Determination of Urinary Calcium-Oxalate Formation Risk with BONN-Risk-Index and EQUIL Applied to a Family

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Apart from environmental and acquired risk factors, a person's genetic predisposition may have a distinct influence on the probability of the onset of urolithiasis. To investigate the family related development of calcium oxalate, CaOx, crystallization risk, we studied urines from three generations of the same family. The paternal line has been suffering from CaOx-urolithiasis for at least two generations; no case of urolithiasis has been reported from the maternal line and the youngest generation.We applicated the BONN-Risk-Index and the computer program EQUIL to determine the crystallization risk of each family member ($n = 7$). We clearly verified by probability calculations of the existence of the two risk groups within the family and showed that one of the siblings of the youngest generation may have inherited the stone-formation risk from its paternal relatives as this person clearly reflects a high risk pattern.

INTRODUCTION

Pathological crystal formation in the urinary tract is the result of a complex interplay of a number of different physicochemical and biochemical processes; at least one of which must be dysfunctional. A wealth of reasons exist for such a dysfunction. Apart from environmental and acquired (and anatomic) risk factors, a person's hereditary predisposition may have a distinct influence on the probability of suffering from urolithiasis. In the case of a genetically caused nephrolithiasis, the genetic defect is often transmitted from the parents to their children.[1−2] In around two-thirds of all stone-formers a metabolic disorder can be diagnosed. The other fraction, the so-called idiopathic stone-formers, show concentrations of the lithogenic substances in both blood and urine,[3] which fall within the normal range.

Focusing on the most common type of urinary calculi, the salts calcium-oxalate-monohydrate (whewellite) and calcium-oxalate-dihydrate (weddellite), the main risk factors associated with formation are hypercalciuria, hyperoxaluria, and hypocitraturia. These phenomena increase with high impact urinary supersaturation in relation to CaOx. For each of these general factors, a number of subgroups indicating the different origins which are involved in the particular pathogenesis can be distinguished.[4,5] Evidence suggests that, in some subgroups, genetic disorders contribute to the development of urolithiasis through a complex interplay with environmental factors. Only a few of the manifestations are caused by a monogenetic defect; they represent, without exception, severe symptoms with very low prevalence.[6−9] Most types of CaOx stone disease are due to a polygenetic etiology;[10,11] however, in these cases the genetic predisposition alone is not sufficient to cause pathological urinary stone-formation. It is mostly a polygenetic defect in combination with unfavorable environmental factors which results in real disease. Idiopathic hypercalciuria, the most common reason for the pathological formation of calcium salts, has been described as reflecting a polygenetic autosomal dominant trait[10,12,13] affecting both sexes equally.

Comprehensive surveys of this topic have recently been published by Goodman et al. (1995)[10] and Baggio (1999);[14] the latter points to the work still required in order to understand the pathogenesis of nephrolithiasis in detail.

In this study, the members of three generations of the same family, from which only the paternal line is affected by a recurrent CaOx-stone disease, were tested for their individual risk to actually form CaOx-stones. The members of the youngest generation are still regarded as "clinically healthy".

The scope of this work is to investigate with examples the CaOx-crystallization risk of the family members and to evaluate whether the paternal line's risk can also be traced to the youngest generation. In a comparative study we quantified the urinary CaOx crystallization risk of test persons based on both the BONN-Risk-Index approach (BRI)[15] and the value of the urinary relative CaOx-supersaturation (RS_{CaOx}) computed by the EQUIL-program.[16−18]

If an increased formation risk is assigned to the youngest generation, prophylactic measures to protect them from stone formation in the future should be taken.

MATERIAL AND METHODS

Seven persons representing three generations of a family were tested for their actual risk of forming calcium oxalate crystals. All persons were highly motivated and showed a high compliance.

Figure 1 illustrates the family relationship, the individual persons' status with respect to urolithiasis, and shows the

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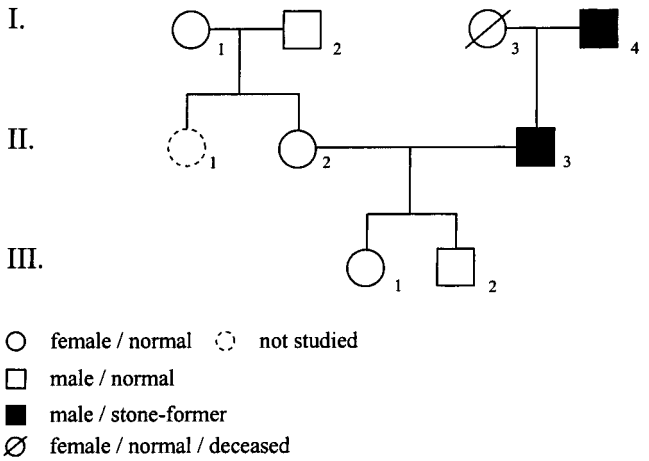

Figure 1. Genealogical tree of the investigated family. The roman numeral of the generation followed by the person's arabic number allows the identification of any person.

Table 1. Person I.3 Deceased (No Stones Reported)[b]

				medication						
person	age	status	BMI	A	B	C	D	E	F	G
I.1	82	H	22.9	−	+	+	+	+	−	−
I.2	85	H	23.2	−	+	+	−	−	−	−
I.4	92	P	29.4	−	+	+	+	+[a]	+[a]	−
II.2	55	H	30.0	−	−	−	+	−	−	+
II.3	64	P	26.4	+	+	+	+	−	−	−
III.1	26	H	22.4	−	−	−	−	−	−	−
III.2	22	H	22.4	−	−	−	−	−	−	−

[a] Acute medication; for details see text. [b] Status: H nonstone-former, P stone-former, BMI: body mass index [kg/m^2], medication (occasionally administered during last six months): A stone related, B blood pressure, C cardiac stimulant, D anticoagulation, E diuretics, F laxatives, G hormones.

identification scheme as a combination of a roman numeral (generation) and an arabic number (person).

In Table 1 further information with respect to age, body mass index, and occasional medication is given. With respect to the instructions given in the manufacturer's package circular and the more detailed doctor's information, no direct and definite relationship between an administered medication and the general stone formation risk was established.

Persons II.2, II.3, III.1, and III.2, on one hand, and persons I.1 and I.2, on the other hand, are living in the same households, following the same diet. Person I.4 is living in a single household. No particularities with respect to the dietary habits existed in any person (e.g. vegetarians, no vitamin supplementation). For control, dietary records were taken at any sampling day. Person II.1 was not available for study; however, no stone history has been reported.

The study was performed during the period from January to March to exclude potential seasonal variations in urinary composition (all test persons were affected by the same meteorological situation as they all live in the conurbation of the City of Bonn, Germany). No dietary restrictions were issued. The sampling days were chosen by chance to ensure that the urine sample taken represents the daily life situation of any proband with respect to both nutrition and physical activity. All volunteers were outpatients and collected 24-h urines in two 12-h fractions (07:00 a.m−07:00 p.m. and 07:00 p.m.−07:00 a.m.) without any chemical pretreatment. During the collection period the urine was stored at 4 °C. A dip-stick test (Combur^{9}Test, *Boehringer*, Mannheim, Germany) was used in order to test the urine samples for nitrite as a marker for urinary tract infection; none of the samples showed a "nitrite positive" reaction.

The initial concentration of ionized calcium, [Ca^{2+}], was measured by a calcium-selective electrode (*Metrohm*, Herisau, Switzerland, relative accuracy ± 3%). Crystallization experiments according to refs 15 and 19 were carried out in order to determine the BRI (no dilution, no preservation, no pH-adjustment of the urine sample). CaOx-particle formation was triggered within 200 mL aliquots of the urine samples by step-by-step addition of ammonium oxalate solution (0.04 N, 0.5 mL/step, 1.5 mL/min). During the experiment, the urine samples were kept at 37 °C and were well mixed by a low shear force stirrer. The onset of CaOx-crystallization was detected by an inline laser probe crystal system analyzer (Messtechnik Schwarz GmbH, Düsseldorf, Germany). The dramatic change in both the particle size distribution and the particle number at crystallization onset enables clear determination of this event. From the initial [Ca^{2+}] and the amount of ammonium oxalate, (Ox^{2-}), which has to be added to the urine sample to detect a CaOx-crystallization, the BRI was calculated as BRI = [Ca^{2+}]/(Ox^{2-}).

Apart from volume and specific weight determination, routine chemical analyses were performed. The following parameters were determined: pH, sodium, potassium, total calcium, magnesium, ammonium, chloride, phosphate, sulfate, creatinine, uric acid, citric acid, and oxalic acid. From these data we calculated 24-h excretion rates and computed the relative supersaturations with respect to CaOx, RS_{CaOx}, using the EQUIL-program.[16−18] Based on the results of the BRI-determinations we tested the existence of distinct risk subgroups within the family with a mathematical probability approach. Additionally, urinary excretion rates were discussed in order to limit the reasons for certain risk expressions.

RESULTS

No crystals were present in the urine samples at the end of the collection period. Table 2 shows the number of samples taken from each person and depicts the detailed results of the BRI-determinations.

The urine volume may be a potential factor influencing the crystallization risk. However, no relevant relation between the urine volume and BRI was observed (r = 0.15). The 24-h creatinine excretions, Cr*, are calculated as a control of metabolic activity. Except for generation II, the intraindividual variations of Cr* are below 9.7% at a mean of 5.6% (II.2 = 14.7%, II.3 = 14.2%). This result is noticeably low, considering the individual living conditions from which they are derived, and it indicates a high degree of intraindividual standardization with respect to both physical activity and nutritional behavior. Table 3 gives the results of the RS_{CaOx} calculations obtained from the 12-h fractions of each proband.

The data of person I.2 are given but are not taken into account for further statistics as his state of health severely deteriorated during the sampling period and immediate medical treatment with furosemide, inter alia, was required. Nevertheless, the calculated mean 24-h BRI and RS_{CaOx} of his two sampling days were 0.67 L^{-1} and 1.36 L^{-1} and 1.44 and 2.40, respectively.

Table 2. Results of BRI [Per Liter] Determinations[b]

	stone formers				nonstone formers									
	I.4		II.3		III.1		III.2		II.2		I.2		I.2	
1	0.12[a]	1.01[a]	1.79	1.63	1.07	2.41	0.10	0.04	0.61	0.17	0.33	0.22	0.65	0.74
2	2.10[a]	0.07[a]	2.31	3.61	0.74	1.01	0.26	0.13	0.74	0.22	0.23	0.10	0.33	0.91
3			1.88	1.09	2.38	0.94	0.09	0.18	0.20	0.12	0.11	0.33	0.27	0.26
4			1.51	1.18	1.20	3.43	0.19	0.19						
5					0.56	2.88								
M	nd	nd	1.87	1.88	1.19	2.13	0.16	0.14	0.52	0.17	0.22	0.22	0.42	0.64
S	*c*	*c*	*c*	*c*	*c*	*c*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*
V	808	901	1218	888	1141	650	1513	1316	1533	1450	653	425	655	473
Cr*	8.76		16.84		13.11		16.21		16.96		7.56		12.92	
SD	0.45		2.41		0.88		0.65		2.49		0.73		0.32	

[a] Patient was treated with furosemide in order to avoid oedema formation; samples have been neglected for further statistics. [b] Person identification according to Figure 1. Left column: fraction 1 (07 a.m. − 07 p.m.), right column: fraction 2 (07 p.m. − 07 a.m.). M: mean BRI-value, S: risk status according to BRI, V: mean urine volume [ml], Cr*: mean 24-h creatinine excretion [mmol/d], SD: standard deviation of Cr* [mmol/d].[c] At risk. [d] Without risk.

Table 3. Relative Urinary Supersaturations with Respect to CaOx[a]

	stone formers				nonstone formers									
	I.4		II.3		III.1		III.2		II.2		I.1		I.2	
1	1.603	1.338	4.667	3.807	6.986	5.600	2.234	2.655	1.345	1.240	4.044	nd	2.652	3.195
2	2.519	2.200	3.387	3.765	2.953	3.166	2.212	1.180	2.315	1.750	2.477	1.755	3.720	3.499
3			nd	2.323	7.257	1.780	1.314	1.125	2.742	1.596	2.050	4.797	1.475	1.995
4			4.269	3.463	7.739	6.438	1.692	1.088						
5					1.585	3.642								
M	nd	nd	4.108	3.340	5.304	4.125	1.863	1.512	2.134	1.529	2.857	3.276	2.616	2.896

[a] Abbreviations as indicated in Table 2. Left column: fraction 1 (07 a.m. − 07 p.m.), right column: fraction 2 (07 p.m. − 07 a.m.). nd, not determined.

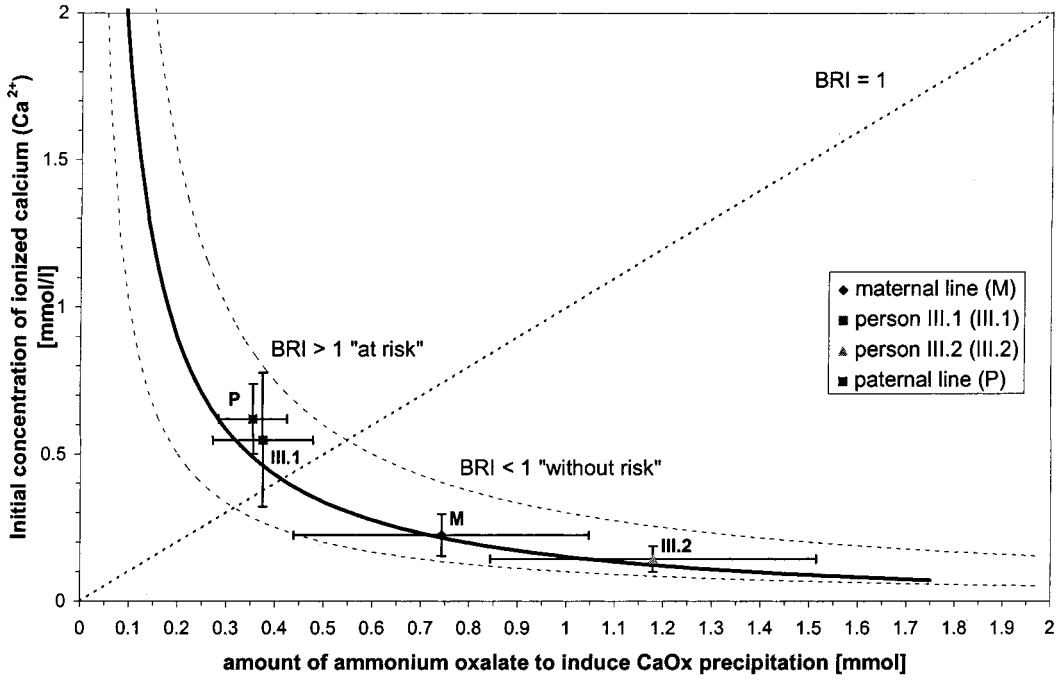

Figure 2. Mean values of (Ox^{2-}) and [Ca^{2+}] and related standard deviations. BRI = [Ca^{2+}]/(Ox^{2-}), i.e., the gradient of the regression line through the origin and the data point.

Considering the results of person II.3 (and with moderation those of person I.4) as the "paternal line" (i.e. stone-formers) and combining person I.1, I.2, and II.2 in the "maternal line" (i.e. healthy persons), the following contrasting results can be obtained by comparing these lines with the individual data from the youngest generation (persons III.1 and III.2).

Figure 2 indicates the distinct distribution of the (Ox^{2-}) and [Ca^{2+}] mean values and related standard deviations derived from both lines and from the youngest generation. Furthermore, the relation of these data to the best-fit hyperbola describing the (Ox^{2-}) vs [Ca^{2+}] distribution of more than 275 urine samples is shown ($r = 0.87$, $p < 0.001$).[15]

The mean value of the 12-h BRI of the paternal line amounts to 1.88 L^{-1} (this clearly indicates "at risk"), and that of the maternal line, on the other hand, amounts to only

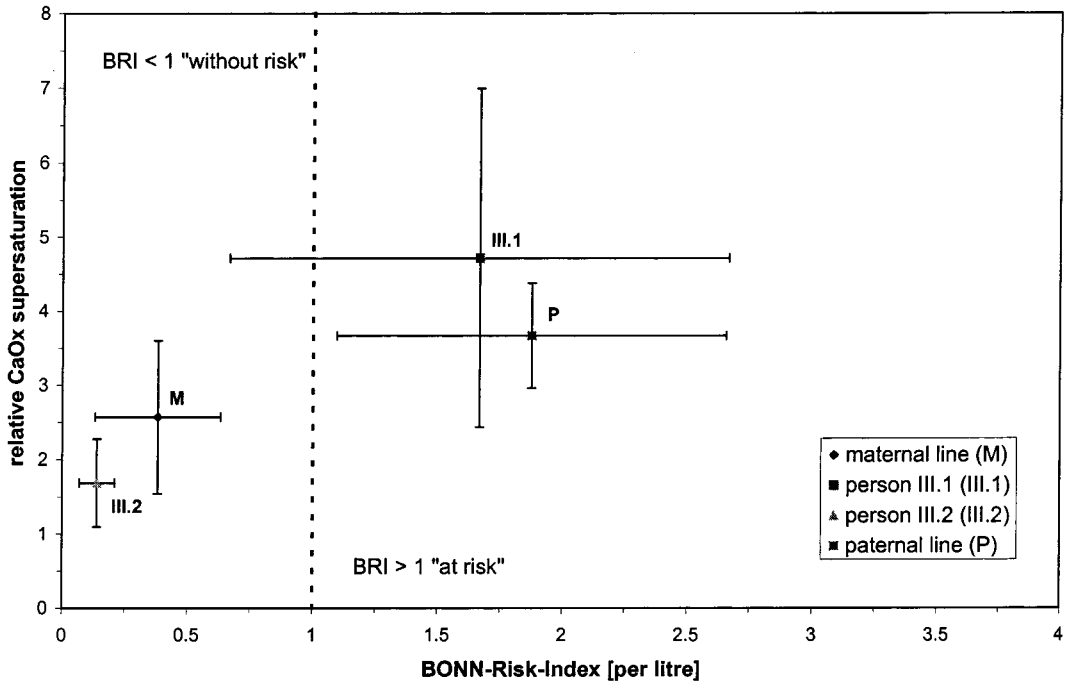

Figure 3. Mean values of BRI vs related mean values of RS_{CaOx} and related standard deviations.

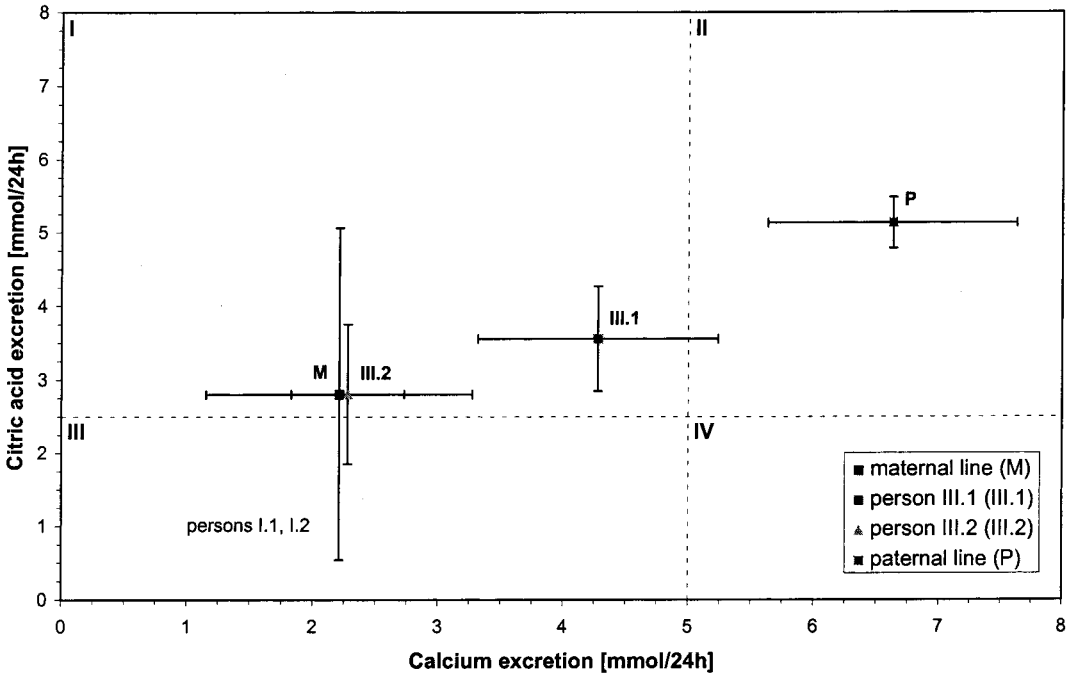

Figure 4. Plot of urinary mean 24-h calcium excretion vs mean 24-h citric acid excretion and related standard deviations. Twenty-four h values calculated from 12-h urine fractions. Broken lines indicate limiting values recommending an urolithiasis related treatment. Fields I−IV: I both parameters are within normal range, II increased calcium excretion, but sufficient citric acid excretion, III decreased excretion of citric acid, but normal calcium excretion, and IV both parameters out of normal range.

0.38 L^{-1} ("without risk"). The youngest generation ($n = 2$) shows different mean and different maximum 12-h urine BRI values. Person III.1's mean risk amounts to 1.67 L^{-1} (3.57 L^{-1}), and person III.2's risk amounts to 0.14 L^{-1} (0.27 L^{-1}). Similarly differentiated results are obtained for RS_{CaOx} as shown in Figure 3. Furthermore, Figure 3 demonstrates the considerably high and direct correlation ($r = 0.89$) between BRI and RS_{CaOx}, confirming the results of.[15]

Figure 4 illustrates the variations of the 24-h calcium excretions, Ca*, and 24-h citric acid excretions, CA*, for the maternal and paternal line in relation to the members of the youngest generation. Four fields (I−IV) can be distinguished by plotting the cutoff values recommending therapeutic measures (Ca* > 5 mmol/d, CA* < 2.5 mmol/d) in case of urolithiasis—these values can also be interpreted as being therapeutically desirable levels of the diurnal excretion of the urinary component under consideration.[20] Samples plotted within field I show normal values, whereas samples plotted into the fields II and III are indicated by an enhanced Ca* and a reduced CA*, respectively. No sample was plotted into field IV. Four of six samples, indicated by Ca* > 5 mmol/d, exceeded Coe's criterion for hypercalciuric urines

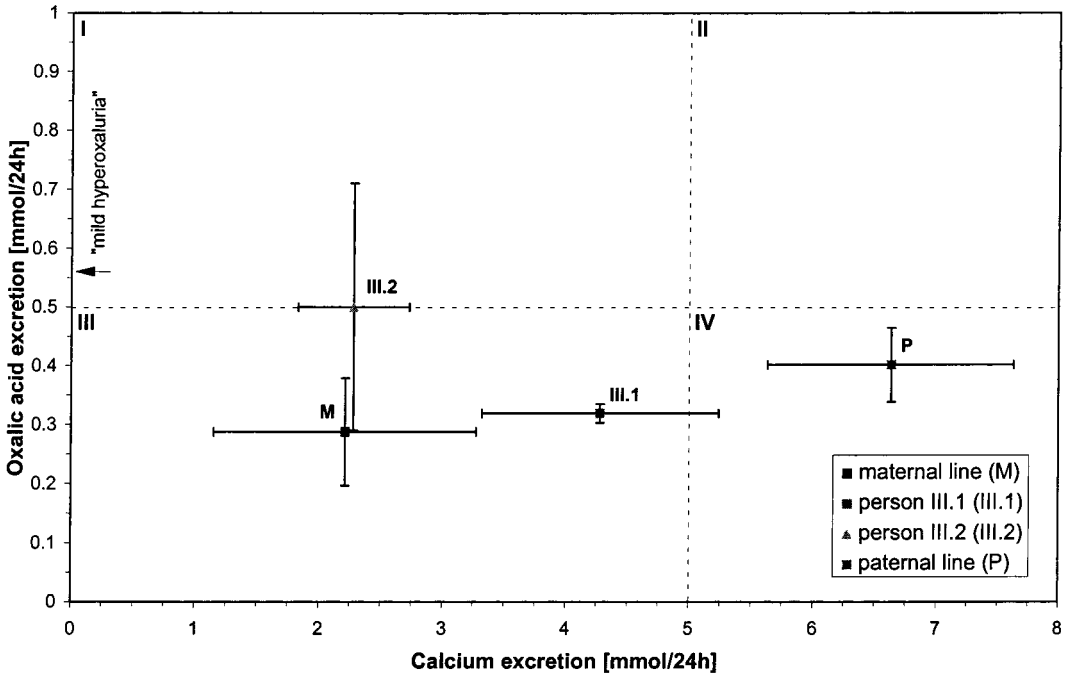

Figure 5. Plot of mean urinary 24-h calcium excretion vs mean 24-h oxalic acid excretion and related standard deviations. Twenty-four h values calculated from 12-h urine fractions. Broken lines indicate limiting values recommending an urolithiasis related treatment. Samples showing 0.56 mmol/d ≤ OA* ≤ 1 mmol/d are termed "mild hyperoxaluric".[5,21]

of Ca* ≥ 0.3952 × Cr* (140 mg Ca* per gram of urinary creatinine).[12]

Figure 5 shows the variations of the urinary oxalic acid excretions, OA*, vs Ca*. As in Figure 4, four discriminative fields with similar meanings can be distinguished; urine samples indicating OA*-values plotting into the range 0.56 mmol/d ≤ OA* ≤ 1 mmol/d are assumed to be "mildly hyperoxaluric".[5,21] Two samples of person III.2 exceeded the recommendation value of 0.5 mmol/d,[22] and one of them showed a "mild hyperoxaluria". No sample was plotted into field II.

Occasionally, slightly elevated values (around 4 mmol/d, in particular in person II.3) for the uric acid excretions at generations II and III have been observed—a risk factor which may promote CaOx salting out under favorable pH-conditions by then providing nuclei which act, on one hand, as seeds for CaOx crystallization and/or, on the other hand, (supplementary) as "surface" for the absorbance of urine inhibitors.[23−25] Whether the increased uric acid excretion is caused by a specific familial nutritional behavior (e.g. excess of animal protein/purine intake or alcoholic beverages) is not evident as an evaluation of the dietary records does not give a clear indication.

DISCUSSION

All results obtained allow inclusion into the existing BRI-concept, which cover the results of more than 275 independent urine analyses. Therefore, the test persons reflect the general situation of risk distribution in a population; they do not represent an unusual group of persons. Only slightly elevated [Ca^{2+}] mean values with respect to the best-fit hyperbola were obtained (Figure 2).

Within the examined family, the expression of the CaOx-stone formation risk can be traced through the three generations. This is valid for the BRI-values as well as for the RS_{CaOx} data, which reflect a similar risk pattern for the investigated persons.

A distinct differentiation between the paternal line and the maternal line with respect to the stone formation risk can be made. The same differentiation can be made with regard to the youngest generation. Where person III.1 reflects a crystallization risk similar to her father (and grandfather), person III.2 has to be assigned at low risk ("without risk" according to BRI), as low as all sampled members of the maternal line. The SD ranges of BRI and RS values of both suggested main groups show no overlap (Figure 3).

To test for the formal correctness of the supposition of the existence of two independent risk groups within the family, we calculated the probabilities of this assumption being true. We presuppose that both groups of data are Gauss-distributed, indicated by mean values $x_1 < x_2$ and standard deviations σ_1 and σ_2. Consider a sample X which belongs with a 50% probability to group 1.

In a first estimate, this sample belongs to group 1 if

$$X \leq \frac{\sigma_1 x_1 + \sigma_2 x_1}{\sigma_1 + \sigma_2}$$

The probability p of this estimation being true can be calculated by

$$p = \psi_0\left(\frac{x_2 - x_1}{\sigma_1 + \sigma_2}\right)$$

with Ψ_0 as the Gaussian error integral. The values of $\Psi_0(x)$ are listed in handbooks of statistics.[26]

Table 4 shows the computed probabilities of p with $0.5 \leq p \leq 1.0$; at $p = 0.5$, no differentiation can be observed. The higher p the higher the probability that the investigated persons/samples belong to different groups and vice versa. The degree of distinction reaches its maximum at $p = 1$.

Table 4. Probabilities *p* of BRI and RS Values from Persons III.1 and III.2 Relating to One of the Two Risk Groups[a]

BRI\RS	M	III.2	III.1	P
M		0.709	0.742	0.736
III.2	0.773		0.853	0.934
III.1	0.848	0.922		0.637
P	0.926	0.979	0.548	

[a] Maternal line, M, and paternal line, P. The lower *p* the lower the degree of distinction. BRI and RS differentiate in the same manner. No differentiation between persons III.1 and P is observed with respect to BRI ($p = 0.548$); the greatest distinction is observed between persons III.2 and P ($p = 0.979$).

The existence of two risk groups as mentioned above is clearly demonstrated.

The differences in the results of person III.1 and person III.2 are of remarkable interest, as these cannot be attributed to different nutritional intake; both share at least two of three meals every day, albeit in different quantities.

All samples of person II.3 exceeded the recommendation value for Ca* (5 mmol/d); however, his CA* is within the normal range. Person III.1, also assigned to be "at risk", shows similar CA* behavior but generally lower urinary Ca*. Only an occasionally exaggerated output of calcium above the 5 mmol/d recommendation value was observed. However, no intraindividual correlation between Ca* and the related BRI or RS_{CaOx} exists in this person, showing an often observed situation where the excretion values of lithogenic substances alone are not sufficient to estimate the actual crystallization risk.

Both persons, II.3 and III.1, show oxalic acid excretions in the same order of magnitude, clearly plotting into the normal range (fields III and IV, Figure 5).

The maternal line and person III.2 show normal values for Ca* ($\approx$2.5 times lower than those of person II.3). Some samples, in particular those of persons I.1 and I.2 of the maternal line, are indicated by a reduced urinary CA* ($<$2.5 mmol/d).

Considering an established, but strong, upper limit of Ca* of nonstone formers (males: $\approx$ 7.5 mmol/d, females $\approx$ 6.2 mmol/d[27]) as discriminative criterion, hypercalciuric urine has only been formed once by person II.3 (BRI $>$ 1). Surprisingly, person III.2, who shows the lowest risk status of all family members, has formed on two occasions a 24-h urine which has to be asigned "(mild) hyperoxaluric" if an OA*-excretion of 0.5 mmol/d is set as limit value. To complete the picture, person I.2, who was taken into account for statistic evaluation, shows values for renal Ca*, CA*, and OA*, which all fit into the normal range.

The OA* values of all persons are in the normal range (except person III.2 whose OA* was moderately increased on two occasions). Thus, a disturbed OA* cannot be the reason for the observed broad intrafamiliar spectra of CaOx-formation risks, although OA* is the most important determinant of urine saturation with respect to CaOx.[16,21,28−31]

Focusing on the test person's values of the urinary calcium excretions, it is possible that this parameter may be the key factor leading to the quite different intrafamilal risk situation. This is all the more so, when considering that there is a decline in calcium absorption with age,[32] which is probably due to a progressive vitamin D deficiency in the elderly. Although 64 years of age, person II.3 still shows the highest values for Ca*, followed by his daughter (person III.1). Both persons show the highest CaOx-crystallization risk in the sense of BRI and RS_{CaOx}. Person III.2—even when considering that he is 4 years younger than his sister—seems not to be affected by elevated values of Ca*.

Ultrasound investigation of the person III.1's kidneys has been performed in order to clarify her actual in-vivo situation. Although without any acute symptoms in the urinary tract, considerable amounts of gravel were identified in both kidneys. As a result, person III.1 has to be considered a "stone-former"—a clear proof of a sucessful risk evaluation in advance of manifestation of a pathological stone-formation. Regarding the genealogical tree (Figure 1) and considering person III.1 as a tested patient, the picture of an autosomal dominant trait with 50% probability of disease transmission from generation to generation seems to be a possible explanation for the intrafamilial distribution of stone-formers and nonstone-formers. In the case of the investigated family, stone disease should be viewed as a family issue, not as an individual one. However, at the moment no apparent reason for the elevated calcium excretions in persons II.3 and III.1 are known. Thus, further investigation have to be performed in order to evaluate the origin—renal, enteric, or resorptive—of the metabolic disorder.

In conclusion it can be stated that a prophylactic examination of the actual CaOx-crystallization risk of the youngest generation of a family with a history of stone-formation can be recommended in order to protect them from pathologic crystal-formation—by, for example, regular monitoring and general dietary measures[33]—in case of an observed enhanced risk. The determination of the BONN-Risk-Index is a suitable, fast and easy to perform method for such a screening. The evaluation of a person's actual calcium oxalate formation risk from his or her unprepared native urine using the BRI approach takes into account their individual native ratio for all urinary constituents—the "true" ratio of the promotoric, inhibitoric, and inert acting substances. Since the pathological excretion ratio of an urinary constituent is often related to a genetic cause, its occurrence through the generations of a family can be followed, even although the substance itself remain unknown. Thus, the BRI-approach can offer important clues for a genetic connection to urinary stone-formation.

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