Urol Res (2012) 40:455–460
DOI 10.1007/s00240-011-0449-8

ORIGINAL PAPER

Quantitative analysis of colonization with real-time PCR to identify the role of *Oxalobacter formigenes* in calcium oxalate urolithiasis

Ertan Batislam · Erdal Yilmaz · Ercan Yuvanc · Ozgul Kisa · Ucler Kisa

Received: 7 July 2011 / Accepted: 20 December 2011 / Published online: 4 January 2012

Abstract The objective of the study was to quantitatively measure the number of *Oxalobacter formigenes* (*O. formigenes*) colonizations in the gastrointestinal tract in calcium oxalate-forming patients with real-time polymerase chain reaction (PCR). Calcium oxalate-forming patients (*n*: 27) were included in the study. Serum calcium, sodium, potassium, urea and creatinine levels, as well as 24 h urine levels of calcium and oxalate were measured. The numbers of *O. formigenes* colonies in stool samples were detected by real-time PCR. One or two metabolic abnormalities were detected in 15 of 27 patients. The *O. formigenes* levels in patients with metabolic disturbance were significantly decreased when compared to the patients with no metabolic abnormalities (*p*: 0.038). The undetectable levels of *O. formigenes* were encountered in one of five patients with hypercalciuria, in three of four patients with hyperoxaluria and in four of six patients with both hypercalciuria and hyperoxaluria. In nine patients with a history of stone recurrence, *O. formigenes* colonization was significantly lower than the patients with the first stone attack (*p*: 0.001). *O. formigenes* formation ceased or significantly diminished in patients with calcium oxalate stones with a coexistence of both hyperoxaluria and hypercalciuria. The measurement of *O. formigenes* colonies by real-time PCR seemed to be an inconvenient and expensive method. For this reason, the real-time PCR measurements can be spared for the patients with stone recurrences and with metabolic abnormalities like hypercalciuria and hyperoxaluria. The exact measurement of *O. formigenes* may also help more accurate programming of *O. formigenes*-based treatments.

Keywords Calcium · Oxalate · Urolithiasis · Oxalobacter formigenes · Real-time PCR

E. Batislam · E. Yilmaz (✉) · E. Yuvanc
Faculty of Medicine, Department of Urology,
University of Kirikkale, Tip Fakultesi, Uroloji AD,
Saglik Sokak, 71100 Kirikkale, Turkey
e-mail: erdaly69@mynet.com

O. Kisa
Department of Medical Microbiology, Gulhane Military Medical Academy and School of Medicine, Ankara, Turkey

U. Kisa
Faculty of Medicine, Department of Biochemistry,
University of Kirikkale, Kirikkale, Turkey

Introduction

The majority of patients with urolithiasis had undergone wide operations and even had nefrectomies before the 1980s [1]. The morbidity decreased after the invention of extracorporeal disintegration techniques and fine developments of endoscopic surgery. The negative impact of these technological innovations has been the ignorance of the developments of medical therapies and preventive modalities. Surgery cures the stone; however, it does not eliminate the causes of the recurrences. Calcium oxalate stones recur 10% at 1 year, 35% at 5 years and 50% at 10 years, when left untreated after the primary treatment [1].

Numerous studies concluded that the modalities against the environmental and metabolic factors decreased the urinary stone recurrence rates [2, 3]. The cost of preventive measures has been far less than the hospital-based treatment cost of the stone. Selective medical therapies that targeted the formation of urolithiasis preclude invasive interventions in many individuals, and, particularly, diet alterations decrease recurrences. One of the most recent forms of diet modifications in the prevention of oxalate-containing stones has been probiotics [4]. *Oxalobacter*

formigenes (*O. formigenes)* has been the mainstay of the probiotic therapies in oxalate stone disease [5–8].

O. formigenes is a non-pathogen, obligate anaerobe bacteria excessively found in the colon [5, 7–9]. It utilizes oxalate as an energy source and decreases oxalate absorption in the colon. Thus, *O. formigenes* reduces the intestinal oxalate absorption, and by this, its urinary excretion [6]. It is suggested that the lack of *O. formigenes* or its diminished existence in the colon leads to calcium oxalate stones [10, 11]. The colonization of *O. formigenes* in the intestines has been proved by polymerase chain reaction (PCR) in the current literature [12]; however, the correct amount of these colonizations could not be retracted. The real-time PCR enables us to measure the colonization quantitatively [13, 14].

The purpose of our study was to detect the numerical levels of *O. formigenes* in the colon of calcium oxalate stone patients and to evaluate the relation with urinary parameters and, eventually, to discuss the possible benefits of detecting the numerical levels of *O. formigenes* in diet programming with a particular view on probiotics.

Materials and method

Patient population

A total of 27 patients with calcium oxalate stone disease who had any kind of treatment resulting in stone passage were included in the study population. The exclusion criteria were active urinary infection, previous intestinal surgery and intestinal disease. None of the patients had received antibiotics or probiotic preparations in the 6 months before enrollment. Patients voluntarily accepted the study with consent. Local ethics committee approval had been obtained before the commence of the study.

Biochemical analysis

All patients had urine analysis and bacteriological culture, plus serum calcium, phosphate, sodium, potassium, chloride, magnesium, blood urea nitrogen (BUN) and creatinine measurements taken. Urine volume, sodium, potassium, phosphate, chloride, calcium, oxalate, citrate, urea and creatinine were measured in 24 h urine. A stool sample was also obtained from all patients. Refrigerated 24-hour urine samples during collection without preservative.

Serum and urine biochemical parameters (calcium, phosphate, sodium, potassium, chloride, magnesium, BUN and creatinine were measured by using an auto analyzer (Hitachi/Roche P800). Urinary citrate and oxalate levels were measured by spectrophotometer with the enzymatic method by using commercially diagnostic kits (Roche Diagnostics and Sigma Diagnostics, respectively).

Isolation *O. formigenes* genomic DNA from human fecal samples

Approximately 200 mg of fecal samples were added rapidly to Universal Transfer Medium (UTM 330CL Diagnostic Hybrid Inc. Athens, OH, USA) containing three 3-mm glass beads and stored at 20°C until used. The DNA extraction from the fecal samples was then performed by the ZR Fecal DNA Kit™ (Zymo Research, USA). The DNA was isolated and purified using fast-spin column technology.

Bacterial strain and the isolation of genomic DNA

The DNA of the *O. formigenes* strain (ATCC 35274) was obtained from PhD Juquan Jiang (Sciences-Urology Surgery, WFU School of Medicine) and was used as the standard throughout this study (1 ng DNA = 4.82×10^5cells). A standard curve for quantitative analysis by the SYBR Green I method was performed with tenfold diluted solutions of DNA from the strain O × B (range of 10^1–10^6). The real-time PCR was found that lower limit of detection was 5×10^3 cells/g stool.

Real-time PCR

The real-time PCR technique was performed as previously described, using 45 cycles [12]. A real-time PCR assay using LightCycler System 1.5 (Roche Diagnostics) and the *LightCycler*® FastStart DNA Master SYBR Green I was developed to detect *O. formigenes* oxalyl-CoA decarboxylase gene. The primers were made by TIB MOLBIOL GmbH Eresburgstraße 22–23 D-12103 (Berlin, Germany). The sequence of forward primer is CGACGACAATG TAGAGTTGACTG (melting temperature 55.8°C) and the sequence of reverse primer is ATCGACGATTTCACGT TCACT (melting temperature 55.5°C) (Gen-Bank Accession No. M77128). The PCR mixture in a final volume of 25 µl contained 0.4 pmol/µl of each primer, 3 mmol/L $MgCl_2$, 1× FastStart DNA Master SYBR Green I (Roche), and 2 µl of extracted DNA template. Reactions were started by initial menstruation at 95°C for 10 min, followed by 45 cycles at 95°C for 10 s, 55°C for 10 s, and 72°C for 20 s. The double-stranded PCR product was measured once every cycle immediately after the 72°C incubation (extension step) by detection of fluorescence associated with the binding of SYBR Green I to the amplification product. Melting curve analysis was performed immediately after the amplification protocol (measuring the fluorescence of SYBR Green I) under the following conditions: 0 s at 95°C (the hold time on reaching temperature), 30 s at 65°C, and 0 s at 95°C. Temperature change rates were 0.1°C per s. Fluorescence data were converted into melting peaks using

the LightCycler software, version 3.5 (Roche Diagnostic). The peak melting temperature obtained represented the specific amplified product. The test results with the melting points around 86.5–90°C were accepted as positive. Each assay was performed using positive and negative controls (DNA from *O. formigenes* and distilled water, respectively) and sterile procedures following contamination-free guidelines to prevent false-positive results.

Statistical analysis

Statistical comparison was made between the patients with and without metabolic abnormalities using Mann–Whitney *U* test. The *O. formigenes* levels of those with and without hyperoxaluria, those with and without hypercalciuria, and those who are with and without both hyperoxaluria and hypercalciuria were compared statistically within the group using the Mann–Whitney *U* test. Again, the statistical comparison of the *O. formigenes* levels of those with and without recurrent stone disease within the group were made using the Mann–Whitney *U* test.

Results

The 27 patients with calcium oxalate stones were predominantly male (70%, $n = 19$). The stone location was in the kidney in 3 patients, in the ureter in 21, and both kidney and ureter in 3. Nine patients had recurrent disease. The ages of patients ranged between 18 and 61 years, with a mean age of 38.12 ± 19.18 years.

Among the 27 calcium oxalate stone formers 5, 4 and 6 of patients were pure hypercalciuria, pure hyperoxaluria and both hypercalciuria and hyperoxaluria, respectively. A metabolic reason could not be detected in 12 patients.

The median 24-hour urinary calcium and oxalate excretion in 15 patients with metabolic disturbance were 379.4 mg/24 h (range 93.8–579.7) and 49.1 mg/24 h (range 14.6–86.4), respectively. Other 12 patients, mean 24-hour urinary calcium and oxalate excretion were 124.8 mg/24 h (range 48.2–218.4) and 20.3 mg/24 h (range 2.8–29.4), respectively.

The average *O. formigenes* levels in 15 patients with metabolic disturbance identified was $3.72 \pm 1.46 \times 10^4$ (range less than 5×10^3cells–1.72×10^5). Other 12 patients had median *O. formigenes* levels of $2.65 \pm 0.89 \times 10^5$ (range less than 5×10^3cells–1.17×10^6). *O. formigenes* levels, urinary calcium and urinary oxalate levels in patients with and without metabolic disturbances presented in Tables 1 and 2, respectively.

The *O. formigenes* levels in patients with metabolic disturbance was significantly decreased when compared to the patients with no metabolic disturbance (p: 0.038). The *O. formigenes* levels measurement was significantly lower in hyperoxaluria patients than in those with no metabolic disturbance (p: 0.033). The *O. formigenes* levels in hypercalcuria patients was significantly decreased when compared to the patients with no metabolic disturbance (p: 0.044). The patients with both hypercalcuria and

Table 1 The *O. formigenes, urinary calcium and urinary oxalate* levels in patients with metabolic disturbance

No	*O. formigenes* cells/g stool	Urinary calcium (mg/24 hours)	Urinary oxalate (mg/24 hours)
1[a]	1.3×10^5	507.0	86.4
2[b, d]	Less than 5×10^3	93.8	77.8
3[c]	Less than 5×10^3	421.1	19.9
4[a, d]	Less than 5×10^3	392,3	76.2
5[c, d]	3.5×10^4	493.7	17.3
6[c]	5.95×10^4	469.8	34.6
7[c, d]	2.6×10^4	376.9	14.6
8[a, d]	Less than 5×10^3	579.7	62.5
9[b, d]	1.72×10^5	134.5	46.3
10[b]	Less than 5×10^3	166.5	49.7
11[a]	9.58×10^4	475.7	50.9
12[a, d]	Less than 5×10^3	521.8	42.2
13[b]	Less than 5×10^3	263.6	59.4
14[c, d]	3.85×10^4	425.8	14.6
15[a, d]	Less than 5×10^3	368.2	84.1

[a] Both hyperoxaluria and hypercalciuria

[b] Pure hyperoxaluria

[c] Pure hypercalciuria

[d] Recurrent stone patient

Table 2 The *O. formigenes, urinary calcium and urinary oxalate* levels in patients with no metabolic disturbance

No	*O. formigenes* cells/g stool[a]	Urinary calcium (mg/24 hours)	Urinary oxalate (mg/24 hours)
1	3×10^4	48.2	2.8
2	3.43×10^5	71.3	18.1
3	8.4×10^4	43.4	9.7
4	Less than 5×10^3	96.6	17.1
5	3.78×10^5	207.2	23.8
6	2.46×10^5	56.3	28.6
7	2.23×10^5	98.2	21.4
8	4.95×10^5	212.5	21.6
9	3.85×10^4	218.4	23.1
10	Less than 5×10^3	213.8	26.6
11	1.17×10^6	76.9	29.4
12	2.02×10^5	155.9	21.3

[a] Larger than limit of detection of 5×10^3cells/g stool

hyperoxaluria also showed low levels of *O. formigenes* colonization (p: 0.021).

In 8 of the 15 patients with metabolic disturbance, the colonization of *O. formigenes* was undetectable. In three of the four patients with pure hyperoxaluria, the levels of *O. formigenes* was not detectable, whereas, the colonization was detected very low in the other patient. While the levels of *O. formigenes* was undetectable in one patient, it was very low in the other four patients with hypercalcuria only. In the six patients with both hyperoxaluria and hypercalcuria, two patient showed decreased *O. formigenes* colonization, whereas, the levels of *O. formigenes* undetectable was seen in four patients.

The *O. formigenes* levels in patients with recurrent stone disease were statistically significantly lower, when compared to that in those who have suffered their first kidney stone attack (p: 0.001). The levels of *O. formigenes* was undetectable in five of these patients, while this was at a quite low level in the other four patients. A metabolic anomaly was present in all of the recurrent stone patients. Three of them had hyperoxaluria, one had hypercalciuria, and five had both hyperoxaluria and hypercalciuria. In two of the three patients with recurrent stone patients and pure hyperoxaluria and in three of the five patients with recurrent stone patients and both hyperoxaluria and hypercalcuria, the levels of *O. formigenes* was not detectable.

Discussion

Hyperoxaluria is an important risk factor in calcium oxalate urolithiasis [6, 15]. The secretory and absorbative pathways of oxalic acid were processed in the proximal and distal segmental parts of the colon [6]. Kleinschmidt et al. were the first to describe the role for *O. formigenes* in these colonic segments in oxalic acid metabolism [16]. *O. formigenes* decreases the oxalate absorption. Various studies implied that *O. formigenes* colonization was decreased in urolithiasis, while some studies established the relation between hyperoxaluria and diminished *O. formigenes* colonization [5, 7, 17–20]. Eventually the *O. formigenes* colonization ratio was 75% in adults [5, 12, 21].

In patients with a history of urolithiasis, *O. formigenes* colonization rates have been 38 and 13%, with two to five and more than five episodes of urolithiasis, respectively [22]. While Kwak et al. found that 45.6% of calcium oxalate patients had *O. formigenes* colonization, Mittal et al. detected the colonization in 33% of the patients with urolithiasis and 65% of the control groups [8, 20].

The reason for the absence of *O. formigenes* colonization in 30–40% of healthy individuals and 60–70% of urolithiasis patients has not been clearly known. However, the antibiotic usage is thought to be a factor influencing the colonization [23, 24]. The *O. formigenes* colonization disappearance causing oxalate excretion in women can be attributed to the excessive usage of antibiotics due to recurrent urinary tract infections. The *O. formigenes* in fecal samples totally ceased after tetracycline application in rats [8].

Almost all of the studies dealing with *O. formigenes* in urolithiasis have used the conventional PCR technique to detect the bacteria in the gastrointestinal system [11, 20, 22, 25], with the exception of a study in which a real-time PCR technique was used to count the number of *O. formigenes* in a healthy study group [12].

In this study, for the first time, a real-time PCR technique was used to identify the exact numbers of colonization in urolithiasis patients. Our study concluded that the number of *O. formigenes* levels have been significantly lower in patients with metabolic disturbances than in patients without metabolic abnormalities.

The other aim of this study was to investigate whether the levels of *O. formigenes* was undetectable in gastrointestinal tract of the patients with hyperoxaluria. The undetectable levels of *O. formigenes* has been found in three of four patients with pure hyperoxaluria and one of the patients with pure hypercalcuria. The same levels of *O. formigenes* was found in four of six patients with both hypercalcuria and hyperoxaluria. Therefore, supporting the value of bacterial counting by real-time PCR, the colonization has not ceased in every patient, and decreased colonization could be a lithogenic factor.

One of the notable findings of our study is the considerably low *O. formigenes* levels in recurrent stone patients. This suggests that one of the most important target groups of *O. formigenes*-based treatments are recurrent stone patients.

The measurement of *O. formigenes* levels with real-time PCR may provide more reliable data in detecting the role for the effect of dietary oxalate on hyperoxaluria. Moreover, dietary supplements can be rationally modified for each patient with these numeric *O. formigenes* values. Recently, probiotic dietary regimens have been recommended to the urolithiasis patients. One of the microorganisms used for the probiotic purpose has been *O. formigenes*. Oral *O. formigenes* vaccines or *O. formigenes* lysate and oxalyl-CoA decarboxilase-containing capsules have been recommended as alternative treatments [21].

In the rats with hyperoxaluria and decreased *O. formigenes* colonization, urinary oxalate could be decreased by *O. formigenes*-derived oxalate degrading enzymes. These rats did not produce excess urinary crystal excretion and calcium oxalate crystallization in nephrons again after the enzyme treatment. The rats did not exhibit any toxicity or antibody production. The rats fed with 1.5% oxalate-containing diet received 2 days of 2×10^9 *O. formigenes*, and oxalate excretion decreased 85%. The same results were

reached by 1% oxalate-containing diet and 10^8 daily doses of *O. formigenes* for 2 weeks [18].

There are a few human studies of this kind. In one study by Duncan et al. [5] a 500 mg *O. formigenes* vaccine HC1 application resulted in a decrease in urinary oxalate excretion. In another study, by Hoppe et al. urinary oxalate decreased 22–48% and 39–92% with CFU frozen cell *O. formigenes* jelly and CFU enteric coated capsules, respectively [17]. A daily dietary dose of 8×10^{11} lactic acid bacteria for 4 weeks also caused a decrease in urinary oxalate excretion [26]. Urinary biomaterial-coated oxalate degrading bacteria has been promising future alternatives [27]. Hoppe et al. have reported that oral *O. formigenes* used in two patients with infantile oxalosis reduced plasma oxalate levels and using it until transplantation would be useful [28]. In another study of Hoppe et al. the researchers compared the use of oral *O. formigenes* (Oxabact) with placebo in patients with primary hyperoxaluria and found that it is not different from placebo in reducing urinary oxalate [29].

The dietary alternatives and *O. formigenes*-based treatments in calcium oxalate stones can particularly be reserved for the patients with hyperoxaluria, both hypercalciuria and hyperoxaluria and recurrent disease, since all calcium oxalate urolithiasis do not show a decrease in *O. formigenes* levels.

Conclusion

O. formigenes colonization either disappeared or greatly diminished particularly in situations with hyperoxaluria and in situations where hyperoxaluria and hypercalciuria coexist as well as, in patients with recurrent calcium oxalate stones. We believe that dietary probiotic arrangements can be made more relevantly through precise measurements with the real-time PCR technique in this group of patients.

References

1. Menon M, Parulkar BG, Drach GW (1988) Urinary lithiasis: etiology, diagnosis and medical management. In: Walsh PC, Retik AB, Vaughan ED Jr, Wein AJ (eds) Campbell's Urology, vol III, 7th edn. W. B. Saunders Co., Philadelphia, pp 2659–2752
2. Goldfarb DS (2009) Prospects for dietary therapy of recurrent nephrolithiasis. Adv Chronic Kidney Dis 16:21–29
3. Borghi L, Meschi T, Maggiore U, Prati B (2006) Dietary therapy in idiopathic nephrolithiasis. Nutr Rev 64:301–312
4. Bruce AW, Reid G (2003) Probiotics and the urologist. Can J Urol 10:1785–1789
5. Duncan SH, Richardson AJ, Kaul P et al (2002) Oxalobacter formigenes and its potential role in human health. Appl Environ Microbiol 68:3841–3847
6. Hatch M, Freel RW (2008) The roles and mechanisms of intestinal oxalate transport in oxalate homeostasis. Semin Nephrol 28:143–151
7. Mittal RD, Kumar R (2004) Gut-inhabiting bacterium *Oxalobacter formigenes*: role in calcium oxalate urolithiasis. J Endourol 18:418–424
8. Mittal RD, Kumar R, Bid HK et al (2005) Effect of antibiotics on *Oxalobacter formigenes* colonization of human gastrointestinal tract. J Endourol 19:102–106
9. Sidhu H, Allison M, Peck AB (1997) Identification and classification of *Oxalobacter formigenes* strains by using oligonucleotide probes and primers. J Clin Microbiol 35:350–353
10. Troxel SA, Sidhu H, Kaul P et al (2003) Intestinal *Oxalobacter formigenes* colonization in calcium oxalate stone formers and its relation to urinary oxalate. J Endourol 17:173–176
11. Mittal RD, Kumar R, Mittal B et al (2003) Stone composition, metabolic profile and the presence of the gut-inhabiting bacterium *Oxalobacter formigenes* as risk factors for renal stone formation. Med Princ Pract 12:208–213
12. Prokopovich S, Knight J, Assimos DG et al (2007) Variability of *Oxalobacter formigenes* and oxalate in stool samples. J Urol 178:2186–2190
13. Larionov A, Krause A, Miller W (2005) A standard curve-based method for relative real-time PCR data processing. BMC Bioinformatics 21(62):1–16
14. Sails AD (2004) Applications of real-time PCR in clinical microbiology. In: Edwards K, Logan J, Saunders N (eds) Real-time PCR: an essential guide. Horizon Biosci, Norfolk, pp 247–326
15. Neuhaus TJ, Belzer T, Blau N et al (2000) Urinary oxalate excretion in urolithiasis and nephrocalcinosis. Arch Dis Child 82:322–326
16. Kleinschmidt K, Mahlmann A, Hautmann R (1994) Oxalate degrading bacteria in the gut: do they influence calcium oxalate stone formation? In: Ryall R, Bais R, Marshall VR (eds) Urolithiasis 2. Plenum Press, New York, pp 439–441
17. Hoppe B, Beck B, Gatter N et al (2006) *Oxalobacter formigenes*: a potential tool for the treatment of primary hyperoxaluria type 1. Kidney Int 70:1305–1311
18. Sidhu H, Schmidt ME, Cornelius JG et al (1999) Direct correlation between hyperoxaluria/oxalate stone disease and the absence of the gastrointestinal tract-dwelling bacterium *Oxalobacter formigenes*: possible prevention by gut recolonization or enzyme replacement therapy. J Am Soc Nephrol 10(Suppl 14):S334–S340
19. Kaufman DW, Kelly JP, Curhan GC et al (2008) *Oxalobacter formigenes* may reduce the risk of calcium oxalate kidney stones. J Am Soc Nephrol 19:1197–1203
20. Kwak C, Kim HK, Kim EC et al (2003) Urinary oxalate levels and the enteric bacterium *Oxalobacter formigenes* in patients with calcium oxalate urolithiasis. Eur Urol 44:475–481
21. Siva S, Barrack ER, Reddy GP et al (2009) A critical analysis of the role of gut *Oxalobacter formigenes* in oxalate stone disease. BJU Int 103:18–21
22. Sidhu H, Holmes RP, Allison MJ et al (1999) Direct quantification of the enteric bacterium *Oxalobacter formigenes* in human fecal samples by quantitative competitive-template PCR. J Clin Microbiol 37:1503–1509
23. Kharlamb V, Schelker J, Francois F et al (2011) Oral antibiotic treatment of helicobacter pylori leads to persistently reduced intestinal colonization rates with *Oxalobacter formigenes*. J Endourol 25:1781–1785
24. Kelly JP, Curhan GC, Cave DR et al (2011) Factors related to colonization with *Oxalobacter formigenes* in US adults. J Endourol 25:673–679
25. Hoppe B, von Unruh G, Laube N et al (2005) Oxalate degrading bacteria: new treatment option for patients with primary and secondary hyperoxaluria? Urol Res 33:372–375

26. Campieri C, Campieri M, Bertuzzi V et al (2001) Reduction of oxaluria after an oral course of lactic acid bacteria at high concentration. Kidney Int 60:1097–1105
27. Watterson JD, Cadieux PA, Beiko DT et al (2003) Oxalate-degrading enzymes from *Oxalobacter formigenes*: a novel device coating to reduce urinary tract biomaterial-related encrustation. J Endourol 17:269–274
28. Hoppe B, Dittlich K, Fehrenbach H et al (2011) Reduction of plasma oxalate levels by oral application of *Oxalobacter formigenes* in 2 patients with infantile oxalosis. Am J Kidney Dis 58:453–455
29. Hoppe B, Groothoff JW, Hulton SA et al (2011) Efficacy and safety of *Oxalobacter formigenes* to reduce urinary oxalate in primary hyperoxaluria. Nephrol Dial Transpl 26:3609–3615