development of stones. Three polymorphic sites exist within the urokinase gene, of which 3'-UTR T/C polymorphism is the most widely studied (18-21). Several case-control studies (18-21) have investigated the possibility that urokinase gene 3'-UTR T/C polymorphism is associated with the formation of urolithiasis. However, the results of these studies were inconsistent. To the best of our knowledge, the present study is the first meta-analysis to assess the association of 3'-UTR T/C polymorphism and urolithiasis susceptibility by synthesizing individual studies.

In the final analysis, a significant association was noted in the 'T vs. C' allele model (HWE OR=3.29; 95% CI, 2.45-4.41; $P=0.000$). Significant results were also observed in the 'TC vs. CC' codominant model (total population OR=2.53; 95% CI, 1.43-4.46; $P=0.001$; Asian OR=2.46, 95% CI, 1.38-4.40; $P=0.002$; male OR=2.98; 95% CI, 1.43-6.21; $P=0.004$; HWE OR=2.46; 95% CI, 1.38-4.40; $P=0.002$; recurrence OR=2.66; 95% CI, 1.51-4.67; $P=0.000$). A significant association of urokinase gene 3'-UTR T/C polymorphism and urolithiasis risk was also evident in the 'TT+TC vs. CC' dominant model (Asian OR=2.51; 95% CI, 1.23-3.77; $P=0.007$; male OR=2.46; 95% CI, 1.21-4.99; $P=0.01$; HWE OR=2.51; 95% CI, 1.23-3.77; $P=0.007$; recurrence OR=1.83; 95% CI, 1.14-2.95; $P=0.01$). It is likely that the rare variant 'T' increases susceptibility to urolithiasis, particularly in Asian populations. A population with a 'T' allele has a 2.51-fold risk of stone susceptibility compared to population groups without 'T' allele. Males with 'T' allele have a 2.46-fold risk of stone susceptibility compared to those without 'T' allele, while for females with or without 'T' allele there is no difference with regard to the risk of urolithiasis. Nevertheless, in the present study, 'T' allele was closely associated with the recurrence of urinary stone.

The specific mechanism regarding whether T/C polymorphism localized in 3'-UTR affects susceptibility to urolithiasis remains to be determined. The 3'-UTR is a particular section of messenger RNA (mRNA) located between the stop codon

and the polyA tail. It is well documented that sequences in the 3'-UTR may effectively regulate gene expression. One feasible model is that 3'-UTR contains binding sites for regulatory proteins. Sequence alterations in the 3'-UTR may change the binding patterns of regulatory proteins and then the stability of mRNA. One example of this model involves the manner in which the translation of the transferring receptor gene is regulated by its 3'-UTR (26). Another feasible model involves miRNA (27,28) recognizing and combining the site located in the 3'-UTR of the gene due to the minor allele. In such a hypothesis, some miRNAs may bind to the sequence containing pathogenic 'T' allele, leading to transcript degradation or translation repression. However, how this polymorphism localizing in 3'-UTR affects the expression and function of urokinase remains to be elucidated.

In the present meta-analysis, we collected published case-control studies to obtain a more reliable association between urokinase gene 3'-UTR T/C polymorphism and urolithiasis. The urokinase gene 3'-UTR 'T' is a potential genetic marker for urolithiasis. Of note, however, is that the final conclusion was based on a moderate sample size. Thus, these results should be confirmed in further studies. Screening genetic markers with a significant association with the risk of urolithiasis presents an important challenge as genotyping of gene-gene and gene-environment factor interaction remains to be clarified.

In conclusion, urokinase gene 3'-UTR T/C polymorphism contributes to the susceptibility of nephrolithiasis, particularly in Asian populations. Additionally, the minor allele 'T' correlates with the recurrence of urolithiasis. Well-designed studies with a large sample size and different population characteristics should be performed to confirm our findings, thereby providing clinical and/or biological support for our results.

Acknowledgements

This study was partly supported by the grant Y2003C10 from the Science Foundation of Shandong (Hainan Liu). Dawei Li received a scholarship (no. 2011622132) from the China Scholarship Council.

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