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Chapter IV

Renal Calcium and Urate Handling and Diet in Idiopathic Calcium Stone Formers

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Abstract

The incidence of calcium stones is increasing world-wide. Dietary changes which impact on urine composition are probably largely responsible. However, despite years of research, we still do not have a clear understanding of the underlying defects in hypercalciuria or hyperuricosuria in idiopathic calcium stone formers or of how diet is implicated. Without this, interventions to prevent stone recurrence are largely empirical and often biased by personal opinion.

From our own stone clinic we reported that male stone formers with idiopathic hypercalciuria reabsorb less of the calcium filtered into the

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kidneys than men with normocalciuria. Similarly, net urate reabsorption of men with idiopathic hyperuricosuria is reduced compared with those with normal urate excretion. This is probably explained by genetic variation in the renal transport of calcium and urate in the general population. In an earlier study, in which we investigated families in which three or more first degree relatives had stones, we confirmed the heritability of hypercalciuria. From our growing knowledge of the numerous genes which are implicated in the renal handling of calcium and urate, and of their close interaction, genetic variation is inevitable. There is increasing interest in this area, but so far few associations have been identified between genetic polymorphisms of renal transporters and hypercalciuria or hyperuricosuria.

From our clinic, we also found subsets of male stone formers with hypercalciuria and hyperuricosuria who had very high creatinine clearance rates, and hence (by inference) glomerular filtration rates. This would lead to delivery of an abnormally high filtered load of calcium or urate to the kidneys. We do not know the explanation. However, one possibility is a high dietary protein intake. There is a large body of evidence that high intakes increase glomerular filtration. From recent studies this might be mediated by enhanced nitric oxide production via the renal arginine-citrulline cycle, which could also influence intrarenal blood flow. This merits further exploration.

As in the case of hypercholesterolemia, idiopathic hypercalciuria and uricosuria may result from interaction between increased genetic susceptibility and dietary excesses, particularly of protein. Whilst identification of those with unfavorable diets and appropriate intervention should reduce calcium or urate excretion of hypercalciuric and hyperuricosuric stone formers, it will not be enough for those at the more severe end of the spectrum for genetic abnormality. They will need additional medication which, hopefully, may be targeted towards specific transporters in the future. With the advances in the technology for rapid through-put DNA analysis, we now have the opportunity to get to the roots of the problem, but it will be fundamental to define the phenotypes of stone formers as precisely as possible if the genetic analyses are to be fruitful.

Introduction

Stones are a common problem world-wide and occur 2-4 times more often in men than women. In America, the life-time risk for stones for men is 12% and for women 5% [1] with estimated annual incidences for men ranging from 100 to 300 per 100,000. [2] Their prevalence has risen over the last 40 years.

[3-5] There is a high recurrence rate, estimated at around 25% at 10y for a first time stone former and 75% for recurrent stone formers. [6] The evidence that hypercalciuria is more common among idiopathic calcium stone formers than in the general population, and that it increases the risk for renal stones is beyond doubt. Precise definition is impossible because urinary calcium excretion is a continuous variable, but using commonly adopted thresholds for urine calcium of >7.50 mmol/24h for men and > 6.25 mmol/24h for women [1], 30- 50% of adult stone-formers have hypercalciuria [3, 7, 8] compared with fewer than 10% in the general population. [3] The underlying cause of hypercalciuria has been investigated intensively. Intestinal calcium absorption is increased, but negative calcium balance develops with dietary calcium restriction which indicates impaired renal conservation of calcium ('renal hypercalciuria') together with increased mobilization of calcium from bone ('resorptive hypercalciuria'). Formerly, patients with idiopathic hypercalciuria were classified as having absorptive, renal or resorptive hypercalciuria and this directed their management. [7-9] However, this categorization was difficult in a clinic setting and interpretation of calcium loading tests used in research clinics to demonstrate absorptive hypercalciuria [10] may have been confounded by co-existing renal hypercalciuria. Indeed, it was suggested that all idiopathic hypercalciuria may be in part renal. [11] In experimental studies on a genetic stone-forming rat all three mechanisms contribute to hypercalciuria, [12] and there is a growing consensus that this is also the case in humans. [1, 3, 8, 9, 13-15] Pedigree and twin studies have demonstrated that hypercalciuria is inherited, with the genetic contribution to urinary excretion estimated at around 50%. [4, 16-20] The heritability of this quantitative trait is polygenic in nature. [15, 18, 19]

Fewer than 20% of individuals with hypercalciuria in the general population have symptomatic stone disease. [7] Hypercalciuria may merely lower the threshold for urolithiasis which is then triggered by other associated factors, such as low urine volume, abnormal urinary pH, and excessive oxalate or urate excretion. These could have a genetic origin and/or result from environmental factors, particularly low fluid intake and diet. They must be controlled in addition to hypercalciuria if interventions to reduce stone recurrence are to be effective. [3, 8]

In view of the numerous variables which may contribute to 'idiopathic' hypercalciuria, investigations to further our understanding of its origin and significance for stone formation must be targeted. We need to identify sub-groups of patients for study who have a homogeneous phenotype with an increased likelihood of having a selected pathological defect.

In this chapter we report findings from our own clinic in Southampton. In an early family study we looked for evidence of linkage between stone formation and intermediate traits associated with stones with four genes which are involved in calcium turnover. The findings have not been previously reported and will be covered in detail. In our other studies we aimed to identify patients from the clinic data base whose predominant abnormality was low renal calcium or urate reabsorption and to look for associated risks for stones. The main findings of three studies which have been published [21-23] will be summarized. Collectively they support the case for a genetic contribution to hypercalciuria, hyperuricosuria and stones in some families, indicate that altered renal tubular handling of calcium and urate is probably a common underlying factor in hypercalciuria and hyperuricosuria, that individuals in whom this is the dominant primary abnormality can be identified in a clinic setting and that their risk factors for stones differ from those of individuals whose dominant disturbance is delivery of an excessive filtered load of calcium or urate to the kidney.

The Southampton Stone Clinic

The Renal Stone Clinic of the Department of Clinical Biochemistry at Southampton General Hospital UK accepts referrals for stone forming patients diagnosed in the local area. Its purpose is to identify and treat factors which can be modified to reduce the risk of stone recurrence. A clinic database, held on Microsoft Access (97-2003), was created in 1996 and includes demographic and biochemical data for most patients investigated for stones who initially presented to the Clinic between June 1990 and March 2007. [21] The data were anonymized for the research studies. At the first attendance, non-fasting blood and a paired fresh random urine sample were collected. With careful verbal and written instruction, patients collected 24h urine samples at home, without refrigeration. From around 1997, patients collected one 24h sample with thymol as preservative. On receipt, the volume was determined from weight and an aliquot was removed for urate analysis without pH adjustment. The urine remaining in the container was then acidified with hydrochloric acid to pH 2.0. Prior to 1997, patients collected two samples: one into hydrochloric acid for the standard tests, and the second into sodium hydroxide for urate analysis if requested. The pH was then adjusted to 2.0 and 7.5, respectively.

The standard tests were: plasma: electrolytes, bicarbonate, creatinine, calcium adjusted for albumin, phosphate, urate and parathyroid hormone (PTH); fresh random urine: pH, phosphate and creatinine concentrations; 24h urine: volume, calcium, oxalate, creatinine and creatinine clearance. 24h urine urate, citrate and magnesium were added variably, often for patients with more severe stone problems. All analyses were carried out at Southampton General Hospital. Stones were analyzed qualitatively, with chemical methods using a Merckognost urinary calculi analysis kit (Merck, Darmstadt, Germany). Non-parametric statistical analyses were used throughout: the two-tailed Mann-Whitney U test and two-sided Fisher's exact test or Chi-square tests to compare differences between groups and Spearman's rank test for correlation.

The Family Study

This study was undertaken between 1997 and 2000. From the clinic database, 25 patients were selected who were recorded as having at least two first-degree relatives with renal stone disease. Selection was biased towards families with young index patients (more likely to have a severe defect and living relatives), affected women (lower incidence of stones than men and possibly a greater likelihood of having a genetic defect), and those living locally. Families of index patients with a known heritable cause of nephrolithiasis were excluded. Fourteen families were finally recruited and participants gave written informed consent. All participants who lived locally provided a blood sample for biochemistry and DNA extraction, a mouthwash for DNA, and fasting and 24h urine samples. Adults living outside Southampton provided a mouthwash sample, fasting urine and if possible, blood for DNA collected locally. Children under 16 years of age provided a mouthwash sample and fasting urine.

To explore the familial basis of renal stone disease, the heritability of stones and relevant biochemical traits were inspected for each kindred. The possibility that these were linked to any of four genes which might be implicated in stone disease was investigated using a microsatellite-based approach for co-segregation analysis. The microsatellite markers were tetranucleotide repeats with between 5 and 9 alleles and heterozygosity frequencies $\geq$ 60%. The selected genes were those for the calcium sensing receptor (CASR; location 3q21-q24), the thiazide-sensitive sodium chloride cotransporter (SLC34A1; 16q13), vitamin D receptor (12q12-q14), and the

sodium–phosphate cotransporter (NPT2; 5q35). Genehunter, a software program that facilitates non-parametric linkage analysis, was used to analyze for linkage between renal stone affection status and the genotyped microsatellite markers in 14 pedigrees. BETA, a software program that facilitates non-parametric linkage analysis for a quantitative trait using allele sharing in sibling pairs, was used to analyze for linkage between quantitative traits associated with renal stone disease and the genotyped microsatellite markers. A LOD score ≈ 3.0, approximates to a significance level of 5% for Mendelian disorders.

Summary of the Findings

Biochemical analyses were undertaken on samples from 14 kindreds from 166 individuals ≥ 18 years of age and 34 aged <18 years. The key relevant data for each family are summarized in Table 1. Samples were obtained for 10 or more members of seven families, but other families were smaller with few surviving members living locally to recruit.

Hypercalciuria

Overall, 32 of 105 individuals (30%) who supplied 24h urine samples had hypercalciuria. The incidence was significantly higher in stone formers than in non-stone formers (45% versus 22%, P 0.016). The frequency varied among the families and was apparently a common abnormality in four of the larger kindreds tested (1, 2, 10, and 11), and possibly important in two other families (13, 14) in each of which two of the only three members tested were hypercalciuric. Hypercalciuria was relatively modest, with median values for men of 9.19 mmol/24h and for women of 7.50 mmol/24h, and only 7 individuals had values > 10.00 mmol /24h. Only 14% of adults had raised fasting urine calcium/creatinine ratios, with no difference between stone formers and non-stone formers, and no apparent segregation with the hypercalciuric kindreds. None of those under 18 years of age had had stones, but four (12%) had raised calcium/creatinine ratios.

Table 1. Abnormal findings in families of 14 stone formers with two or more first degree relatives with stones

Family (no. of stone formers tested)	Inc. 24h calcium (no. with stones)	†n	Inc. Ca/crt ratio (no. with stones)	n	<18y with inc Ca/crt (no. with stones)	n	Inc. 24h oxalate (no. with stones)	n	Low 24h citrate (no. with stones)	n	Low cit/crt ratio (no. with stones)	n	<18y with low cit/crt (no. with stones)	n	TmPO4 < 0.80 mmol/l (no. with stones)	n	Inc. 1,25-DHCC (no. with stones)	n	Inc. PTH (no. with stones)	n
1 (4)	4 (3)	9	2(1)	14	0	6	3(2)	9	0	9	3(0)	14	0	6	6(4)	8	3(0)	9	1(1)	9
2 (3)	6 (1)	10	7(1)	17	1	1	2(0)	10	1(1)	10	4(1)	16	1	1	2(1)	9	4(1)	6	0	9
3 (4)	1 (1*)	4	1(1*)	5	0	2	0	4	0	4	1(1)	5	0	2	3(3*)	4	0	4	1(1*)	4
4 (3)	1 (1*)	4	1(1*)	4	1	3	0	4	1(1*)	4	2(1)	4	0	3	2(1*)	4	3(2*)	4	1(1*)	4
5 (2)	0	3	0	5	none tested		0	3	0	3	1(0)	5	none tested		0	2	2(2)	3	0	3
6 (3)	1 (0)	13	2(0)	17	1	6	2(0)	13	0	13	3(1)	17	0	6	5(2)	12	2(0)	11	2(1)	12
7 (2)	0	6	0	8	none tested		1(0)	6	1(0)	6	2(1)	7	none tested		3(2)	5	3(2)	5	0	5
8 (2)	1 (1)	2	1(0)	5	0	1	0	2	0	2	2(1)	5	0	2	2(2)	2	1(1)	2	0	2
9 (1)	1 (1)	4	0	4	none tested		0	4	1(0)	4	2(0)	4	none tested		0	3	2(0)	4	0	4
10 (5)	4 (2)	7	2(1)	9	1	2	1(1)	7	1(1)	7	4(1)	10	0	2	1(1)	8	5(3)	8	0	8
11 (3)	5 (2)	10	2(0)	16	0	3	3(0)	10	2(0)	10	2(0)	17	0	3	2(1)	9	5(2)	9	1(0)	9
12 (5)	4 (3)	27	4(1)	39	0	6	3(0)	27	6(1)	25	**16(1)	39	2	6	4 (2)	17	6(3)	27	2(0)	25
13 (3)	2 (1)	3	2(1)	11	0	1	2(1)	3	2(1)	3	4(2)	11	1	1	2(1)	3	2(1)	3	0	3
14 (3)	2 (2)	3	0	8	0	2	0	3	0	3	2(1)	7	1	2	1(1)	3	0	3	0	3
Totals: abnorm (tested)																				
sf	18(40) 45%		7(41) 17%				4(40)10%		5(40) 13%		11(42) 26%				21(39) 54%		17(38) 45%		4 (40) 10%	
non-sf	14(65) 22%		17(125)14%		4 (33) 12%		13 (65) 20%		10(63) 16%		36(119)30%		5(34) 15%		12(50) 24%		21(60) 35%		4(60) 7%	
all	32(105)30%		24(166)14%				17(105)16%		15(103)15%		47(161)29%				33(89) 41%		38(98) 39%		8(100) 8%	

† Number of samples analyzed; * Results indicated that this stone former had primary hyperparathyroidism; ** 10 members of this kindred had fasting citrate/creatinine ratios < 0.10 mmol/mmol (ref ≥ 0.16); ref for calcium/ creatinine ratio for adults ≤ 0.59 mmol/mmol; <18years ≤ 0.69 mmol/mmol; Abbreviations: inc. increased; Ca/crt urine calcium/creatinine ratio; cit/creat: citrate/creatinine ratio; 1,25-DHCC 1,25-dihydroxycholecalciferol; PTH parathyroid hormone; sf stone formers; non-sf non-stone formers.

Table 2. Comparison of data for all adults ≥ 18 y with hypercalciuria (men urine calcium > 7.50 mmol/24h; women > 6.25 mmol/24h) with those with normocalciuria[†]

	MEN 24h calcium > 7.50 mmol median; range (n)	**MEN** 24h calcium ≤ 7.50 mmol median; range (n)	P[††] high v normal	**WOMEN** 24h calcium > 6.25 mmol median; range (n)	**WOMEN**24h calcium ≤ 6.25 mmol median; range (n)	P high v normal
Total in group	16	33		13	40	
stones	11 (69%)	14 (42%)	0.128	6 (46%)	8 (20%)	0.080
Plasma						
Calcium mmol/l	2.41; 2.25-2.54 (15)	2.36; 2.24-2.61 (30)	0.288	2.32; 2.22-2.52 (13)	2.36; 2.20-2.59 (38)	0.043
Phosphate mmol/l	1.03; 0.71-1.38 (15)	1.02; 0.80-1.68 (30)	0.399	1.00; 0.80-1.27 (13)	1.14; 0.71-1.40 (38)	0.230
PTH pmol/l	3.3; 1.4-15.8 (15)	3.3; 1.1-10.6 (30)	0.952	3.1; 1.1-7.6 (13)	2.7; 0.6-8.1 (38)	0.762
1,25 DHCC pmol/l	120; 61-161 (15)	85; 46-182 (30)	0.044	126; 70-180 (13)	98; 38-151 (36)	0.029
24h urine						
Oxalate mmol/24h	0.46; 0.29-0.67 (16)	0.33; 0.12-0.67 (33)	0.002	0.36; 0.23-0.76 (13)	0.32; 0.14-0.94 (40)	0.121
Calcium mmol/24h	9.19; 7.53-16.81 (16)	5.24; 1.38-7.50 (33)	<0.001	7.50; 6.44-18.58 (13)	3.32; 1.17-6.24 (40)	<0.001
Citrate mmol/24h	4.06; 1.10-8.49 (16)	2.59; 3.77-21.92 (32)	0.018	3.48; 1.72-7.19 (13)	2.77; 0.35-5.74 (39)	0.148
Volume l/24h	1.83; 1.28-3.02 (16)	1.53; 0.78-2.65 (33)	0.002	1.69; 0.76-3.44 (13)	1.49; 0.49-3.91 (40)	0.347
Creatinine mmol/24h	15.9; 9.5-21.2 (16)	12.4; 3.8-21.9 (33)	0.001	12.6; 8.11-21.67 (13)	8.72; 3.6-16.0 (40)	0.002
Creatinine clearance l/24h	178; 70-263 (15)	125; 36-274 (30)	0.003	164; 104-289 (13)	122.9; 37-196 (38)	0.002
Random fasting urine						
Calcium/creat mmol/mmol	0.48; 0.28-0.99 (16)	0.33; 0.04-0.82 (33)	0.003	0.65; 0.18-0.76 (11)	0.31; 0.09-0.84 (40)	0.013
Citrate/creat mmol/mmol	0.20; 0.09-0.54 (16)	0.16; 0.06-0.49 (33)	0.062	0.31; 0.06-0.59 (12)	0.27; 0.04-0.67 (39)	0.617
$TmPO_4$/GFR mmol/l	0.78; 0.51-1.25 (14)	0.78; 0.50-1.68 (26)	0.755	0.94; 0.58-1.18 (12)	0.97; 0.48-1.53 (34)	0.491

[†] All ≥ 18 years of age, including family members and spouses, stone formers and non-stone formers.[††] Mann-Whitney U-test P <0.05 statistically significant. Abbreviations as for Table 1.

Table 3. Comparison of data for all adults with $TmPO_4$ /GFR below 0.80 mmol/l (low) and ≥ 0.80 mmol/l†

	MEN TmPO4/GFR <0.80 mmol/l median; range (n)	**MEN** TmPO4/GFR ≥ 0.80 mmol/l median; range (n)	P†† low v normal	**WOMEN** TmPO4/GFR <0.80 mmol/l median; range (n)	**WOMEN** TmPO4/GFR ≥ 0.80 mmol/l median; range (n)	P low v normal
Total in group†††	23	18		8	38	
stones	14 (61%)	9 (50%)	0.539	5 (63%)	9 (24%)	0.044
Age years	58; 27-89 (23)	43; 23-74 (18)	0.004	45; 29-60 (8)	41; 18-84 (38)	0.761
Plasma						
Bicarbonate mmol/l	28; 23-33 (23)	28; 22-32 (18)	0.426	26; 24-27 (8)	27; 21-31 (37)	0.027
Calcium mmol/l	2.40; 2.28-2.61 (23)	2.35; 2.24-2.52 (18)	0.151	2.34; 2.23-2.36 (8)	2.35; 2.20-2.59 (38)	0.151
Phosphate mmol/l	0.93; 0.71-1.10 (23)	1.17; 0.96-1.40 (18)	<0.001	0.85; 0.71-1.00 (8)	1.17; 0.87-1.40 (38)	<0.001
Urate mmol/l	0.32; 0.20-0.49 (23)	0.29; 0.22-0.48 (18)	0.252	0.30; 0.20-0.35 (8)	0.19; 0.09-0.41 (37)	0.008
Creatinine μmol/l	100; 76-101 (23)	87; 76-105 (18)	0.006	76; 54-82 (8)	74; 56-97 (38)	0.816
PTH pmol/l	4.0; 1.1-15.8 (23)	2.8; 1.3-5.9 (18)	0.009	3.6; 2.2-7.6 (8)	2.7; 0.6-8.1 (38)	0.164
1,25-DHCC pmol/l	108; 46-150 (22)	85; 52-159 (17)	0.497	97; 65-146 (8)	105; 38-180 (36)	0.670
24h urine						
Calcium mmol/24h	6.68; 1.38-16.81 (23)	6.36; 3.34-12.47 (17)	0.935	7.41; 2.99-18.58 (8)	3.67; 1.17-8.68 (38)	0.012
Citrate mmol/24h	3.01; 0.05-11.27 (23)	3.03; 0.70-8.49 (17)	0.935	3.98; 2.08-7.19 (8)	2.68; 0.35-5.74 (37)	0.030
Creatinine clearance l/24h	147; 36-263 (23)	164; 113-274 (17)	0.113	164; 101-289 (8)	134; 37-205 (38)	0.021
Volume l/24h	1.60: 0.78-2.64 (23)	1.59; 0.93-2.63 (17)	0.805	1.54; 0.66-3.44 (8)	1.52; 0.49-3.91 (38)	0.685
Fasting urine pH	5.95; 5.2-7.1 (20)	6.05; 5.0-8.3 (16)	0.610	5.60; 4.8-6.3 (7)	6.05; 5.0-7.2 (32)	0.056

† All ≥ 18 years of age, including family members and spouses, stone formers and non-stone formers.Two stone formers (one male, one female) and one female non-stone former with primary hyperparathyroidism have been excluded; †† Mann-Whitney U-test , P <0.05 statistically significant. †††23/41 (56%) of the men and 8/46 (17%) of the women had TmPO4/GFR <0.80 mmol/l (P< 0.001). Abbreviations as for Table 1.

Association of Hypercalciuria with Other Intermediate Traits

Hyperoxaluria

Mild hyperoxaluria (median 0.61 mmol/24h, observed range 0.51-0.94 mmol/24h) was observed in 14% of samples and did not differ between stone formers and non-stone formers. However, the increases showed clustering with the hypercalciuric families (1, 2, 11 and 13) and 24h oxalate excretion was significantly higher in hypercalciuric than normocalciuric men (medians 0.46 and 0.33 mmol/24h respectively, P 0.002, Table 2).

Hypocitraturia

Only 15% of individuals had a low 24h urine citrate excretion and there was no difference between stone formers and non-stone formers. Contrary to expectation, values were significantly *higher* for hypercalciuric than normocalciuric men (median values 4.06 and 2.59 mmol/24h, P 0.018) but this was not the case for women (Table 2). Low fasting urine citrate/creatinine ratios were a common finding (29% of adults) with no difference between stone formers and non-stone formers, and were observed in five of 34 subjects (15%) <18 y of age. Twenty of the 47 low values in adults were from families 12 and 13 (with incidences of 41% and 36%, respectively). If these families are excluded, the incidence for the remaining families was 24%.

Phosphaturia

The maximum renal tubular reabsorption of phosphate ($TmPO_4$/GFR) is a practical means of assessing overall tubular capacity for tubular phosphate reabsorption which is independent of GFR. [24] Low values are indicative of phosphaturia. Low values for $TmPO_4$/GFR (<0.80 mmol/l) were observed in 41% of subjects and in 12 of the 14 families. In four families (3, 6, 7 and 8) low $TmPO_4$/GFR was the most frequent abnormality. The incidence was significantly higher in men than women (56 % versus 17% P <0.001). In both men and women $TmPO_4$/ GFR correlated significantly with plasma phosphate (men r 0.91, 95% confidence intervals CI 0.83, 0.95 n= 41 P<0.001; women r 0.83, CI 0.71, 0.91 n=46, P<0.001), in men with age (r -0.42 CI -0.65,-0.12 n= 41, P 0.006; Figure 1), and in women with plasma urate (r -0.35 CI –0.59,-0.05 n= 45, P 0.019). There was no significant correlation with 24h urine calcium (for men r 0.07, P 0.654; women r -0.25, P 0.098). Among men, $TmPO_4$/GFR <0.80 mmol/l had similar frequency among stone formers and non-stone formers. Men with values <0.80 mmol/l were older than those with a normal value and had higher plasma creatinine and PTH levels, perhaps a reflection of

age. In contrast, compared with women with normal $TmPO_4$/GFR, more women with $TmPO_4$/GFR <0.80 mmol/l were stone formers (63% versus 24%; P 0.044, Table 3), they had a small, but significant, reduction in plasma bicarbonate, increased plasma urate which was not associated with an increase in plasma creatinine, and significantly higher 24h calcium and citrate excretion and creatinine clearance. These increases were not explained by differences in 24h urine volume. It is feasible that these differences and phosphaturia were manifestations of a common underlying disturbance.

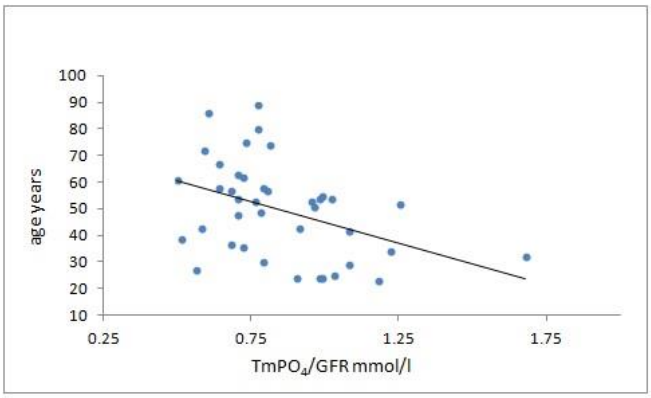

Figure 1. Correlation of TmPO4/GFR with age for all men ≥ 18 years of age in the family study. These included stone formers and non-stone formers, family members and spouses. Spearman's correlation coefficient r -0.42; 95% CI -0.65, -0.12 P 0.006.

Plasma 1,25-dihydroxycholecalciferol (1,25-DHCC)

We did not have our own reference data, and used the upper reference limit of 110 pmol/l provided with the assay kit. In view of the high frequency of raised values recorded (39%) this may have been too low. There was a similar incidence of raised values in men and women, and high values were not confined to the hypercalciuric families (Table 1). More men with raised values were stone formers than men with normal levels (68% versus 36%; P 0.039). A notable observation was that the 24h urine calcium excretion of both men and women with raised 1,25-DHCC was significantly higher than that of individuals with normal values (median calcium excretion for men 7.50 and 4.97 mmol/24h, P 0.004; for women 5.06 and 3.19 mmol/24h, P 0.014). This was not explained by differences in 24h urine volume: for men, the median volumes were 1.61 and 1.60 l/24h, P 0.679, and for women with raised 1,25-DHCC, 1.49 versus 1.60 l/24h, P 0.429. Fasting urine calcium/creatinine ratios

were also higher but not significantly different (median values for men 0.42 and 0.32 mmol/mmol, P 0.070; for women 0.40 and 0.30 mmol/mmol, P 0.077). There were no differences in age, plasma phosphate, creatinine, creatinine clearance, PTH or $TmPO_4$/GFR. The combined data for stone forming and non-stone forming men showed significant correlation between 1,25-DHCC and 24h urine calcium excretion : r 0.56, CI 0.30, 0.74 n=44, P<0.001, and fasting calcium/creatinine ratio: r 0.44, CI 0.16, 0.65 n=46, P 0.002. For women, there was significant correlation with 24h urine calcium: r 0.41, CI 0.14, 0.62, P 0.004, but not with calcium/creatinine ratio (P 0.101). There was no significant correlation in men or women between 1,25-DHCC and plasma calcium, phosphate, PTH or $TmPO_4$/GFR and hence there was no apparent explanation for increased 1,25-DHCC levels.

Plasma PTH

PTH was increased in only 8 individuals (8% of those tested) and was therefore a relatively uncommon finding. In three of the subjects, the biochemical findings were consistent with primary hyperparathyroidism. In two others who were normocalcemic and elderly (86y and 80y) the increases may have been attributable to ageing.

DNA Microsatellite Linkage Analyses

None of the LOD scores for linkage of any of the six microsatellite markers with stone formation, hypercalciuria or phosphaturia were indicative of linkage disequilibrium. This is strong, although not conclusive, evidence that in these pedigrees the genes for the CASR, SLC34A1, vitamin D receptor and NPT2 are not susceptibility loci for these abnormalities.

However, an analysis of the individual quantitative traits among all sib pairs in the study identified a possible link between fasting urinary citrate/creatinine ratio and the D5S614 marker for the *NPT2* gene with a summated LOD score of 2.54. There was also a weak positive signal with 24-hour urinary citrate and D5S614. When the analysis of linkage was restricted to the two hypocitraturic families a LOD score of 4.99 was obtained for linkage between fasting urinary citrate/creatinine ratio and D5S614. This link was supported by a weak positive signal between fasting urinary citrate/creatinine ratio and D5S1354 (LOD score 0.6). Retrospectively, it was then possible to show that all the evidence for linkage between D5S614 and fasting citrate/creatinine ratio arose from a single family (Family 12) with a LOD score of 6.56. Subsequently, we demonstrated that four other microsatellite markers located close to the *NPT2* gene were also in linkage

disequilibrium with a locus controlling fasting urinary citrate/creatinine ratio in this family. A mutation scan of the *NPT2* gene would have been the logical next step to look for an abnormality and, if confirmed, functional studies of the cloned gene. Unfortunately these investigations were not readily available at the time of the study.

Overview of the Observations for Hypercalciuria

The findings from this small study confirm that hypercalciuria is a familial abnormality in some, but not all, stone-forming kindreds, that it increases the risk for stones, and is sometimes associated with hyperoxaluria which may also be familial and, of some surprise, in men with increased urinary citrate which is believed to protect against calcium stones. From a large epidemiological study Curhan also reported, as a new and inexplicable observation, that 24h urine citrate was increased in male stone formers with a family history of stones. [18] In our families, there was no demonstrable linkage of hypercalciuria with the genes for the CASR, SLC34A1, vitamin D receptor and NPT2. Phosphaturia, as evidenced by low $TmPO_4$/GFR values, was common, but was a non-specific abnormality since the frequency was similar among normocalciuric individuals. $TmPO_4$/GFR decreased with age in men. There was no demonstrable linkage of low $TmPO_4$/GFR with the *NPT2* phosphate transporter gene. Plasma 1,25-DHCC was higher in hypercalciuric than normocalciuric subjects. This increase could not be attributed to differences in PTH or plasma phosphate.

Studies from the Stone Clinic Database

We have recently analyzed the data for stone formers investigated in the clinic from 1990 to July 2007, and now summarize the published findings from three of the investigations. [21-23] There were clearly sex differences for many parameters, particularly those related to creatinine, and we have analyzed the data for men and women separately in all of our studies.

Study 1: An Overview of the Data for the Full Clinic Cohort

We reviewed the data for the full patient cohort of 816 women and 1983 men (ratio 1:2.43). [21] Important observations were differences in the frequencies of stone risk factors between men and women, and changes with age. Relevant results are summarized in Table 4. They confirm the high familial incidence of stones and showed a significantly higher frequency of women with a first degree affected relative (24%) than men (19%). Overall, the observations are similar to those reported from other large clinics. As is well-recognized, [2, 4, 8, 25] hypercalciuria, hyperoxaluria and hyperuricosuria were more common in men. Excretion of calcium, oxalate and urate were significantly correlated. The frequency of hypocitraturia was high using our lower reference limit of 1.60 mmol/24h, derived in-house from healthy volunteers. Bias in patient selection may be partly responsible since 24h urine citrate was not measured routinely, but more often in recurrent stone formers or patients with underlying clinical disorders associated with hypocitraturia. Applying diagnostic thresholds of 2.0 and 1.7 mmol/24h, two studies reported hypocitraturia in 30% and 32.7% of recurrent stone formers. [8, 26] One large study found no difference in citrate excretion between stone formers and controls. [27] These observations make the finding of *raised* urinary citrate levels in hypercalciuric subjects in our family study even more surprising. Low values for $TmPO_4$/GFR, found frequently among our patients, are discussed below.

We compared the data for hypercalciuric and normocalciuric patients with no evident underlying clinical disorder to cause stones (‘idiopathic’ stone formers). Table 5 presents indices which were significantly different. Hypercalciuria was associated with higher excretion of oxalate and urate, but also with small increases in the 24h urinary volume. Errors in timing of the 24h collections might have over-estimated the calculated 24h oxalate and urate excretion. However, these were large patient groups and there is no reason why hypercalciuric individuals should have made mistakes more often than normocalciuric patients. The calcium *concentration* of their urine was significantly higher. Age, plasma calcium, phosphate, PTH, and $TmPO_4$/GFR did not differ significantly.

Table 4. Overview of relevant data from the stone clinic data base

Variable Median; 2.5-97.5 percentiles no.	Women		Men		Women & men compared P
Final cohort: n=2799	816 (29%)		1983 (71%)		1:2.43
Age (years)	49; 20-79	811	49; 23-77	1968	
Age at first stone episode (years)	44; 28-75	805	43;28-73	1968	
Recurrent stones	184 (23%)		663 (33%)		<0.001
No. (%) with first degree relatives with stones one relative two relatives three or more relatives total	 153 (19%) 26 (3%) 14 (2%) 193 (24%)		 314 (16%) 49 (3%) 10 (<1%) 373 (19%)		 0.004
Plasma Creatinine (µmol/l) *Ref :women 53-97µmol/l* *men 80-115µmol/l*	77; 56-116	760	93; 72-129	1787	<0.001
Phosphate: mmol/l *Ref 0.70-1.50 mmol/l*	1.02; 0.68-1.34	778	0.92; 0.60-1.29	1889	<0.001
$TmPO_4$/GFR: mmol/l *Ref: 0.80-1.35 mmol/l* No. <0.80 mmol/l No. <0.70 mmol/l	0.91; 0.54-1.48 165 (29%) 73 (13%)	563	0.82; 0.46-1.35 638 (46%) 341 (25%)	1380	<0.001 <0.001 <0.001
24h urine †	n=597		n= 1490		
Calcium: mmol/24h Ref: women 2.00-6.25mmol/24h men 2.00-7.50 mmol/24h no. above ref range	5.20; 1.62-12.27 186 (31%)	593	7.10; 2.35-14.59 646 (43%)	1486	<0.001 <0.001

Table 4. (Continued)

Variable Median; 2.5-97.5 percentiles	no.	Women	no.	Men	Women & men compared P
Oxalate: mmol/24h *Ref: < 0.50 mmol/24h* no. above ref range		0.32; 0.18-0.65 38 (7%)	564	0.40; 0.21-0.73 239 (17%)	1423	<0.001 <0.001
Uric acid: mmol/24h *Ref: women < 4.50 mmol/24h* *men < 4.80 mmol/24h* no. above ref range		3.18;1.64-6.27 23 (10%)	222	3.93; 2.01-6.90 159 (22%)	708	<0.001 <0.001
Citrate: mmol/24h *Ref: 1.52- 7.00 mmol/24h* no. below ref range		1.73; 0.29-6.25 64 (42%)	152	1.62; 0.38-4.58 172 (46%)	372	0.051 0.439
Creatinine: mmol/24h *ref: men ≥10.60 mmol/24h* *women ≥7.00 mmol/24h*		10.00; 7.20-15.41	597	15.30; 10.90-22.00	1490	<0.001
Creatinine clearance: l/24h *ref: men 135-200 l/24h* *women 120-180 l/24h*		134.0; 70.2-223.8	528	165.0; 98.0-242.0	1267	<0.001

† After excluding samples with low 24h urine creatinine and hence probably incomplete collections (men <10.60 mmol/24h, n= 175 ; women <7.00 mmol/24h, n= 80) and with no recorded 24h urine creatinine (men 318; women 139); $TmPO_4$/GFR= renal threshold of phosphate concentration. Statistically significant differences between men and women: P<0.05, Mann-Whitney U test for continuous variables; Chi square with Yate's correction or Fisher's exact test for contingency comparisons. Adapted from Walker et al. Ann Clin Biochem 2013;50: 127-39 [21] Reproduced with permission from Sage Publications Ltd.

Table 5. Comparison of data for idiopathic stone formers with hypercalciuria and normocalciuria

	MEN urine calcium > 7.50 mmol/24h Median; range[†] (n)	**MEN** urine calcium ≤ 7.50 mmol/24h Median; range (n)	P[††] high v normal	**WOMEN** urine calcium > 6.25 mmol/24h Median; range (n)	**WOMEN** urine calcium ≤ 6.25 mmol/24h Median; range (n)	P high v normal
Total in group[†††]	276	347		133	278	
Age with first stone years	41; 10-79 (276)	43; 9-78 (347)	0.035	42; 10-76 (131)	42; 9-94 (276)	0.661
Age at the clinic years	46; 20-80 (276)	48; 19-92 (347)	0.070	45; 18-76 (131)	45; 15-94 (276)	0.761
Plasma						
Calcium mmol/l	2.37; 2.13-2.54 (276)	2.37; 2.14-2.55 (347)	0.526	2.35; 2.14-2.53 (133)	2.36; 2.11-2.59 (278)	0.237
Phosphate mmol/l	0.93; 0.34-1.34 (270)	0.93; 0.52-1.46 (335)	0.747	1.00; 0.66-1.36 (128)	1.03; 0.60-1.63 (270)	0.179
PTH[††††] pmol/l	2.9; 1.0-7.3 (208)	3.0; 1.0-7.2 (267)	0.240	2.9; 1.2-7.3 (99)	3.3; 0.7-7.3 (195)	0.539
24h urine						
Calcium mmol/24h >10.00mmol/24h	9.30;7.56-19.40 (276) 101 (36%)	5.80; 2.21-7.50 (347)	<0.001	7.80; 6.30-18.60 (133) 17 (13%)	4.60; 2.00-6.24 (278)	<0.001
Calcium mmol/l	9.30;1.80-13.30 (276)	5.80;0.67-9.36 (347)	<0.001	7.80;1.50-13.86 (133)	4.60;0.70-11.29 (278)	<0.001
Oxalate mmol/24h >0.50 mmol/24h	0.41; 0.09-0.87 (272) 45 (17%)	0.38; 0.13-1.45 (338) 40 (12%)	<0.001	0.32; 0.12-0.90 (123) 11 (8%)	0.31; 0.08-1.29 (265) 12 (5%)	0.002
Urate mmol /24h Increased: m > 4.8; f >4.5 mmol/24h	3.99;1.22-10.18 (114) 28 (25%)	3.78; 1.86-8.40 (145) 23 (16%)	0.048	3.52; 1.41-7.80 (37) 8 (16%)	3.10; 1.54-7.00 (102) 8 (8%)	0.058
Citrate mmol/24h <1.60 mmol/24h	1.88; 0.44-6.00 (66) 25 (38%)	1.66; 0.36-4.95 (75) 35 (47%)	0.315	1.97; 0.68-7.00 (30) 12 (40%)	2.18; 0.30-6.98 (70) 26 (37%)	0.925
urine volume l/24h	1.95; 0.73-5.23 (272)	1.73; 0.63-5.08 (344)	<0.001	1.84; 0.76-4.75 (130)	1.69; 0.48-4.17 (278)	0.043

Table 5. (Continued)

	MEN urine calcium > 7.50 mmol/24h Median; range[†] (n)	**MEN** urine calcium ≤ 7.50 mmol/24h Median; range (n)	P[††] high v normal	**WOMEN** urine calcium > 6.25 mmol/24h Median; range (n)	**WOMEN** urine calcium ≤ 6.25 mmol/24h Median; range (n)	P high v normal
Random urine						
pH	6.20; 4.30-7.78 (258)	6.10; 4.50-8.00 (337)	0.045	6.04; 4.85-7.70 (124)	6.00; 4.50-8.00 (261)	0.990
$TmPO_4$/GFR mmol/l <0.80 mmol/l <0.70 mmol/l	0.82; 0.24-1.60 (198) 87 (44%) 43 (22%)	0.86; 0.29-1.99 (256) 99 (39%) 48 (19%)	0.112	0.91; 0.54-1.82 (88) 26 (30%) 13 (15%)	0.92; 0.25-2.12 (188) 45 (24%) 13 (7%)	0.527

[†] Median and observed ranges; [††] Mann-Whitney U-test; P <0.05 statistically significant.

[†††] 276/ 623 (44%) men & 133/411 (32%) of the women had hypercalciuria (P <0.001); [††††] Three PTH assay kits were used; only the results for the first kit used to 2001 are shown.

Table 6. Comparison of data for hypercalciuric male stone formers with filtered calcium in the lowest and highest quintiles (n = 55 per quintile)

	Lowest quintile median; 2.5-97.5 percentiles no. of observations		**Highest quintile median; 2.5-97.5 percentiles no. of observations**		**Lowest v highest quintile p**
Age of first stone (years)	44; 19-71	55	37; 19-60	55	0.012
Age at clinic (years)	52; 29-75	55	40; 24-60	55	<0.001
Positive family history	11 (20%)	55	15 (27%)	55	0.501
Recurrent stones	22 (40%)	55	19 (35%)	55	0.694
Plasma					
Ultrafiltrable calcium (mmol/l) ref 1.29-1.53	1.41; 1.30-1.49	55	1.43; 1.33-1.50	55	0.007

	Lowest quintile median; 2.5-97.5 percentiles no. of observations		**Highest quintile median; 2.5-97.5 percentiles no. of observations**		**Lowest v highest quintile p**
PTH † (pmol/l) ref <7.3	2.9; 1.3-6.7	40	2.8; 1.4-5.0	43	0.553
Urine					
Calcium (mmol/24h) ref 2.00-7.50	8.70; 7.67-12.50	55	10.50; 7.77-18.58	55	<0.001
Filtered calcium (mmol/24h)	194.8; 140.2-211.1	55	318.1; 296.0-366.4	55	<0.001
Reabsorbed calcium (mmol/24h)	184.5; 131.2-202.2	55	307.7; 285.2-357.0	55	<0.001
Oxalate (mmol/24h) ref <0.50	0.35; 0.17-0.53	54	0.45; 0.32-0.75	54	<0.001
Urate (mmol/24h) ref <4.80	3.41; 2.23-6.00	26	4.73; 3.51-6.89	20	<0.001
Creatinine (mmol/24h)	13.20; 10.87-18.16	55	19.70; 15.44-23.29	55	<0.001
Creatinine clearance (l/24h)	140.0; 96.7-154.7	55	224.1; 201.5-253.3	55	<0.001
Volume (l/24h)	2.03; 0.85-4.44	55	1.81; 1.06-3.60	55	0.490
pH	6.25; 5.13-7.44	54	6.07; 5.10-7.55	52	0.656
$TmPO_4$/GFR (mmol/l) ref 0.80-1.35	0.85; 0.60-1.14	43	0.83; 0.60-1.29	41	0.816
Stone composition	**n = 30**		**n = 25**		
calcium oxalate	4 (13%)		5 (20%)		0.717
calcium oxalate phosphate	24 (80%)		17 (68%)		0.363

PTH parathyroid hormone; † three assay kits for PTH were used with differing sensitivities; only the results obtained up to December 2001 using the first assay kit are shown. Statistical comparisons: Mann-Whitney U test for continuous variables; Fisher's exact test for contingency comparisons; $P < 0.05$ statistically significant. Adapted from Walker et al: J Clin Pathol 2014; 67(4): 355-360.[22] Reproduced with permission from BMJ Publishing Group Ltd.

Table 7. Data for hyperuricosuric male stone formers: all 30 with filtered urate below the median for normouricosuria ('low filtered urate') compared with the 30 with the highest filtration ('high filtered urate')

	Hyperuricosuria† Low filtered urate median; 2.5-97.5 percentiles	n	Hyperuricosuria High filtered urate median; 2.5-97.5 percentiles	n	Low v. high filtered urate P
Age of first stone years	37; 15-69	30	41; 16-61	29	0.617
Age at clinic years	45; 27-70	30	47; 26-62	30	0.469
First degree relative	6 (20%)	30	2 (7%)	30	0.254
Recurrent stones	14 (47%)	30	13 (43%)	30	1.000
eGFR ml/min/m^2	80; 51-110	30	85; 62-123	30	0.141
Plasma					
Urate mmol/l	0.29; 0.17-0.43	30	0.46; 0.37-0.59	30	<0.001
Calcium mmol/l	2.35; 2.22-2.57	30	2.39; 2.30-2.51	29	0.097
Creatinine µmol/l	96; 74-136	30	91; 70-116	30	0.169
Biochemical risk factors					
Urine urate mmol/24h	5.60; 4.89-8.53	30	5.84; 4.90-8.29	30	0.171
Filtered urate mmol/24h	47.4; 29.3-51.6	30	96.7; 85.2-115.7	30	<0.001
Percentage of urate reabsorbed	87.0; 74.5-90.3	30	93.6; 91.7-95.6	30	<0.001
Urine oxalate mmol/24h Urine oxalate >0.50 mmol/24h	0.42; 0.23-0.94 7 (25%)	28	0.53; 0.31-0.71 15(56%)	27	0.014 0.029
Urine calcium mmol/24h Urine calcium >7.50 mmol/24h	8.17; 4.45-15.54 20 (67%)	30	7.79; 3.29-16.51 15 (50%)	30	0.554 0.295
Urine volume l/24h	1.84; 0.71-3.50	30	1.80; 1.24-3.85	30	0.965
Random urine pH	5.99; 5.00-7.52	28	5.64; 4.92-7.17	28	0.206
$TmPO_4$/GFR mmol/l†† <0.70 mmol/L	0.78; 0.50-1.00 8 (40%)	20	0.94; 0.64-1.21 4 (22%)	18	0.019 0.307
Creatinine clearance l/24h >200 l/24h	154.5; 112.2-225.5 5 (17%)	30	219.5; 157.3-266.6 22 (73%)	30	<0.001 <0.001

† Hyperuricosuria: urinary urate >4.80 mmol/24h; ††$TmPO_4$/GFR renal threshold of phosphate concentration; Statistical comparisons: Mann-Whitney U test for continuous variables; Fisher's exact test for contingency comparisons; $P < 0.05$ statistically significant. Adapted from Walker et al., Urolithiasis 2014; 42 (4) 291-300 [23]. Reproduced with kind permission from Springer Science + Business Media.

Phosphaturia

Our clinic is unusual in that we estimate $TmPO_4$ /GFR. This investigation is seldom included in front-line clinic screening protocols for stone risk factors. After excluding patients with abnormal PTH concentrations, $TmPO_4$/GFR of 44% of men and of 24% of women was below a reference limit of 0.80mmol/l [24] ($P<0.001$), and of 22% of men and 8% of women was < 0.70mmol/l ($P <0.001$). The incidence was no higher in hypercalciuric than normocalciuric stone formers, which is in keeping with the lack of an association with hypercalciuria observed in our family study. Rendina *et al.* identified a sub-set of stone formers with evidence of a renal phosphate leak on the basis of fasting levels of plasma phosphate below the reference range, $TmPO_4$/GFR <0.70 mmol/l and a normal plasma PTH concentration. [28] In our cohort, 6% of men and 1.6% of women had similar biochemical abnormalities and probably fell within this category, although the samples were usually non-fasting. Compared with stone formers with normal PTH, plasma phosphate and $TmPO_4$/GFR >0.80 mmol/l, the men with a phosphate leak were older and had higher plasma creatinine and lower plasma calcium concentrations (2.35; 2.20-2.50 mmol/l, n = 72; controls 2.38; 2.22-2.54 mmol/l, n = 618; P = 0.001). In a similar comparison for women, the seven with a phosphate leak had lower plasma bicarbonate concentrations (median 25 range 23-28 mmol/l, n = 7; controls 28, range 23-34 mmol/l, n = 303; P = 0.001), and more often had a first degree relative with stones (71% versus 28% P = 0.008). There were no other significant differences. It is interesting that in our family study, women with low TmPO4/GFR also had a small decrease in plasma bicarbonate.

Data for phosphaturia among stone formers are sparse. [8] Phosphaturia cannot be diagnosed from measurement of 24h urine phosphate, unless this is undertaken during metabolic balance studies, since this largely reflects dietary phosphate intake. One study reported lower $TmPO_4$/GFR in calcium stone formers with normal PTH compared with normal subjects, [29] and another found evidence of a phosphate leak (see above) in 20% of stone formers. [28] The explanation for the high frequency of phosphaturia found in stone patients is unknown. In our clinic, collection of non-fasting samples may have inflated the incidence. Among patients with low $TmPO_4$/GFR values, Prié *et al.* identified two with mutations of the gene for NPT2a, one with renal stones and the other with osteoporosis, and in seven other phosphaturic patients, found mutations of the *NHERF-1* gene (see below). [30, 31] Activity of NPT2a is controlled by PTH, by pH (activity is decreased at low pH) and by fibroblast growth factor-23 (FGF23). FGF23 is a phosphaturic hormone produced in

bone which binds to the apical membrane of the proximal tubules with a renally-produced protein, klotho, and suppresses NPT2 activity. [32] Rare mutations of the *FGF23* gene cause phosphaturia, [32] but we are not aware of any reports linking gene polymorphisms with stones. A polymorphism of the klotho gene has been associated with an increased risk for renal stones. [33] *NPT2a* and *NHERF-1* mutations appear to occur too rarely to explain the high frequency of phosphaturia in stone formers, and it seems more likely that this abnormality is a response to some other biochemical disturbance, possibly of H^+ secretion.

Study 2: Investigation of Male Clinic Patients with Idiopathic Hypercalciuria [22]

In this data base study, our first objective was to segregate male stone formers with idiopathic hypercalciuria due to impaired renal tubular handling of calcium (renal hypercalciuria) from those filtering increased amounts of calcium into the kidneys. This distinction will be fundamental for future meaningful genetic studies. Our second aim was to find out whether associated risk factors for stone production differed between these two groups of men.

Study 2 (a) Identification of Male Stone Formers with Renal Hypercalciuria

Because we wanted to minimize the effects of variations in plasma calcium levels on the amount of calcium delivered to the kidney, we selected men from the data base who had normal levels of plasma ultrafiltrable calcium, which we estimated as 60% of total plasma calcium adjusted for albumin. We obtained this figure from reported observations in experimental animals, and healthy adult volunteers and stone formers. [11, 34, 35] Measurement of ultrafiltrable calcium of large numbers of clinic patients is not feasible, and measurement of ionized calcium excludes complexed calcium. With normal plasma PTH and bicarbonate levels and good renal function errors will be very small.

Men were included if they had idiopathic calcium stones, no disorder or drug treatment associated with abnormalities of ionized plasma calcium or calcium excretion, did not have diabetes mellitus or gout, had two well-functioning kidneys with normal plasma creatinine and bicarbonate, estimated GFR (eGFR) $\geq$ 60 ml/ min/ 1.73 m^2 and no structural abnormality of the urinary tract, if their 24h urine sample was probably complete (creatinine $\geq$

10.60 mmol/24h), [27] and urine calcium was >2.00 mmol/24h. We estimated the amount of calcium filtered daily as ultrafiltrable plasma calcium x creatinine clearance, which we used as an approximation for glomerular filtration rate (GFR). The amount of calcium reabsorbed per 24h was the difference between filtered calcium and 24h urine calcium. The final study cohort of 623 men comprised 276 (44%) with hypercalciuria and 347 with normocalciuria. They had similar plasma concentrations of ultrafiltrable calcium (P 0.598). At each level of filtered calcium there was wide inter-individual variation in the percentage of filtered calcium which was reabsorbed (Figure 2). As predicted from the calculation, for any given amount of filtered calcium, hypercalciuric men reabsorbed less calcium than normocalciuric men—a new observation for a clinic population, which is only demonstrable by relating filtered calcium to excreted calcium. This observation implies that reduced renal tubular reabsorption is a common abnormality in idiopathic hypercalciuria. Others have found that renal calcium reabsorption is reduced in small groups of carefully selected stone formers with idiopathic hypercalciuria [7, 11, 36, 37] Ninety three (34%) of the hypercalciuric men had values for filtered calcium below the median for men with normocalciuria. It was inferred that reduced reabsorption of filtered calcium was the dominant abnormality in these men.

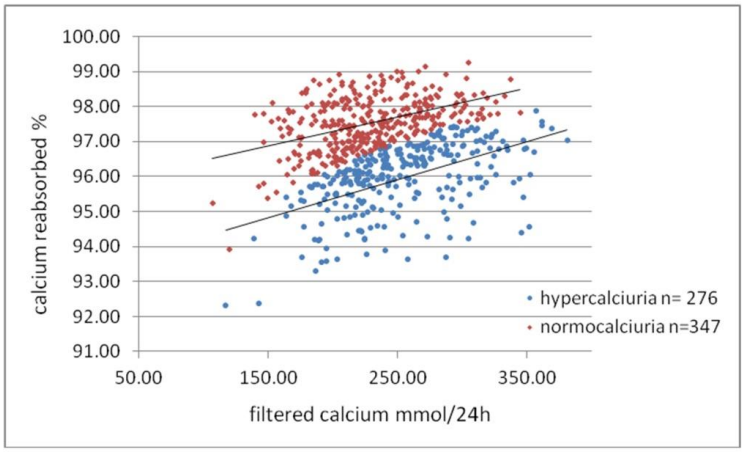

Figure 2. Correlation between filtered calcium and percentage reabsorbed by 623 male stone formers with idiopathic calcium stones. Spearman's coefficient r (95% CI): for hypercalciuric men: r 0.56, CI 0.47, 0.52, P <0.001; for normocalciuric men r 0.43, CI 0.34, 0.52, P < 0.001. Walker et al. 2014, J Clin Pathol 67 (4): 355-360[22]. Figure reproduced with permission from BMJ Publishing Group Ltd.

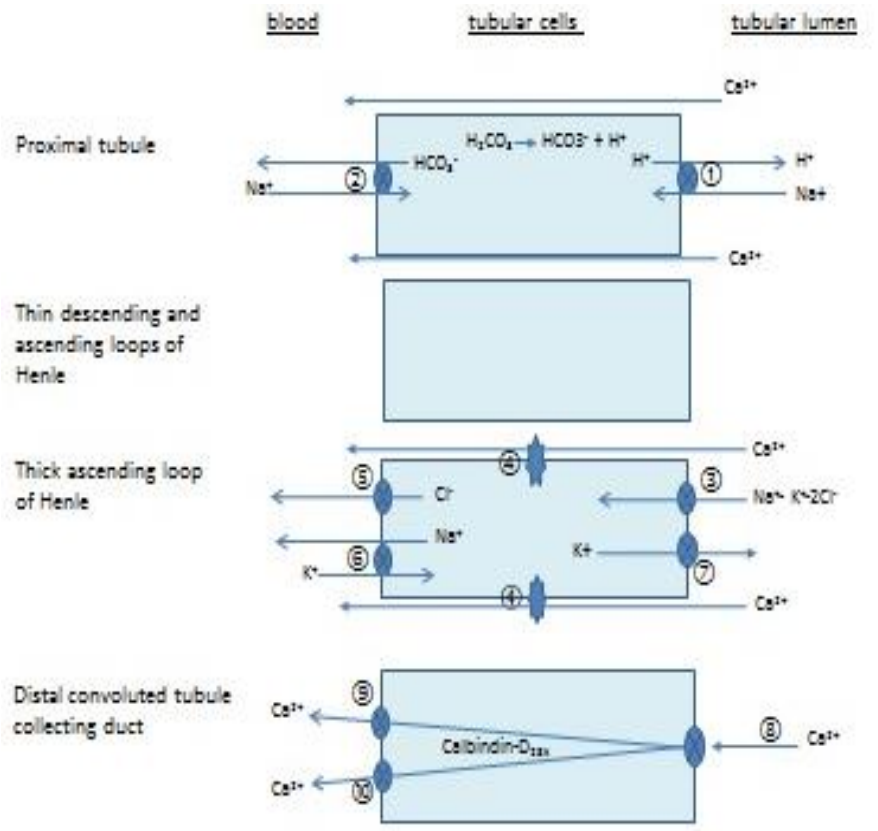

Figure 3. Reabsorption of filtered calcium by the renal tubules. Approximately 65% is reabsorbed in the proximal tubule by the paracellular route, none in the thin descending and ascending loops of Henle, around 25% by the paracellular route in the thick ascending loop of Henle, and 8-10% by trans-cellular transport in the distal nephron. Key:
1 NHE3 (Na^+/H^+ antiporter)*
2 NBCe1 ($HCO3^-$ Na^+ exchanger)*
3 NKCC2 (NA-K-Cl cotransporter 2)*
4 Claudin-16 (paracellin-1)
5 Chloride channel CLC-Kb
6 Na^+-K^+-ATPase
7 ROMK (ATP-sensitive inwardly rectifying K channel)
8 TRPV5 (transient receptor potential vanilloid member 5 protein)
9 PMCA (plasma membrane Ca^{2+} ATPase)
10 NCX (Na^+ / Ca^{2+} exchanger)
* These transporters are also involved in H^+ secretion and NH_3 buffering

Study 2 (b) Comparison of Stone Risk Factors of Men with Hypercalciuria due to Reduced Renal Reabsorption of Calcium and Those with a High Filtered Load

We then compared stone risk factors of the 55 hypercalciuric men in the lowest filtration quintile with the 55 in the highest (Table 6). The recurrence rate for stones and incidence of a first degree relative with stones were high, and similar, in both groups. The men with high calcium filtration reabsorbed a very large proportion of the filtered load but not enough to achieve a normal 24h urinary calcium excretion. Compared with men in the lowest quintile, they had higher 24h urine calcium, oxalate and urate excretion and, of greater significance for stone risk, higher urinary concentrations per liter of calcium, oxalate and urate. These increases could account for their younger age at their first stone episode and at the time of referral to the clinic. The incidence of phosphaturia was similar and high in both groups, and plasma PTH and urine pH were not significantly different.

Mechanism of Decreased Renal Reabsorption of Calcium

Genetic variation in the renal handling of calcium seems the most likely explanation for decreased calcium reabsorption in hypercalciuria. Because of the stringent selection criteria for this study, other possible causes [38, 39] were considered to be highly unlikely, although we cannot exclude a high dietary sodium consumption [8, 39] since urinary sodium excretion was not recorded on the database. A familial risk for stones has been well documented. [4, 7, 15, 18, 19] One study reported a frequency of 16.8% of a first degree affected relative of men and 22.7% of women with stones compared with 6% of controls. [40] There was a high incidence of patients with first degree relatives with stones in our cohort, and heritability was clearly evident in our family study. Our findings in the family study strongly support inheritance of hypercalciuria.

Calcium Reabsorption by the Renal Tubules

Reabsorption of calcium by the renal tubules is now known to occur through the highly co-ordinated activities of a growing number of identified renal transport processes. [4, 12, 13, 15, 41] Others will almost certainly be discovered. Calcium reabsorption is closely integrated with the transport of H^+ ions, phosphate and other anions and some transporters are structurally associated through binding to common intracellular scaffolding proteins. [42, 43] Figure 3 presents a simplified overview. Briefly, circulating ionized and

complexed calcium is filtered into the kidneys and normally around 98% is subsequently reabsorbed back into the blood. Most (around 65%) is reabsorbed in the proximal tubules, none in the thin ascending and descending loops of Henle, around 25% in the thick ascending limb of loop of Henle (TAL) and 8-10% in the distal convoluted tubule and collecting duct. It is in the distal nephron that calcium reabsorption is finely controlled by PTH and 1,25-DHCC. Calcium sensing receptors (CASR), located on the apical membranes of the cells lining the proximal tubules and inner medullary collecting duct, and the basolateral membranes of cells in the TAL and the distal nephron, monitor luminal and blood calcium levels and regulate calcium reabsorption. In the proximal tubule most calcium is reabsorbed by the paracellular route driven by the lumen positive potential difference generated by the Na^+/H^+ antiporter NHE3 which is the principal sodium proximal tubular transporter. Absorption from the TAL similarly is paracellular, in this case driven by a lumen positive voltage generated by the bumetamide-sensitive Na^+-K^+-Cl^- cotransporter (NKCC2). Intracellular Na^+ and Cl^- concentrations are maintained by the activities of Na^+/K^+-ATPase and the chloride channel CLC-Kb. K^+ is transported back into the tubular lumen by the ATP-sensitive inwardly rectifying potassium (ROMK) channel creating the voltage which drives Ca^{2+} into the blood through claudin-16 (paracellin -1) tight junctions. In the distal nephron calcium is actively reabsorbed. It enters the cells by the transient receptor potential vanilloid member 5 protein (TRPV5) channel, is transported through the cells bound to calbindin-D_{28k} and exported via the Na^+/Ca^{2+} exchanger (NCX) and plasma membrane Ca^{2+} ATPase (PMCA). [12, 44]

Genetic Abnormalities which Cause Hypercalciuria

Hypercalciuria, nephrocalcinosis and nephrolithiasis are features of some inherited monogenic disorders, [4, 12, 13, 15, 41] but these are exceptionally rare and not likely to be encountered in a stone clinic. However, common polymorphisms of these and other so far unidentified genes which have a small variable effect on transporter function probably account for the inherited variation in calcium excretion in the general population. The profile of activities of the ‘package’ of transporter genes inherited will determine the susceptibility to hypercalciuria perhaps through a threshold effect. Co-inheritance of genes with polymorphisms associated with excretion of other stone risk factors, for example phosphate, oxalate or urate, will add to the genetic risk for stone formation. The search for polymorphisms of familiar renal calcium transporters has been unproductive so far. Two studies found a

potential susceptibility locus near the vitamin D receptor gene but others found no evidence of linkage. An Italian study found a gain of function polymorphism of the CASR gene in women which was associated with an increased risk of hypercalciuria, but other studies reported negative findings, and others found no evidence of an association with the 1α-cholecalciferol hydroxylase gene. [4, 12, 13, 15, 41] In one large kindred there was linkage of absorptive hypercalciuria with the soluble adenyl cyclase gene, but the mechanism by which an abnormality of this enzyme would increase calcium excretion is unknown. [45] In our family study, we found no evidence of linkage of the genes for the CASR, thiazide-sensitive sodium chloride cotransporter, vitamin D receptor and NPT2 sodium-phosphate cotransporter-2 with hypercalciuria or phosphaturia. The linkage of NPT2 to fasting hypocitraturia in one kindred, indicates possible interaction of this pH dependent gene with renal citrate metabolism, which may be via H^+ secretion. An exciting fairly recent development has been the discovery of NHERF-1 and NHERF-2 which are members of a protein family called the Sodium-Hydrogen-Exchanger Regulatory Factor. They contain tandem domains (PDZ domains) which are recognized by a range of proteins. In rat and mouse kidney, NHERF-1 is highly expressed in the renal proximal tubule. It binds to a range of proteins which include NHE3, NPT2, the sodium-bicarbonate transporter and the PTH receptor. Binding with NHERF-1 targets NPT2 to the apical membrane and is essential for its activity, inhibits NHE3 and initiates degradation of the PTH receptor. NHERF-1 is estrogen-inducible. NHERF-1 knock-out mice have phosphaturia, hypercalciuria, hyperuricosuria, raised 1,25-DHCC and a tendency to low PTH. NHERF-2 is localized principally in the distal nephron and binds to TRPV5, the outer medullary potassium channel and H^+-ATPase. [42, 46-48] In our family study, hypercalciuria was significantly associated with raised 1,25-DHCC levels which could not be explained by hypophosphatemia or increases in plasma PTH and which might be consistent with an abnormality of NHERF-1.

Mechanisms Causing Hyperfiltration of Calcium

Higher calcium filtration of men in the top quintile was explained largely by their higher creatinine clearance which was above an upper reference limit of 200l/24h in 54 (98%) of cases. Inaccurately timed collections might have accounted for this, [49] but was an unlikely explanation for the group as a whole. [22]

Raised creatinine clearance was observed in one other study of hypercalciuric male recurrent stone formers on a free choice diet. Compared

with normocalciuric recurrent stone formers and controls, they had increased renal mass and 24h urinary creatinine excretion and a higher ratio of plasma 1,25-DHCC to PTH which is an index of regulation of 1,25-DHCC production. [50] In another study of stone formers with idiopathic hypercalciuria, creatinine clearance decreased after 15 days of dietary protein restriction, but not significantly. There was a significant decrease in the ratio of plasma 1,25-DHCC to PTH. [51] There is a large body of evidence to show that high intakes of protein and amino acids increase GFR in animals and humans, whether this is measured by creatinine clearance or by other techniques which avoid interference from dietary creatine. [52-57] In short-term studies of healthy humans ($\leq$ 3 weeks) the increase in GFR ranged from negligible to 20%. The increase was greater on diets maintained for months or years. [57] Acute protein loading causes a rapid increase in GFR which, in one study, reached a peak by 2.5h. [54] This has been associated with changes in intra-renal perfusion, [55] and in animal models, high protein feeding causes parallel changes in GFR and renal plasma flow indicating a hemodynamic response. In rats, renal size increases due to enlargement of both the renal tubules and glomeruli. [57] Despite extensive investigation, the mechanisms which cause the changes in intra-renal blood flow are still unclear. It has been proposed that increased production of the vasodilator nitric oxide (NO) from L-arginine in the kidneys may be responsible. [56, 57] Infusion of L-arginine or amino acid mixtures into isolated perfused kidneys increases renal blood flow, [57] and administration of an inhibitor of NO synthesis, N^{G}-monomethyl-L-arginine, via the renal artery into rats infused with amino acids significantly reduced the GFR and renal plasma flow. The NO hypothesis is attractive in view of our increased understanding of the processes which link dietary protein ingestion with renal NO synthesis. [58] In the kidneys, NO is produced by the arginine-citrulline–nitric oxide cycle. [59] The arginine substrate for this cycle derives mainly from citrulline which is synthesized in the small intestine from ingested amino acids and from endogenously produced glutamine. Endogenous glutamine production is increased after protein ingestion. Citrulline is transported from the intestine to the kidneys where it is converted to arginine and exported to the circulation or metabolized via the arginine-citrulline–nitric oxide cycle. High protein consumption could account for the high creatinine clearance values observed in our study through a true increase in GFR, exaggerated by a spurious increase due to a high creative intake from meat, [49] and hemodynamic changes within the kidney might have reduced the renal reabsorption of calcium. There is now a large body of evidence that high protein consumption increases urinary calcium

excretion, [51, 60, and 61] and reduced renal absorption may be a contributory factor. However, this is speculative since, although discussed with the patients, protein intakes were not calculated. Dietary excesses together with increased GFR might also explain the higher oxalate and urate excretion in this group. [3, 8, 50] These abnormalities are frequently associated with a high protein intake. [51] Dietary protein restriction for 15 days led to significant decreases in urinary excretion of calcium, urate and oxalate. [51]

Study 3. Comparison of Risk Factors of Hyperuricosuric Male Stone Formers with High and Low Filtered Urate [23]

Using a similar approach to study 2 for hypercalciuric men, we investigated 153 male idiopathic stone formers who had hyperuricosuria. [23] As for hypercalciuria, there was a wide inter-individual variation in the net reabsorption of urate (the difference between the amounts of urate filtered and excreted per 24h) at each level of filtered urate, and the net percentage of urate reabsorbed was lower than for normouricosuric men (Figure 4). These findings indicate that a reduced capacity for net urate reabsorption is a common underlying abnormality in hyperuricosuria.

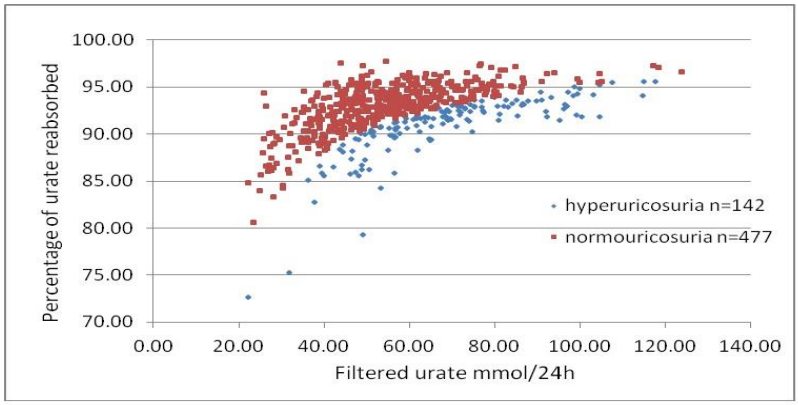

Figure 4. Correlation between filtered urate and net percentage reabsorption by 142 hyperuricosuric male stone formers with 477 normouricosuric stone formers after excluding those with clinical disorders or medications known to alter urate turnover. Spearman's coefficient r (95% CI): for hyperuricosuric men: r 0.89, CI 0.85, 0.92, P <0.001; for normouricosuric men r 0.70, CI 0.65, 0.75, P < 0.001. From Walker et al. 2014, Urolithiasis 42 (4) 291-300 [23]. Figure reproduced with kind permission of Springer Science + Business Media.

Thirty (21%) of the hyperuricosuric men had values for filtered urate which were below the median value for 477 male stone formers with normouricosuria. They were identified as a group whose hyperuricosuria was likely to be due to reduced net renal reabsorption of urate and were designated as low filtrators. In Table 7 their risk factors for stones are compared with those of the 30 men with the highest urate filtration ('high filtrators'). In both groups there was a high incidence of stone recurrence. Only 7% of high filtrators had a first degree relative with stones compared with 20% of low filtrators, but the difference was not statistically significant. In contrast to the findings in study 2 for hypercalciuria, there were no differences in age at the first stone episode or clinic assessment. However, there were differences in their factors for stone risk. High filtrators had higher urinary oxalate excretion, and low filtrators had lower $TmPO_4$ /GFR values. Calcium and urate excretion and urine pH were similar. Higher filtration was explained both by higher plasma urate concentrations and higher creatinine clearance. This was > 200l/ 24h in 75% of men. Most of the large excess of filtered urate was reabsorbed and 24h urate excretion of high filtrators was no higher than of low filtrators.

Mechanisms of Decreased Net Renal Reabsorption of Urate

Net renal urate reabsorption is decreased by medications, an expansion of plasma volume and genetic deficiencies of transport proteins for urate in the kidneys. We excluded patients taking uricosuric drugs, few patients had significantly impaired renal function (eGFR < 60 ml/min/m^2) and none had recorded inappropriate antidiuretic hormone secretion. [62, 63]

Renal Urate Excretion

Almost all the urate excreted in urine is produced endogenously as an end product of purine metabolism. It circulates in the blood as sodium urate which is filtered freely into the kidneys. In the proximal tubules, most of the filtered urate is reabsorbed into the blood but some is also secreted into the tubules. These processes occur in the proximal tubules. Normally around 8–12 % of the filtered load is finally excreted in the urine. [62, 64, 65] Urate reabsorption and secretion involves complex interaction between several transporters. Some have been identified and characterized. URAT1 is the major apical membrane transporter which reabsorbs urate. It is functionally coupled to two lactate transporters. The organic anion exchanger OAT4 also contributes to urate reabsorption. GLUT9 (isoform 1; coded by the *SLC2A9* gene) is thought to be the major urate transporter in the basolateral membrane which transfers urate into the blood and interstitial fluid. Another 10 or more proteins may also be

involved in urate transport, in some cases coupled with transport of other anions including phosphate, oxalate, lactate and sulfate. [43, 66-68] Some, including URAT1 are tethered to a scaffolding protein, PDZK1, in the proximal tubules together with other transporters and these may function as a molecular complex. [43] So far, only two rare inherited deficiencies of renal urate transporters are known to cause heavy hyperuricosuria, in both cases with very low plasma urate levels: in URAT1 deficiency urate reabsorption from the renal filtrate is severely impaired; in GLUT 9 deficiency transport of urate from the proximal tubules into the renal interstitium is grossly reduced. [43, 64, 66, 69, 70] It is unlikely that any of our patients had these defects. As yet, no gene polymorphisms have been identified which are associated with hyperuricosuria. Variants of the *SLC2A9* gene associated with low fractional excretion of urate and gout have been reported. [65] There are numerous theoretical possibilities for interaction of transporters. For example, changes in ionization of anions (possibly triggered by abnormal H^+ secretion) might have a secondary effect on urate transport. Primary hyperuricosuria is likely to be polygenic in the majority of cases and due to the summation of inherited gene polymorphisms which individually have a weak effect.

Mechanisms Causing Hyperfiltration of Urate

Hyperfiltration in our study groups was explained by increased creatinine clearance and, in contrast to the hypercalciuric men in study 2, also to an increase in plasma urate. There was no evidence that the increased creatinine clearance was due to inaccurate urine collection in the hyperfiltration group as a whole. [23] A high protein intake could explain the increased GFR, and hemodynamic changes within the kidney might also reduce the renal reabsorption of urate (see the discussion above for hypercalciuric men). It could also explain the increased urinary oxalate excretion observed in the high filtrators. In one study, dietary protein restriction for 15 days reduced urate and oxalate excretion significantly, as well as decreasing calcium excretion. [51] The raised plasma urate levels of high urate filtrators compared with low filtrators may be indicative of a higher dietary intake of animal protein.

Conclusion

Kidney stones are pathological products. They indicate that there is a problem somewhere in the handling and/ or excretion of minerals or anions but

rarely point to the cause. Hypercalciuria or hyperuricosuria are clues to the site of the primary problem but cannot identify it since they are merely biochemical end-points which may have multiple origins. In the majority of cases they appear to arise from a mis-match between the diet and the genetic constitution of an individual. Mineral absorption and excretion involves the interaction of numerous proteins, and polymorphisms of their genes will modify their activity. Family and population studies show that the inherited factors contribute around 50% to the risk for hypercalciuria, and that inheritance is polygenic. The capacity of an individual to handle minerals will depend upon the combined activities of the 'package' of genes inherited and their protein products. This in turn will determine dietary tolerance. With low capacity, hypercalciuria will develop even on a standard diet. The risk will increase with increasing dietary excesses. The escalation in stone formation world-wide is almost certainly a reflection of dietary changes. From our own studies we suggest that a higher protein intake may be an important contributor. With the dramatic recent developments in our understanding of mineral and organic anion turnover, and in technology enabling rapid through-put DNA analysis, we now have the opportunity to get to the root of the problems. However, it will be fundamental to define the phenotypes of stone formers as precisely as possible if the genetic analyses are to be fruitful. Stone formers could prove to be an exciting group for elucidating renal tubular transport mechanisms. Better understanding must surely expand the therapeutic options to prevent stone recurrence, which are currently very limited and have not progressed for decades.

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Ethics: The Southampton family and data base studies were approved by the local ethics committee, now the Southampton & South West Hampshire Research Ethics Committee.

Competing interests: Neither author has competing interests to declare

References

[1] Coe FL, Evan A, Worcester E. Kidney stone disease. *J Clin Invest* 2005; 115: 2598-608.

[2] Hughes P. Kidney stones epidemiology. *Nephrology* 2007; 12: S26-S30.
[3] Robertson WG. *Urinary tract calculi.* In: Nordin BEC, Need AG, Morris HA, eds. Metabolic Bone and Stone Disease. Edinburgh: Churchill Livingstone, 1993: 249-311.
[4] Sayer JA. The genetics of nephrolithiasis. *Nephron Exp Nephrol* 2008; 110: e37-e43.
[5] Pearle MS, Calhoun EA, Curhan GC. *Urolithiasis.* In: Litwin MS, Saigal CS, eds. Urologic Diseases in America. Washington, DC: US Government Publishing Office, 2004; NIH No. 04-5512: pp 3-39.
[6] Tiselius H-G. Who forms stones and why? *European Urology Supplements* 2011;10: 408-14.
[7] Coe FL, Favus MJ. *Neprolithiasis*. In: Brenner BM, Rector FC, eds. The Kidney, 4th edn. Philadelphia: WB Saunders Company, 1991; vol 2: 1728-67.
[8] Tiselius H-G. *Aetiological factors in stone formation.* In: Davison AM, Cameron JS, Grünfeld J-P, et al. eds. Oxford Textbook of Clinical Nephrology 3rd Ed. Oxford: Oxford University Press, 2005: 1199-1223.
[9] Sakhaee K, Maalouf NM, Sinnott B. Kidney stones 2012: pathogenesis, diagnosis, and management. *J Clin Endocrinol Metab* 2012; 97: 1847-60.
[10] Levy FL, Adams-Huet B, Pak CYC. Ambulatory evaluation of nephrolithiasis: an update of a 1980 protocol. Am J Med 1995; 98: 50-9.
[11] Worcester EM, Gillen DL, Evan AP et al. Evidence that postprandial reduction of renal calcium reabsorption mediates hypercalciuria of patients with calcium nephrolithiasis. *Am J Physiol Renal Physiol* 2007; 292: F66-75.
[12] Frick KK, Bushinsky DA. Molecular mechanisms of primary hypercalciuria. *J Am Soc Nephrol* 2003; 14: 1082-95.
[13] Worcester EM, Coe FL. New insights into the pathogenesis of idiopathic hypercalciuria. *Semin Nephrol* 2008; 28: 120-32.
[14] Pearle MS, Roehrborn CG, Pak CY. Meta-analysis of randomized trials for medical prevention of calcium oxalate nephrolithiasis. *J Endourol* 1999; 13: 679-85.
[15] Moe OW, Bonny O. Genetic hypercalciuria. *J Am Soc Nephrol* 2005; 16: 729-45.
[16] Coe FL, Parks JH, Moore ES. Familial idiopathic hypercalciuria. *N Engl J Med* 1979; 300: 337-40.
[17] Pak CYC, McGuire J, Peterson R et al. Familial absorptive hypercalciuria in a large kindred. *J Urol* 1981; 126: 717-19.

[18] Curhan GC, Willett WC, Rimm EB, et al. Family history and risk of kidney stones. *J Am Soc Nephrol* 1997; 8: 1568-73.

[19] Griffin DG. A review of the heritability of idiopathic nephrolithiasis. *J Clin Pathol* 2004; 57: 793-6.

[20] Stechman MJ, Loh NY, Thakker RV. Genetics of hypercalciuric nephrolithiasis: renal stone disease. *Ann NY Acad Sci* 2007; 1116: 461-84.

[21] Walker V, Stansbridge EM, Griffin DG. Demography and Biochemistry of 2800 patients from a renal stones clinic. *Ann Clin Biochem* 2013; 50: 127-39.

[22] Walker V, Cook P, Griffin DG. Male hypercalciuric stone formers with low renal calcium reabsorption. *J Clin Pathol* 2014; 67: 355–60.

[23] Walker V, Cook P, Griffin DG. Characterisation of risk factors for stones in hyperuricosuric men attending a stone clinic. *Urolithiasis* 2014; 42: 291-300.

[24] Walton RJ, Bijvoet OLM. Nomogram for derivation of renal threshold phosphate concentration. *Lancet* 1975; 2: 309-10.

[25] Hess B, Hasler-Strub U, Ackermann D, et al. Metabolic evaluation of patients with recurrent idiopathic calcium nephrolithiasis. *Nephrol Dial Transplant* 1997; 12: 1362-8.

[26] Mossetti G, Vuotto P, Rendina D, et al. Association between vitamin D receptor gene polymorphisms and tubular citrate handling in calcium nephrolithiasis. *J Internal Med* 2003; 253: 194-200.

[27] Curhan GC, Willett WC, Speizer FE, et al. Twenty-four-hour urine chemistries and the risk of kidney stones among women and men. *Kidney International* 2001; 59: 2290-8.

[28] Rendina D, Mossetti G, De Filippo G, et al. Fibroblast growth factor 23 is increased in calcium nephrolithiasis with hypophosphatemia and renal phosphate leak. *J Clin Endocrinol and Metab* 2006; 91: 959-63.

[29] Prié D, Ravery V, Boccon-Gibod L, et al. Frequency of renal phosphate leak among patients with calcium nephrolithiasis. *Kidney Int* 2001; 60: 272-6.

[30] Prié D, Huart V, Bakouh N et al. Nephrolithiasis and osteoporosis associated with hypophosphatemia caused by mutations in the type 2a sodium-phosphate cotransporter. *N Engl J Med* 2002; 347: 983-91.

[31] Karim Z, Gérard B, Bakhou N, et al. NHERF1 mutations and responsiveness of renal parathyroid hormone. *N Engl J Med* 2008; 359: 1128-35.

[32] Prié D, Ureña Torres P, Friedlander G. Latest findings in phosphate homeostasis. *Kidney International* 2009; 75: 882-9.

[33] Telci D, Dogan AU, Ozbek E et al. KLOTHO gene polymorphism of G395A is associated with kidney stones. *Am J Nephrol* 2011; 33: 337-43.

[34] Schuette SA, Zemel MB, Linkswiler HM. Studies on the mechanism of protein-induced hypercalciuria in older men and women. *J Nutr* 1980; 110: 305-15.

[35] Suki WN, Rouse D. *Renal transport of calcium, magnesium and phosphorus.* In: Brenner BM, Rector FC, eds. The Kidney, 4th edn. Philadelphia: WB Saunders Company, 1991; vol 1: 380-423.

[36] Edwards NA, Hodgkinson A. Studies of renal function in patients with idiopathic hypercalciuria. *Clin Sci* 1965; 29: 327-38.

[37] Peacock M, Nordin BEC. Tubular reabsorption of calcium in normal and hypercalciuric subjects. *J Clin Pathol* 1968; 21: 353-8.

[38] Moe OW, Huang C-L. Hypercalciuria from acid load: renal mechanisms. *J Nephrol* 2006; 19 Suppl 9: S53-61.

[39] Borghi L, Schianchi T, Meschi T et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med* 2002; 346: 77-84.

[40] Lavan JN, Neale FC, Posen S. Urinary calculi: Clinical, biochemical and radiological studies in 619 patients. Med J Aust 1971; 2 : 1049-61

[41] Eggerman T. Review: genetics of urolithiasis European Medical Journal 2014. emjreviews.com/wp-content/uploads/Review-Genetics-of-Urolithiasis (accessed 29 12 2014).

[42] Weinman EJ, Mohanlal V, Stoycheff N et al. Longitudinal study of urinary excretion of phosphate, calcium, and uric acid in mutant NHERF-1 null mice. *Am J Physiol Renal Physiol;* 290; F838-43.

[43] Anzai N, Jutabha P, Amonpatumrat-Takahashi S, et al. Recent advances in renal urate transport: characterization of candidate transporters indicated by genome-wide association studies. *Clin Exp Nephrol* 2012; 16: 89–95.

[44] Sayer JA, Carr G, Simmons NL. Nephrocalcinosis: molecular insights into calcium precipitation within the kidney. *Clin Sci* 2004; 106: 549-61.

[45] Reed BY, Gitomer WL, Heller HJ et al. Identification and characterization of a gene with base substitutions associated with the absorptive hypercalciuria phenotype and low spinal bone density. *J Clin Endocrinol Metab 2002*; 87: 1476-85.

[46] Shenolikar S, Voltz JW, Minkoff CM et al. Targeted disruption of the mouse NHERF-1 gene promotes internalization of proximal tubule sodium-phosphate cotransporter type IIa and renal phosphate wasting. *PNAS* 2002; 99: 11470-5.

[47] Weinman EJ, Cunningham R, Wade JB et al. The role of NHERF-1 in the regulation of renal proximal tubule sodium-hydrogen exchanger 3 and sodium-dependent phosphate cotransporter 2a. *J Physiol* 2005; 567: 27-32.

[48] Ardura J A, Wang B, Watkins SC et al. Dynamic Na^+-H^+ exchanger regulatory factor-1 association and dissociation regulate parathyroid hormone receptor trafficking at membrane microdomains. *J Biol Chem* 2011; 286: 35020-9.

[49] Levey AS, Madaio MP, Perrone RD. *Laboratory assessment of renal disease: clearance, urinalysis, and renal biopsy.* In: Brenner BM, Rector FC, eds. The Kidney, 4th edn. Philadelphia:W.B. Saunders Company, 1991; vol 1: 919-68.

[50] Hess B, Ackermann D, Essig M et al. Renal mass and serum calcitriol in male idiopathic calcium renal stone formers: role of protein intake. *J Endocrinol & Metab* 1995; 80: 1916-21.

[51] Giannini S, Nobile M, Sartori L, et al. Acute effects of moderate dietary protein restriction in patients with idiopathic hypercalciuria and calcium nephrolithiasis. *Am J Clin Nutr* 1999; 69: 267-71.

[52] Kim Y, Linkswiler HE. Effect of level of protein intake on calcium metabolism and on parathyroid and renal function in the adult human male. *J Nutr* 1979; 109: 1399–1404.

[53] Hegsted M, Linkswiler HM. Long-term effects of level of protein intake on calcium metabolism in young adult women. *J Nutr* 1981; 111: 244–51.

[54] Bosch JP, Saccaggi A, Lauer A et al. Renal functional reserve in humans. Effect of protein intake on glomerular function rate. *Am J Med* 1983; 75: 943-50.

[55] Krishna GG, Newell G, Miller E et al. Protein-induced glomerular hyperfiltration: role of hormonal factors. *Kidney Int* 1988; 33: 578–83.

[56] Tolins JP, Raij L. Effects of amino acid infusion on renal hemodynamics. Role of endothelium-derived relaxing factor. *Hypertension* 1991; 17: 1045-51.

[57] King AJ, Levey AS. Dietary protein and renal function. *J Am Soc Nephrol* 1993; 3: 1723-37.

[58] Walker V. Ammonia metabolism and hyperammonemic disorders. *Advances in Clinical Chemistry* 2014; 67: 73-150.

[59] Erez A, Nagamani SCS, Shchelochkov OA et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. *Nat Med* 2011; 17: 1619–26.

[60] Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. *Am J Clin Nutr* 2003; 78 (suppl): 584S-92S.

[61] Margen S, Chu J-Y, Kaufmann NA et al. Studies in calcium metabolism. I.The calciuretic effect of dietary protein. *Am J Clin Nutr* 1974; 27: 584-9.

[62] Shekarriz B, Stoller M. Uric acid nephrolithiasis: current concepts and controversies. *J Urol* 2002; 168: 1307–14.

[63] Fenske W, Störk S, Koschker A-C et al. Value of fractional uric acid excretion in differential diagnosis of hyponatremic patients on diuretics. *J Clin Endocrinol Metab* 2008; 93: 2991–97.

[64] Tasic V, Hynes AM, Kitamura K et al. Clinical and functional characterization of URAT1 variants. *PLoS One* 2011; 6(12): e28641. doi:10.1371/journal.pone.0028641.

[65] Wright AF, Rudan I, Hastie ND, et al. A 'complexity' of urate transporters. *Kidney Int* 2010; 78: 446-52.

[66] Bobulescu IA, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. Adv Chronic Kidney Dis 2012; 19: 358–71.

[67] Eraly SA, Vallon V, Rieg T et al. Multiple organic anion transporters contribute to net renal excretion of uric acid. *Physiol Genomics* 2008; 33: 180–92.

[68] Wu W, Dnyanmote AV, Nigam SK. Remote communication through solute carriers and ATP binding cassette drug transporter pathways: an update on the remote sensing and signaling hypothesis. *Mol Pharmacol* 2011; 79: 795–805.

[69] Enomoto A, Kimura H, Chairoungdua A et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature* 2002; 417:447–52.

[70] Vitart V, Rudan I, Hayward C et al. SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. *Nat Genet* 2008; 40: 437–42.