OXFORD UNIVERSITY PRESS | Nephrology Dialysis Transplantation

This is the overview page

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Journal:	*Nephrology Dialysis Transplantation*
Manuscript ID	NDT-01851-2017.R1
Manuscript Type:	Original Article - Clinical Research
Date Submitted by the Author:	n/a
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Keyword list:	bone mineral density, FGF-23, nephrolithiasis, phosphatemia, vitamin D

Dinstinct phenotype of kidney stone formers with renal phosphate leak

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Running head: Phosphate leak and kidney stones

Key words: Kidney stone, phosphate, brushite, vitamin D, PTH

25 Pages, 4 Tables, 3 Figures, 242 abstract words, 3116 manuscript words

ABSTRACT

Background: Hypercalciuria is the most frequent metabolic disorder encountered in kidney stone formers. Reduced renal phosphate reabsorption (i.e. renal phosphate leak) was proposed to be a driver of hypercalciuria in calcium stone formers. However, the phenotype of stone formers with renal phosphate leak remains poorly defined, and the association of renal phosphate leak with stone history, stone composition and bone mineral density has not been studied.

Methods: To fill these knowledge gaps, we conducted a cross-sectional analysis in a cohort of 555 idiopathic calcareous stone formers. The ratio of tubular maximum reabsorption of phosphate to glomerular filtration rate (TmP/GFR) was used to evaluate renal phosphate transport.

Results: Multivariable regression analyses revealed a negative association of PTH, a positive association of 25(OH) and $1,25(OH)_2$ vitamin D but no association of FGF23 with TmP/GFR. Stone formers with low TmP/GFR had their first stone event at a younger age and were more likely to have a positive family history of kidney stones. In addition, urinary calcium excretion and prevalence of brushite stones were significantly higher in stone formers with low TmP/GFR. However, bone mineral density, measured by dual-energy x-ray absorptiometry, was not associated with TmP/GFR in stone formers.

Conclusions: Renal phosphate handling has a strong heritable component in stone formers and correlates with PTH, 25-VitD and 1,25-VitD, but not with FGF23 levels. Furthermore, a low TmP/GFR (i.e. a renal phosphate leak) is associated with higher urinary calcium excretion and an increased prevalence of brushite stones.

INTRODUCTION

Nephrolithiasis is a worldwide healthcare problem with a current lifetime risk of ~18.8 % in men and ~9.4 % in women in Western civilizations [1]. 80-90 % of stones are composed of calcium oxalate, calcium phosphate or a mixture of both. Hypercalciuria is the most frequent metabolic abnormality encountered in idiopathic calcium stone formers (SF) [2]. Supersaturation of urinary calcium oxalate and calcium phosphate are two of the major driving forces for calcium stone formation. Although many inherited and acquired systemic diseases underlie calcium stone formation, most stones are designated broadly as idiopathic by exclusion [3]. The prevalence of a renal phosphate leak, i.e. hypophosphatemia in conjunction with inappropriately low renal tubular phosphate reabsorption, has been reported to be as high as 53 % in recurrent SF but also present but much less prevalent in healthy individuals [4-8]. Hypophosphatemia-stimulated $1,25(OH)_2$ with secondary augmentation of intestinal calcium absorption has been postulated to cause hypercalciuria (absorptive hypercalciuria type III) in a subgroup of calcium SF [7, 9-11]. However, the evidence supporting such a mechanism has been inconclusive. With the exception of one study [4], no differences in urinary calcium excretion or $1,25(OH)_2$ vitamin D levels were detected between SF with and without renal phosphate leak [7, 8, 12]. Compared to healthy non-stone forming controls, $1,25(OH)_2$ vitamin D levels have been reported as increased [4] or unchanged [12, 13] in SF with renal phosphate leak.

The mechanisms responsible for renal phosphate leak in idiopathic SF remain unclear. A previous study suggested that the renal phosphate leak was closely linked to the renal stone burden at the time of metabolic work up [6]. In contrast, more recent data suggest that the primary abnormality encountered in SF with renal phosphate leak is either a frankly elevated or inappropriately normal level of fibroblast growth factor 23 (FGF23) [12]. The latter was proposed to be partially caused by an allelic variant of the *FGF23* gene (c.C716T; p.T239M)

that results in higher FGF23 secretion and increased activation of the FGF receptor/ERK signaling pathway [13].

Thus, the phenotype of SF with renal phosphate leak remains poorly defined at the moment and the role of potential confounders (age, sex, BMI, renal function, comorbidities) largely unexplored. Furthermore, the association of renal phosphate leak with stone composition, stone frequency and bone mineral density (BMD) has not been studied rendering significant impediment for clinicians to recognize and understand this disorder. To address these issues, we conducted a cross-sectional analysis in our cohort of idiopathic calcium SF.

MATERIALS AND METHODS

Study population and protocol

The study was conducted with patients recruited at the Division of Nephrology and Hypertension at the Bern University Hospital, Bern, Switzerland with approval of the Ethical Committee of the Kanton Bern. All participants provided written informed consent and the study was conducted in accordance with the Declaration of Helsinki. Patients were seen at the Division between March 2004 and June 2016. Inclusion criteria for this study were: informed consent, age ≥ 18 years and at least one calcium stone episode. Calcium stone was defined as i) at least one stone analysis with a calcium containing stone or ii) radiopaque stone by plain abdominal X-ray or iii) > 500 Hounsfield unit stone on computed tomography. Patients were excluded from the study analysis based on the following criteria: primary hyperparathyroidism or history of parathyroidectomy; sarcoidosis; primary or enteric hyperoxaluria; chronic pancreatitis; chronic diarrhea; complete distal renal tubular acidosis (dRTA); known cystinuria or elevated 24 h cystine excretion; uric acid nephrolithiasis; chronic liver disease or more than threefold elevated liver enzymes; BMI < 16 or > 40 kg/m^2; history of anorexia nervosa or bulimia, active malignant diseases; pregnancy during the study visit; history of solid organ transplantation; hypocortisolism; hyperthyroidism with a TSH < 0.1 mU/l; total plasma calcium >2.5 mmol/l with a PTH >65 pg/ml; administration of the following drugs: over-the-counter or prescribed calcium and/or vitamin D supplements, phosphate, bisphosphonates, cinacalcet, denusomab, teriparatide, systemic glucocorticoids or mineralocorticoids, antiepileptics, carboanhydrase inhibitors, diuretics. All variables employed for study analyses were verified by manual review of the original patient charts by NAD, DL, LS, CM and DGF. In the final analysis, 555 SF were included.

Data collection and measurements

Patients collected two 24 h urines on a random outpatient diet and a spot urine and a fasting blood draw were performed in the morning after the second 24 h urine collection. All blood parameters were determined from the same blood draw. Plasma FGF23 was measured at the laboratory of TECOmedical AG (Sissach, Switzerland) by the second generation C-terminal assay (Immutopics, San Clemente, CA) with plasma samples frozen at the time of sampling and stored at – 80° C. All other urine and blood analyses were performed at the Central Laboratory of the Bern University Hospital as single measurements immediately after sampling. Assay characteristics for the measurements of FGF23, PTH, 25-OH Vitamin D, and $1,25(OH)_2$ vitamin D, were recently described [14]. The glomerular filtration rate GFR was estimated by the creatinine-based CKD-EPI 2009 equation [15]. Urinary creatinine excretion was used as the criterion for completeness of 24 h urine collections in individuals with normal renal function [16, 17]. Percentiles 2.5 and 97.5 of the 24 h creatinine excretion were calculated for each 24-hours urine collection using a linear regression model recently published [18]. Completeness of 24 h urine collections was assumed for each subject if the total 24 h creatinine excretion was within percentiles 2.5 and 97.5. The mean value of both 24 h urine collections was used for the calculation of 24 h urinary excretions.

Corrected plasma calcium was calculated by the formula: Ca_{corr} (mmol/L) = plasma calcium measured (mmol/L) + 0.025 × (40 – plasma albumin (g/L)) in case of a plasma albumin ≤40 g/L. Tubular fractional reabsorption of phosphorus (TRP) was calculated by the formula: TRP (%) = (100 – $FePO_4$ (%)). Tubular maximum reabsorption of phosphorus per glomerular filtration rate (TmP/GFR in mmol/l) was calculated with the algorithm derived by Kenny and Glen [19, 20]. If TRP was ≤ 86%, then the formula: TmP/GFR (mmol/l) = TRP × plasma phosphate (mmol/l) was used. If TRP was > 86%, then the formula: TmP/GFR (mmol/l) = (0.3 × TRP)/(1 – 0.8 × TRP) × (plasma phosphate (mmol/l)) was used. Both TRP and TmP/GFR were calculated using 24 h urine samples. Diabetes was defined as reported,

treated, fasting glycemia ≥7 mmol/L (≥126.13 mg/dl), or random glycemia ≥11.1 mmol/L (≥200 mg/dl). Hypertension was defined as reported, treated, a mean systolic BP ≥140 mmHg, or a mean diastolic BP ≥90 mmHg. Osteodensitometry was performed at the Department of Osteoporosis at the University Hospital of Bern, Switzerland at the time of biochemical work-up by dual-energy X-ray absorptiometry (DEXA; Hologic QDR 4500A, Hologic, Bedford, MA, USA) at the lumbar spine, the non-dominant femoral neck, the proximal femur and the distal tibia diaphysis and epiphysis as described [21, 22].

Statistical analysis

All statistical analyses were conducted using the R software, version 3.2.2 [23]. The shape of the distribution of each continuous variable was visually inspected and transformations were applied where appropriate to ensure normality for statistical analyses. All statistical tests were two-sided and a p value <0.05 was considered statistically significant. To estimate associations of renal phosphate handling as a continuous phenotype, i.e. TmP/GFR, with baseline characteristics, laboratory parameters, kidney stone and bone turnover parameters, we applied univariable mixed effects linear and logistic regression analyses. Due to the physiological seasonal variation of vitamin D levels, calendar day of blood sampling was taken as random effect into account in all mixed effects regression models. Independent associations of urinary phosphate leak with variables of interest were analyzed by multivariable mixed effects linear and logistic regression containing sex, age, BMI, GFR (CKD-EPI), diabetes mellitus (yes or no) and hypertension (yes or no). Multivariable regression models were visualized using the R package visreg, which allows to show the relationship between TmP/GFR and characteristics of interest while holding the effect of all other co-variables in the model constant [24]. For visualization, regression models were re-created without including a random effect.

RESULTS

Characteristics of study population

We included adult SF with idiopathic calcium stones in the study. A total of 555 SF met the inclusion criteria (age ≥ 18 years, written informed consent, at least one episode with a calcium containing kidney stone) and had no exclusion criterion (for details see Materials and Methods).

Demographic and anthropometric characteristics, stone analyses and bone density measurements of study participants are shown in Table 1. The majority of patients were male (77 %), median age was 43 years and most patients had recurrent stone disease (82 %). Dual-energy X-ray absorptiometry (DEXA)-based bone density measurements were available in 423 of 555 patients (76.2 %). Blood and urinary parameters of participants are depicted in Table 2. The distribution of TmP/GFR in our cohort of calcareous SF is shown in Fig. 1.

Association analyses

Renal phosphate handling in SF was assessed by the ratio of tubular maximum reabsorption of phosphate to glomerular filtration rate (TmP/GFR). We first performed association analyses by mixed effects linear regression with TmP/GFR as outcome variable and baseline characteristics, blood and urine parameters as predictor variables (Table 3). In both univariable and multivariable analyses, calendar day of blood sampling was taken as random effect into account. Multivariable analyses were adjusted for the covariables age, sex, BMI, GFR, diabetes and hypertension. Male sex (β: -0.06, $p<0.001$) and arterial hypertension (β: -0.05, $p<0.01$) were found to be inversely associated with TmP/GFR in multivariable analyses. Consistent with previous reports, we found a positive association of TmP/GFR with GFR (β: 0.0013, $p=0.021$). There was a strong negative association of PTH (β: -0.002, $p<0.001$) and a strong positive association of 25(OH) vitamin D (β: 0.001, $p<0.01$) with TmP/GFR. Given the

fact that vitamin D status strongly influences PTH secretion, we performed a second multivariable analysis where the association of PTH with TmP/GFR was additionally adjusted for 25(OH) vitamin D levels. As shown in Table 4, adjusting for 25(OH) vitamin D greatly weakened the association of PTH with TMP/GFR to borderline significance (β: -0.001, p=0.037).

In multivariable analyses we also observed a weak positive correlation of $1,25(OH)_2$ vitamin D (β: 0.0004, $p<0.05$) with TmP/GFR, but no association was found for FGF23 (β: <0.0001, p=0.87). Interestingly, there was also an inverse association of 24 h urinary calcium excretion (β: -0.006, $p<0.05$) and a positive association of 24 h urinary oxalate excretion (β: 0.05, $p<0.01$) with TmP/GFR. In contrast, 24 h urinary glycolate excretion, a marker of endogenous oxalate metabolism, did not correlate with TmP/GFR (β: -0.04, p=0.13). Figure 2 shows visualization of multivariable regression analyses for the associations of PTH, FGF23, $25(OH)_2$ vitamin D, $1,25(OH)_2$ vitamin D and 24 h urinary calcium and oxalate excretion with TmP/GFR.

We next performed association analyses with TmP/GFR as predictor variable and kidney stone history, stone analysis and bone parameters as outcome variables (Table 4). After multivariable adjustment, renal phosphate leak (i.e. a low TmP/GFR) was associated with a higher frequency of positive family history of renal stone disease (OR: 0.3, $p<0.05$) and with a significantly increased likelihood for younger onset of a first stone event (OR: 8.2, $p<0.01$). However, we observed no significant association of TmP/GFR with stone recurrence frequency. Interestingly, renal phosphate leak (low TmP/GFR) was associated with an increased risk for brushite containing stones (OR 0.006, $p<0.01$) with a concomitant reduced risk for calcium oxalate containing stones (OR 219, $p<0.01$). BMD measured by dual-energy x-ray absorptiometry (DEXA) at the lumbar spine, femoral neck, tibia diaphysis and epiphysis as well as the bone turnover marker alkaline phosphatase did not reveal a significant association with TmP/GFR. Figure 3 shows again visualization of multivariable regression

models for the association of TmP/GFR with family history of stone disease, age of first stone event, probability of calcium oxalate, calcium phosphate, apatite or brushite containing stones.

DISCUSSION

Classically, SF are dichotomized with respect to renal phosphate handling [4-8, 12, 25]. The analysis of our cohort of 555 idiopathic calcium SF demonstrates that TmP/GFR does not have a bimodal distribution but is rather a continuous trait, which is compatible with a previous study [4]. For our association analyses, we choose to treat renal phosphate handling, i.e. TmP/GFR, as a continuous variable. Our results reveal a strong heritable component of kidney stone disease in SF with low TmP/GFR, suggesting that genetic factors play an important role in renal phosphate handling in SF. Support for this notion comes from Mendelian forms of calcareous nephrolithiasis associated with sodium/ phosphate co-transporter IIa and IIc gene mutations (*SLC34A1* and *SLC34A3,* respectively) and recent genome-wide association studies and candidate gene sequencing both reported an association of sequence variants in the *SLC34A1* gene with kidney stone disease [26-29].

We failed to confirm previous reports of an association of FGF23 with TmP/GFR in SF [12, 13]. The reason for this is not clear and maybe due to different genetic background or diets (outpatient versus controlled) of patients studied [4, 12]. Another explanation maybe the fact that we measured FGF23 with the second generation C-terminal assay for FGF23, which detects both intact FGF23 and C-terminal fragments thereof and thus may overestimate "bioactive" FGF23 [30, 31]. Hence, it is possible that we missed an association of "bioactive" intact FGF23 with TmP/GFR. However, a recent study using an intact FGF23 assay also failed to observe an association of FGF23 with fractional phosphate excretion in SF [32]. Instead of FGF23, our multivariable regression analyses indicate that PTH, 25(OH) vitamin D and $1,25(OH)_2$ vitamin D all showed a significant association with renal phosphate handling in SF. The cause of increased PTH in SF with phosphate leak cannot be deduced from our cross-sectional analysis. The finding that adjustment for the co-variable 25(OH) vitamin D greatly attenuates the association of PTH with TmP/GFR suggests that low 25-OH-Vitamin

D3 levels may drive a secondary PTH elevation and thus TmP/GFR reduction in stone formers. If this is indeed the case can only be confirmed by a prospective study.

Our analyses reveal that urinary calcium excretion is increased but $1,25(OH)_2$ vitamin D decreased in SF with low TmP/GFR. This suggests that not $1,25(OH)_2$ vitamin D but other, as of yet unidentified factors, are involved in the increase of urinary calcium excretion in the setting of low TmP/GFR. Interestingly, we also observed lower urinary oxalate excretion in SF with renal phosphate leak whereas urinary excretion of the metabolic oxalate precursor glycolate was unaltered, indicating that the difference may be caused by reduced gastrointestinal oxalate absorption or enhanced gastrointestinal oxalate secretion compared to SF with high TmP/GFR. In line with these biochemical urinary alterations in SF with low TmP/GFR, our results demonstrate for the first time an increased prevalence of brushite stones and a reduced prevalence of calcium oxalate stones in SF with renal phosphate leak.

Chronic hypophosphatemia causes osteomalacia with elevated alkaline phosphatase and reduced BMD [33]. Our results are surprising in that they reveal no reduction of BMD and no alteration of alkaline phosphatase in SF with low TmP/GFR. Together with our observation of elevated PTH in SF with low TmP/GFR, these results may indicate the presence of a resistance to PTH action at the level of the bone. Clearly, measurements of not only total but bone-specific alkaline phosphatase activity and of additional bone turnover markers are needed to further substantiate this claim.

To our knowledge, this is by far the largest study to date analyzing the phenotype of SF with respect to renal phosphate handling. Our results demonstrate that the clinical phenotype of reduced renal phosphate reabsorption in SF has a strong heritable component and correlates with higher PTH, 25-VitD and 1,25-VitD, but not with FGF23 levels. Furthermore, our data reveal that SF with renal phosphate leak is associated with elevated urinary calcium and an increased prevalence of brushite stones.

ACKNOWLEDGEMENTS

We thank Orson Moe and Khashayar Sakhaee for helpful discussions and critical reading of the manuscript.

CONFLICT OF INTEREST STATEMENT

DGF has served as a consultant for Otsuka Pharmaceuticals and has received unrestricted research funding from Novartis, Abbvie and Otsuka Pharmaceuticals.

AUTHOR' CONTRIBUTIONS

NAD, BV and DGF conceived and planned the study. NAD, DL, LS, CM and DGF performed manual chart review. NAD and DGF performed analyses. NAD and DGF wrote the manuscript with input from all authors. All authors approved the final version of the manuscript.

FUNDING

DGF was supported by the Swiss National Centre of Competence in Research NCCR TransCure, the Swiss National Science Foundation (grants #31003A_135503 and #31003A_152829), by a Medical Research Position Award of the Foundation Prof. Dr. Max Cloëtta. DGF and NAD were supported by an unrestricted research grant from Abbvie.

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TABLES

Table 1. Baseline characteristics of study population. The number (N) of participants is indicated for each characteristic. Categorical variables are described by percentage, continuous variables are described by their mean ± SD or median (25^{th}-75^{th} percentile).

Characteristics	N	% or Mean ± SD or Median (25^{th}-75^{th})
Age, y	555	43.4 (34.5-53.9)
Male, %	425	76.6
Body mass index, kg/m2	555	25.8 (23.2-28.9)
Diabetes, %	34	6.1
Hypertension, %	267	48.1
Kidney stone history		
Positive family history of stones, %	253	46.5
Patients with stones available for analysis, %	445	81.1
Age at first stone event, y	527	32.7 (24.7-41.6)
Patients with stone recurrence, %	452	82.2
Number of stone events		
1 event, %	98	17.8
2 events, %	160	29.1
3 events, %	116	21.1
≥4 events, %	176	32
Stone composition (containing)		
Calcium oxalate, %	418	93.3
Calcium phosphate, %	176	39.3
Apatite, %	154	34.4
Brushite, %	15	3.3
Bone density parameters		
T score lumbar spine	422	-0.5 ± 1.1
T score femur neck	423	-0.5 ± 1.1
T score tibia diaphysis	418	0.4 ± 1.1
T score tibia epiphysis	417	-0.6 ± 1
Z score lumbar spine	423	0 ± 1.1
Z score femur neck	424	0.2 ± 1
Z score tibia diaphysis	418	0.6 ± 1.1
Z score tibia epiphysis	417	-0.2 ± 0.9

Table 2. Blood and urine parameters of study population. The number (N) of patients is indicated for each characteristic. Continuous variables are indicated by their mean ± SD or by their median (25^{th}-75^{th} quantile).

Parameter	N	Mean ± SD or Median (25^{th}-75^{th})
Blood		
Creatinine, µmol/l	555	78 (68-88)
GFR, ml/min per 1.73m^2 BSA	555	99.1 (86.7-111.1)
FGF23, RU/ml	259	66.3 (52.6-90)
PTH, pg/ml	553	39 (29.3-47.3)
25-OH-Vitamin D3, nmol/l	372	40.7 (27.9-58)
1,25-OH-Vitamin D3, pmol/l	540	97 (73-123)
Calcium total, mmol/l	555	2.34 ± 0.1
Phosphate, mmol/l	555	1.01 ± 0.17
Alkaline phosphatase, units/l	553	64 (54-75)
Urine		
Sodium, mmol/24 h	527	184 (140-233)
Potassium, mmol/24 h	526	62.3 (49.1-78.4)
Calcium, mmol/24 h	527	5.94 (4.23-8.38)
Phosphate, mmol/24 h	527	30.3 ± 10.1
TmP/GFR, mmol/l	555	0.85 ± 0.19
pH, 24 h	463	5.97 (5.44-6.62)
Citrate, mmol/24 h	517	2.71 (1.74-3.69)
NGIA, mmol/24 h	471	34.6 (17.3-49.8)
Oxalate, µmol/24 h	517	0.37 (0.26-0.54)
Glycolate, µmol/24 h	462	0.36 (0.16-0.54)
Sulfate, mmol/24 h	459	22 (17-27.6)

Table 3. Association of baseline characteristics and blood and urine parameters with TmP/GFR. Models were calculated by mixed effects linear regression taking calendar day of blood sampling as random effect into account. Univariable models contain the predictor variables listed with TmP/GFR as outcome variable. Multivariable models were adjusted for the covariables age, sex, BMI, GFR, diabetes, and hypertension. For PTH a second multivariable model with additional adjustment for the covariable 25-OH-Vitamin D3 was generated. The number of subjects in each model (N), the β coefficient and its 95% confidence interval (CI), and the *p* value of the predictor variable are indicated.

	Univariable Models				Multivariable Models			
Predictor variable	**N**	**β**	**95% CI**	***p***	**N**	**β**	**95% CI**	***p***
Baseline characteristics								
Age, y	555	-0.0013	-0.0024;-0.0002	0.024	555	0.0010	-0.0005;0.0026	0.20
Sex, Male	555	-0.0737	-0.1087;-0.0387	<0.001	555	-0.0626	-0.0975;-0.0277	<0.001
Body mass index, kg/m2	555	-0.0039	-0.0074;-0.0004	0.030	555	-0.0009	-0.0045;0.0028	0.64
GFR, ml/min per 1.73m^2 BSA	555	0.0014	0.0006;0.0022	<0.001	555	0.0013	0.0002;0.0024	0.021
Diabetes, % present	555	-0.0232	-0.0859;0.0396	0.47	555	-0.0182	-0.0802;0.044	0.57
Hypertension, % present	555	-0.0689	-0.0985;-0.0393	<0.001	555	-0.0540	-0.0868;-0.0211	0.0014
Blood parameters								
FGF23, RU/ml	259	0.0001	-0.0001;0.0002	0.28	259	0.0000	-0.0001;0.0001	0.87
PTH, pg/ml	553	-0.0019	-0.0029;-0.001	<0.001	553	-0.0018	-0.0027;-0.0008	<0.001
PTH, pg/ml additionally adjusted for 25-OH-Vitamin D3, nmol/l	-	-	-	-	372	-0.0013	-0.0026;-0.0001	0.037
25-OH-Vitamin D3, nmol/l	372	0.0009	0.0002;0.0016	0.014	372	0.0010	0.0003;0.0018	0.0052
1,25-OH-Vitamin D3, pmol/l	540	0.0005	0.0001;0.0009	0.011	540	0.0004	0;0.0008	0.040
Calcium total, mmol/l	555	0.1598	0.0034;0.3159	0.045	555	0.2216	0.0684;0.3746	0.0049
Urine parameters								
Sodium, mmol/24 h	527	-0.0001	-0.0003;0.0001	0.48	527	0.0001	-0.0002;0.0003	0.58
Potassium, mmol/24 h	526	-0.0004	-0.0011;0.0003	0.27	526	0.0002	-0.0005;0.0008	0.67
Calcium, mmol/24 h	527	-0.0075	-0.0126;-0.0025	0.0037	527	-0.0064	-0.0116;-0.0012	0.017
Uric acid, µmol/24 h	527	0	0;0	0.0022	527	0	0;0	0.055
pH, 24 h	463	-0.0076	-0.0302;0.0146	0.51	463	-0.0162	-0.039;0.0066	0.17
Citrate, mmol/24 h	517	-0.0035	-0.0133;0.0064	0.49	517	-0.0020	-0.0119;0.0079	0.69
NGIA, mmol/24 h	471	-0.0001	-0.0006;0.0004	0.72	471	-0.0001	-0.0006;0.0004	0.80

Oxalate, µmol/24 h	517	0.0420	0.0017;0.0821	0.040	517	0.0528	0.0134;0.0922	0.0089
Glycolate, µmol/24 h	462	-0.0437	-0.0914;0.004	0.073	462	-0.0370	-0.0841;0.0101	0.13
Sulfate, mmol/24 h	459	-0.0016	-0.003;-0.0003	0.021	459	-0.0007	-0.0021;0.0007	0.34

Table 4. Association of TmP/GFR with kidney stone and bone turnover parameters. Models were calculated by mixed effects logistic regression and by mixed effects linear regression taking calendar day of blood sampling as random effect into account. Univariable models for outcome variables listed contain TmP/GFR as predictor variable. Multivariable models were adjusted for the covariables age, sex, BMI, GFR, diabetes and hypertension. The number of subjects in each model (N), the odds ratio (OR) or β coefficient and its 95% confidence interval (CI), and the *p* value of the predictor variable are indicated.

	Univariable Models				Multivariable Models			
Outcome variable	**N**	**OR/β**	**95% CI**	***p***	**N**	**OR/β**	**95% CI**	***p***
Kidney stone history								
Positive family history of stones[a]	544	0.399	0.15;1.07	0.067	544	0.289	0.105;0.798	0.017
Age at first stone event, y[a]	527	1.71	-4.25;7.65	0.57	527	8.2	2.77;13.6	0.0033
Patients with stone recurrence	550	0.207	0.059;0.725	0.014	550	0.356	0.092;1.37	0.13
Number of stone events								
1 event	550	4.82	1.38;16.9	0.014	550	2.81	0.72;10.956	0.14
2 events	550	0.688	0.247;1.92	0.47	550	0.656	0.227;1.89	0.44
3 events	550	0.741	0.237;2.32	0.61	550	0.844	0.252;2.83	0.78
≥4 events	550	0.624	0.227;1.72	0.36	550	0.884	0.294;2.66	0.83
Calcium oxalate, present	448	58.2	2.61;1286	0.010	448	219	5.37;8913	0.0044
Calcium phosphate, present	448	0.756	0.232;2.47	0.64	448	0.462	0.126;1.7	0.24
Apatite, present	448	0.923	0.282;3.02	0.89	448	0.579	0.16;2.1	0.41
Brushite, present	448	0.011	0;0.261	0.0051	448	0.006	0;0.157	0.0021
Bone parameters								
T score lumbar spine	422	0.186	-0.41;0.78	0.54	422	0.263	-0.331;0.855	0.39
T score femur neck	423	0.355	-0.231;0.941	0.24	423	0.341	-0.222;0.903	0.24
T score tibia diaphysis	418	0.005	-0.589;0.603	0.99	418	0.279	-0.315;0.874	0.36
T score tibia epiphysis	417	-0.225	-0.762;0.311	0.41	417	-0.040	-0.557;0.474	0.88
Z score lumbar spine	423	0.307	-0.318;0.93	0.33	423	0.412	-0.184;1.01	0.18
Z score femur neck	424	0.343	-0.224;0.908	0.24	424	0.554	-0.001;1.11	0.052
Z score tibia diaphysis	418	0.082	-0.514;0.677	0.79	418	0.352	-0.229;0.941	0.25
Z score tibia epiphysis	417	-0.193	-0.715;0.327	0.47	417	-0.011	-0.522;0.499	0.97
Alkaline phosphatase, units/l[b]	553	-0.176	-0.653;0.3	0.47	553	0.076	-0.406;0.558	0.76

[a]not adjusted for age, [b]square root transformed

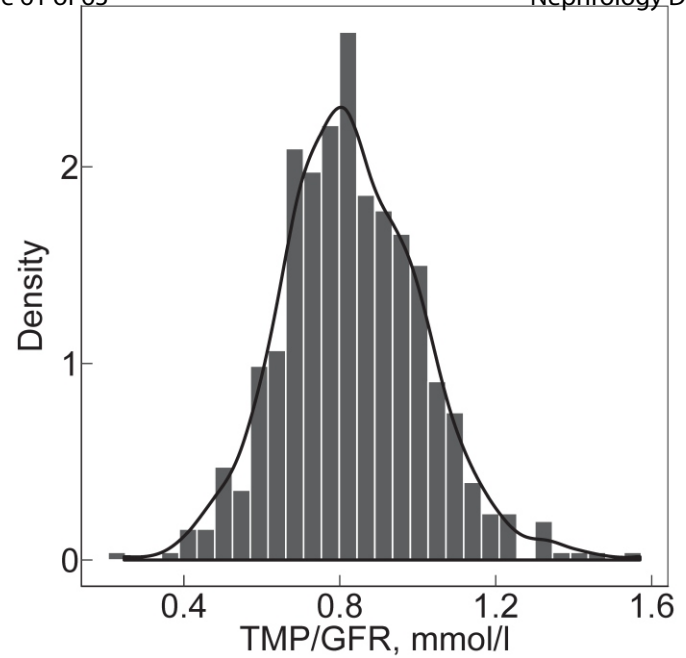
FIGURE LEGENDS

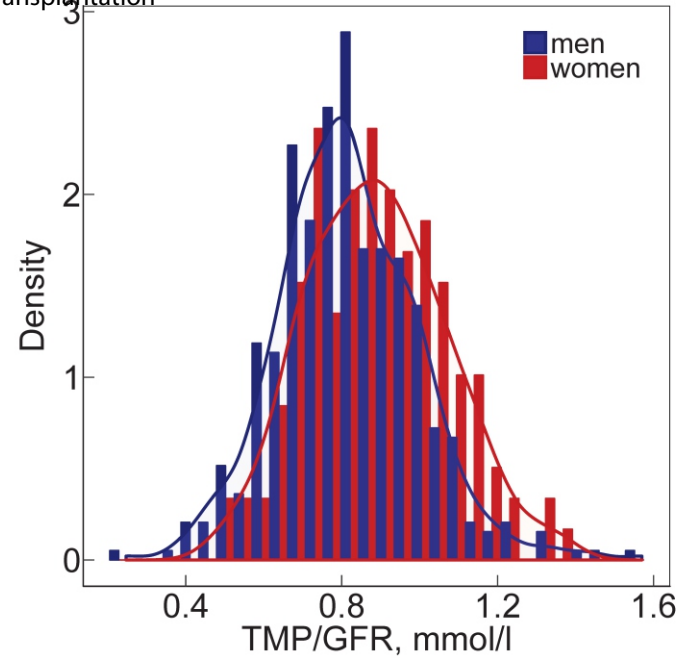
Figure 1. Distribution of TmP/GFR in study population.

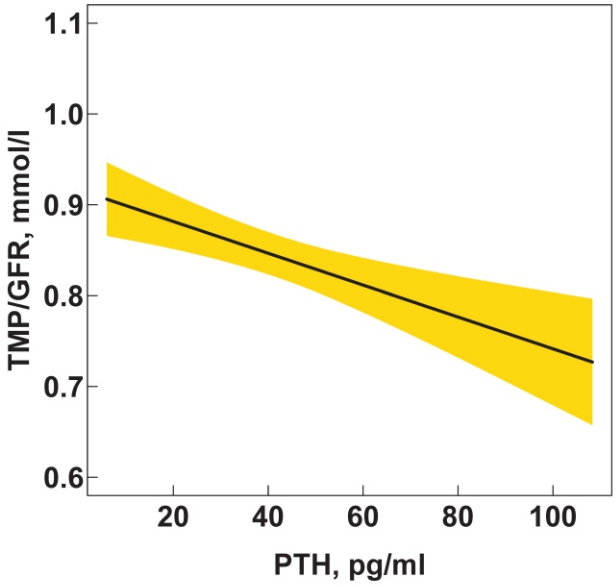
Kernel density plot histogram of TmP/GFR in the study cohort of 555 idiopathic calcium SF. Left panel: entire cohort. Right panel: cohort separated in men (blue) and women (red).

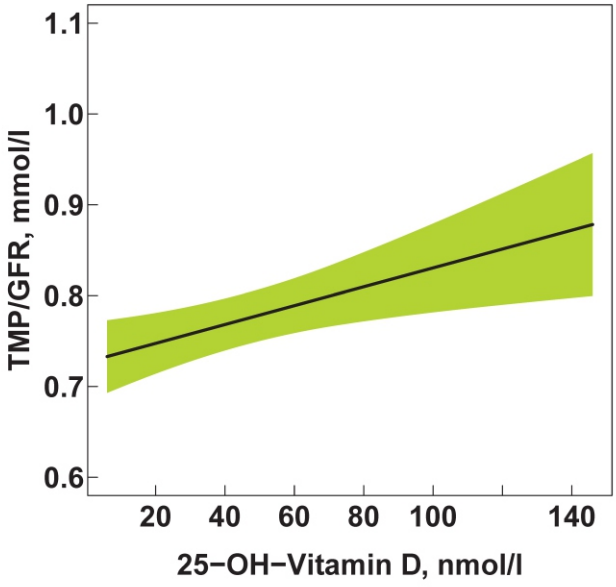
Figure 2. Visualization of selected multivariable regression models from Table 3. All models were calculated by linear regression and are visualized using the R package visreg. Solid lines indicate regression lines and the shaped area represents the 95 % confidence interval.

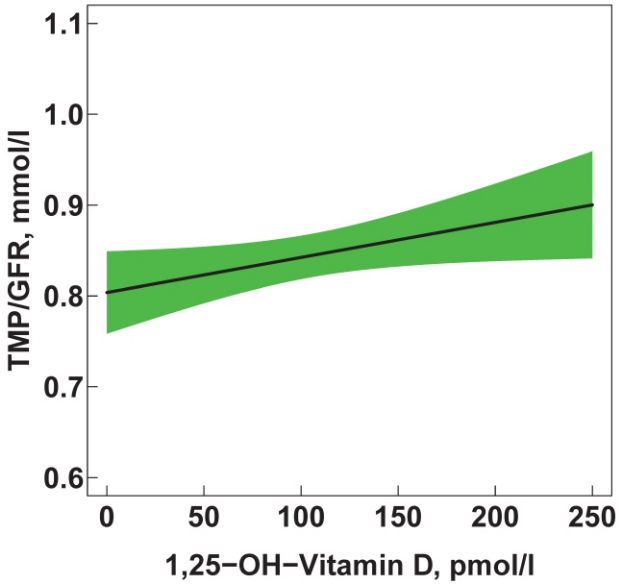
Figure 3. Visualization of selected multivariable regression models from Table 4. For dichotomous outcome variables, figures were created by an inverse logistic transformation of the linear regression line and 95 % confidence bands derived from the logistic regression models. The vertical axis for dichotomous outcome variables in logistic regression models is labelled with the probability scale and the horizontal axis with the predictor variable of interest.

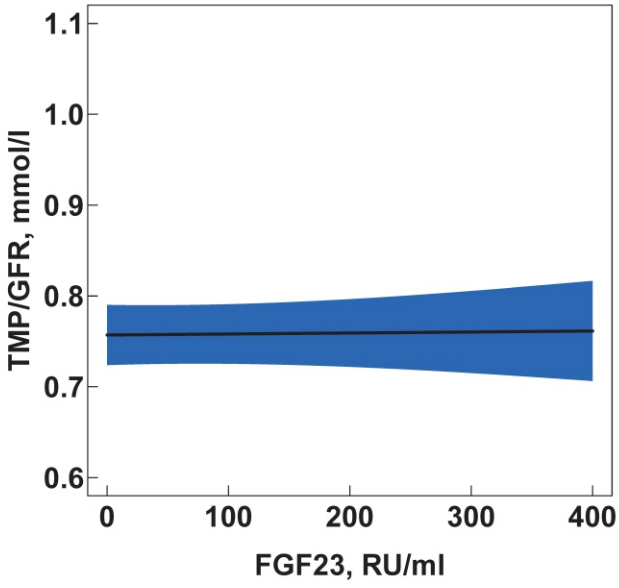

Density
0
1
2
0.4
0.8
1.2
1.6
TMP/GFR, mmol/l

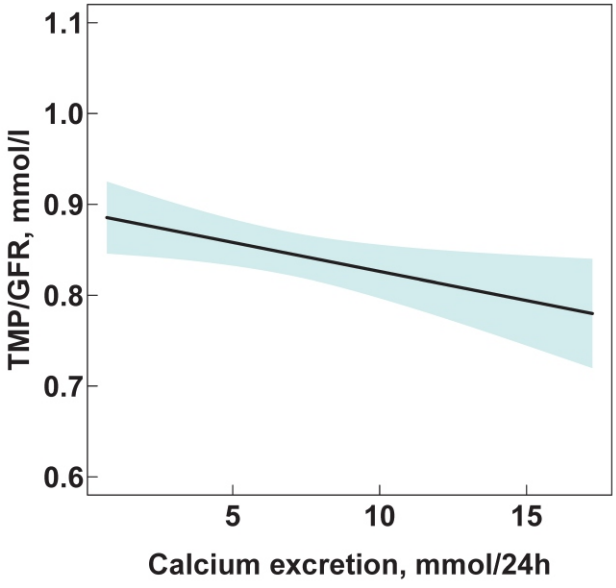

men
women
Density
0
1
2
3
0.4
0.8
1.2
1.6
TMP/GFR, mmol/l

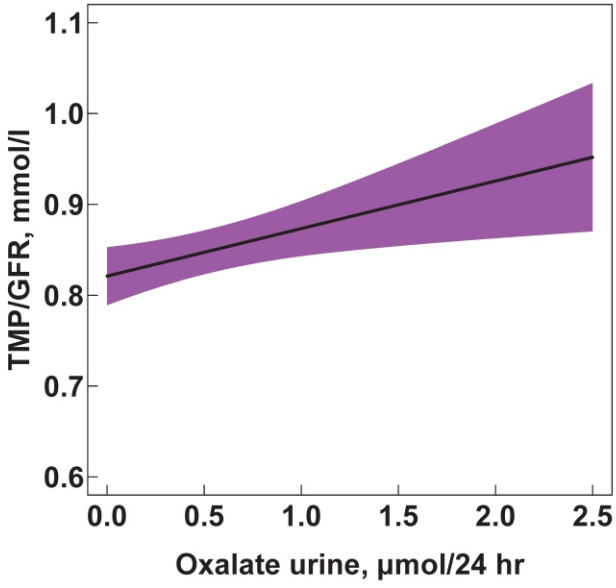

TMP/GFR, mmol/l
1.1
1.0
0.9
0.8
0.7
0.6
20
40
60
80
100
PTH, pg/ml

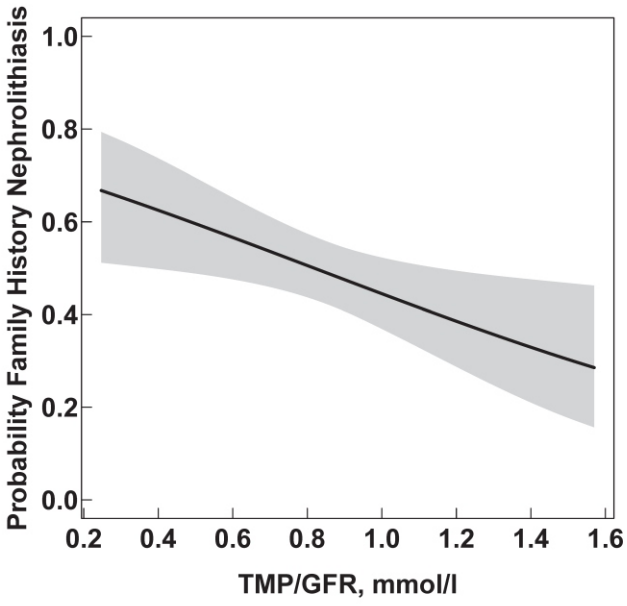

TMP/GFR, mmol/l
1.1
1.0
0.9
0.8
0.7
0.6
20
40
60
80
100
140
25−OH−Vitamin D, nmol/l

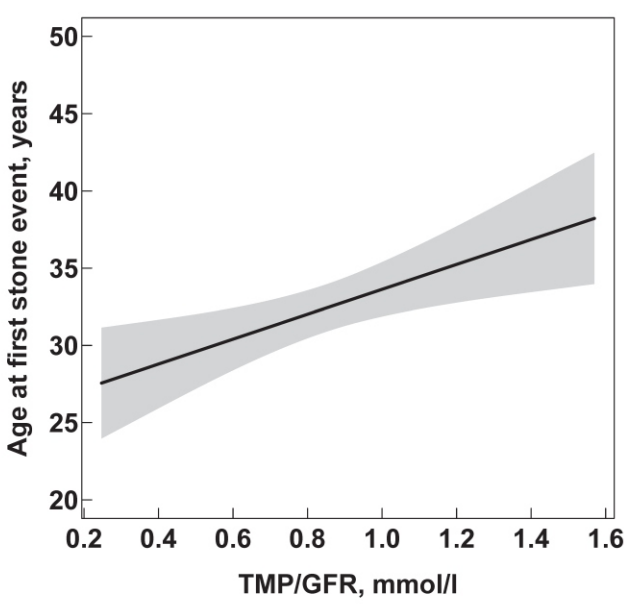

TMP/GFR, mmol/l
1.1
1.0
0.9
0.8
0.7
0.6
0
50
100
150
200
250
1,25−OH−Vitamin D, pmol/l

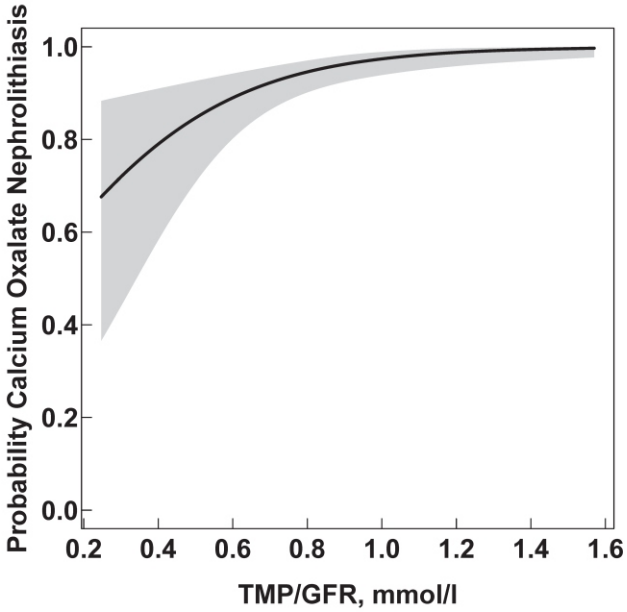

TMP/GFR, mmol/l
1.1
1.0
0.9
0.8
0.7
0.6
0
100
200
300
400
FGF23, RU/ml

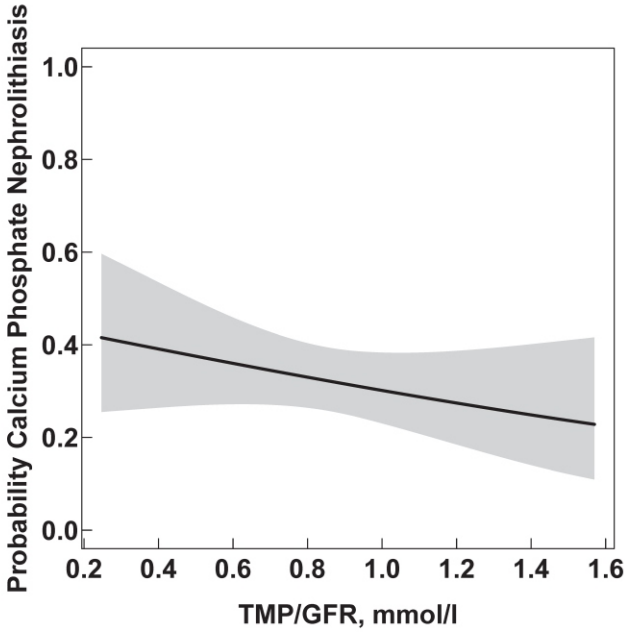

TMP/GFR, mmol/l
1.1
1.0
0.9
0.8
0.7
0.6
5
10
15
Calcium excretion, mmol/24h

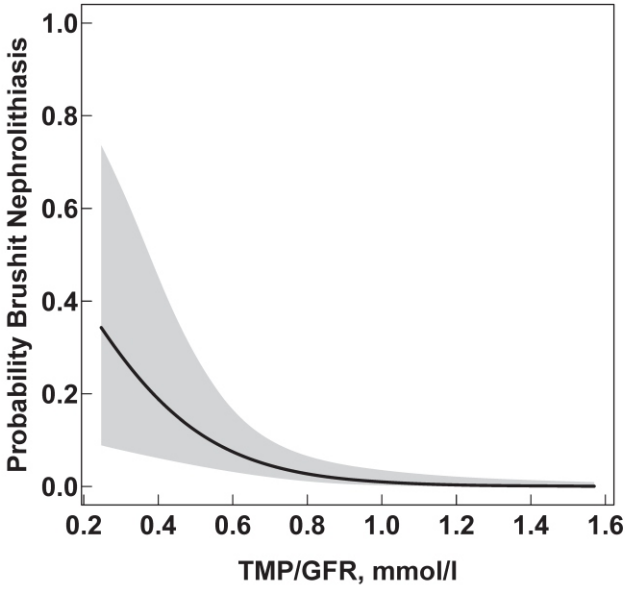

TMP/GFR, mmol/l
1.1
1.0
0.9
0.8
0.7
0.6
0.0
0.5
1.0
1.5
2.0
2.5
Oxalate urine, µmol/24 hr

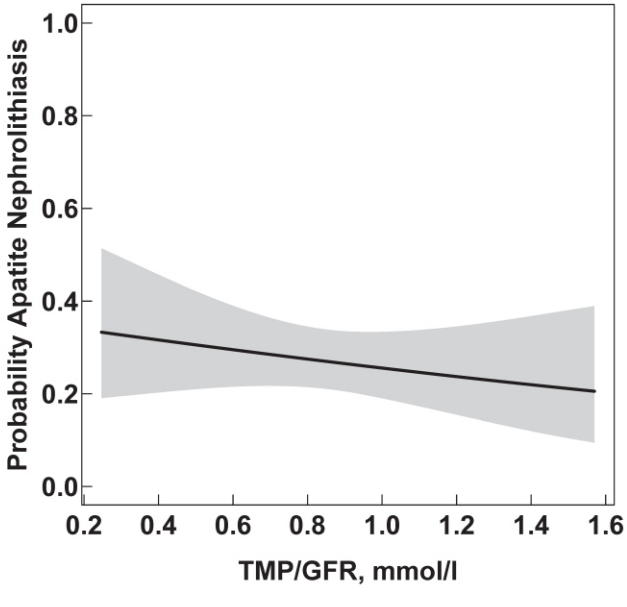

Probability Family History Nephrolithiasis
1.0
0.8
0.6
0.4
0.2
0.0
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6
TMP/GFR, mmol/l


Age at first stone event, years
50
45
40
35
30
25
20
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6
TMP/GFR, mmol/l


Probability Calcium Oxalate Nephrolithiasis
1.0
0.8
0.6
0.4
0.2
0.0
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6
TMP/GFR, mmol/l


Probability Calcium Phosphate Nephrolithiasis
1.0
0.8
0.6
0.4
0.2
0.0
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6
TMP/GFR, mmol/l


Probability Brushit Nephrolithiasis
1.0
0.8
0.6
0.4
0.2
0.0
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6
TMP/GFR, mmol/l


Probability Apatite Nephrolithiasis
1.0
0.8
0.6
0.4
0.2
0.0
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6
TMP/GFR, mmol/l