IDENTIFYING A SINGLE LOCUS IN THE POLYGENIC COMPLEX OF IDIOPATHIC OXALATE STONE DISEASE

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Abstract

The genetic basis for urinary calcium excretion was selected as a potential paradigm for an individual genetic component in the polygenic complex responsible for susceptibility to oxalate stone disease. Analyses of mean urinary calcium excretion among healthy volunteers supported the inference that a principal genetic factor influencing calcium excretion is a codominant pair of alleles, one for higher, one for lower excretion. Three basic requirements for an individual polygenic component satisfied by the hypothesized pair of genes are: 1) between-individual differences significantly exceeded differences within-individuals, 2) data were compatible with a Hardy-Weinberg equilibrium test, and 3) families exhibited segregation compatible with a pair of codominant genes.

Several studies have inferred that hypercalciuria is an autosomal dominant trait. However, these studies did not fully satisfy requirements for such a trait in that they had a deficiency of hypercalciuric offspring from hypercalciuric parents and included some hypercalciuric offspring born to normocalciuric parents. Reinterpretation using the codominant hypothesis resolved these discrepancies. Data from one study provided independent support for the codominant hypothesis in that matings of the 3 types capable of producing hypercalciuric offspring occurred in the proportions expected when our estimates of gene frequency were applied to their data.

Key Words: Calcium excretion, inheritance, polygenes, codominance, gene frequency.

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Introduction

Progress in the understanding of genetic contributions to complex disorders like calcium oxalate stone disease has been very slow. One factor accounting for this Sisyphean progress is that most often these disorders have been treated as black boxes ever since Fisher (1918) demonstrated that discrete Mendelian units could give rise to both qualitative and quantitative inheritance. The primary statistical tools for studying complex diseases have been variance and covariance analyses (Murphy, 1979) which generally result in estimates of heritability rather than in the identification of specific genes involved in the disease process. Another factor slowing progress is the absence of paradigms. The principal genetic paradigm for diseases today is, of course, monogenic (autosomal dominant, recessive and X-linked recessive) inheritance and some of its attributes and terminology have been carried into the polygenic domain by many investigators. For example, terms like defective, abnormal or mutant genes, dominance, recessivity, epistasis, and lack of penetrance have specific meanings and are useful descriptors in the monogenic realm but are either inappropriate, currently untestable or rare phenomena in the polygenic domain.

The most fundamental distinction between monogenic and polygenic inheritance relates to the nature of causes in the two realms. In the monogenic domain, the causes are always necessary (barring phenocopies) and generally sufficient. For example, receiving a double dose of the recessive gene for Tay-Sachs disease at conception is *necessary* for the disease to occur. No one who has a single or double dose of the normal allele is known to have Tay-Sachs disease. A double dose of that gene is also generally *sufficient* to produce this fatal disorder; no special environment or agent other than the passage of time being required for the disease to become evident. These facts account for the very high correlation between the presence of a double dose of the gene and the occurrence of the disease. On the other hand, in polygenic disorders, none of the environmental or genetic components is *individually* either a necessary or a sufficient cause (Edwards, 1969). With regard to hypercalciuria, for example, the absence of

hyper-calciuria in some oxalate stone formers implies that hypercalciuria is not a necessary cause of stone disease. The fact that about 10% of persons in the general population are hypercalciuric and some of these persons will never form stones is evidence that hypercalciuria is not a sufficient cause (Goodman *et al*., 1995). Hence, the presence of a single or double dose of the allele for susceptibility and presence of oxalate stone disease exhibit a low correlation. Appreciation of this fundamental distinction between monogenic and polygenic inheritance and its corollaries is critical to perceiving what data we collect can tell us.

Our purposes are 1) to describe our efforts to dissect out the gene pair influencing urinary calcium excretion, 2) to review the genetic literature on calcium oxalate stone disease in the light of our findings, and 3) to discuss the implications of our findings on clinical and research activities. Several of the characteristics distinguishing monogenic from polygenic inheritance are presented in an appendix as well as a brief glossary.

A Genetic Hypothesis for Hypercalciuria

The polygenic complex creating susceptibility to oxalate stone disease (Resnick *et al*., 1968) implies the existence of individual genic components at two or more loci. We chose urinary calcium excretion as probably reflecting one such component for three reasons. First, two groups of calcium excretors had been fairly well delineated, hyper- and normocalciurics. Secondly, several studies had demonstrated a significant excess of hypercalciurics among stone formers suggesting a causal role for this trait. Thirdly, Coe *et al*. (1979) and Mehes and Szelid (1980) had presented evidence suggesting hypercalciuria was an autosomal dominant trait. However, classic studies of polygenic traits (Wright, 1978) indicated that the genes involved generally have small, additive effects. Since the additivity implies codominance, we chose the simplest hypothesis consonant with additivity, that a codominant pair of alleles influences calcium excretion. A pair of codominant alleles would produce three genotypes giving rise to three distinguishable phenotypes. We assumed that hypercalciurics, being extreme, are probably those with a double dose of an allele for higher calcium excretion and that the third presently unrecognized class was hidden among the normocalciurics. Knowing that urinary calcium excretion is highly variable, even within individuals, we hoped to minimize that variability by collecting multiple 24-hour urines, settling on 3 as the largest number of specimens we could persuade volunteers to carefully collect. We were also conscious of the need for a large number of normal subjects if we hoped to detect a third class of excretors, setting 100 as our goal.

In order to establish the genetic basis for a trait such as hypercalciuria, three criteria must be satisfied. First, variability between individuals must be significantly greater than variability within. If it is not, the variation is due entirely to environmental factors, i.e., even if genetic factors underlie the trait, they presumably are the same in all individuals tested. The second criterion is satisfaction of the Hardy-Weinberg equilibrium equation. If the frequency of the allele Ca^L for lower calcium excretion is p and that of its alternative allele Ca^H for higher excretion is q, then the Hardy-Weinberg equation states that the three phenotypes expected, low, intermediate and high (hypercalciuric) excretors, will occur in the population in the proportions ($p^2 + 2pq + q^2 = 1$). Satisfaction of this relationship is no less significant than tests for segregation but is exceedingly difficult if one of the alleles is rare. Obviously, Hardy-Weinberg tests also require large numbers for reliable estimates of gene frequencies. The third criterion to be satisfied is segregation, requiring study of family groups. We studied parents and siblings of a few subjects included in our large sample.

It should be pointed out that it is generally accepted that there are two different physiological responses that may lead to high calcium excretion. One is thought to result from hyperabsorption in the gut whereas the other results from decreased reabsorption of filtered calcium. Distinctions between these two types of hypercalciuria in families (Holmes *et al*., 1994) and subsequent unpublished studies have revealed that the locus we are describing here is the one associated with absorptive hypercalciuria. Fasting or renal hypercalciuria may be inherited as an autosomal recessive trait but further work is needed.

In order to correct partially for differences in body size, calcium excretion was expressed in mg calcium/ g creatinine. Without some such correction it seemed probable that detecting a third excretor group would be very unlikely because variability in body size would be confounded with variation due to genetic factors. Use of the creatinine denominator also would partially correct for incomplete urine specimens. It is possible that the third class has been previously undetected for these reasons. Some investigators prefer to utilize concentration as an index to characterize calcium excretion because of its direct bearing on the supersaturation of urine with calcium oxalate. However, as this index is almost entirely influenced by environmental variables related to fluid intake and sweat production, it is not appropriate for use in genetic classifications.

Test of the Hypothesis

In order to test the first criterion, an analysis of variance was performed on the mean calcium excretions of the 101 subjects studied as shown in Table 1. Clearly, the between-individual variation significantly exceeds the variance within individuals, almost 78% of the total

variability being attributable to between individual variation. This finding per se does not establish that genetic factors play a role in calcium excretion but is compatible with that inference. If large differences between individuals in their calcium intakes occurred, one could obtain the same result providing intakes for each person were very constant. However, our data do satisfy the first criterion and cannot be readily explained by differences in dietary intake since no significant differences in calcium intake in the excretor subgroups were detected (Holmes *et al.*, 1994).

In the past, calcium excretors have been divided into hypercalciuric and normocalciuric individuals. The right hand arrow in Fig. 1, at 0.18 g Ca/g creatinine, was selected to separate hypercalciurics from normocalciuric subjects. The existence of two subgroups among the normocalciurics was inferred in part because an incompletely dominant gene pair would yield three subgroups. Further, the distribution of normocalciurics had a range which was about double that of the hypercalciurics. Finally, a dip was seen near the mean for normocalciurics where one would expect the highest frequencies. The left hand arrow at 0.1 g Ca/g creatinine in Fig. 1 was used to separate intermediate ($Ca^L Ca^H$) from low excretors ($Ca^L Ca^L$). We have used Ca^L to denote the allele for lower excretion and Ca^H, the allele for higher excretion to avoid using either different letters for genes at the same locus (e.g., L and H) or upper and lower case letters (e.g., Hh and hh), the latter usage implying dominance. The numbers of low, intermediate and high excretors provide the basic data for testing the second criterion, the Hardy-Weinberg equilibrium. Gene frequencies were estimated from our data as shown in Table 2A. From the derived frequencies, p (Ca^L) = 0.68 and q (Ca^H) = 0.32, one can calculate the expected proportions and numbers of individuals in the three classes and compare them with the observed numbers, as shown in Table 2B. The fit between observed and expected numbers gives no basis for rejecting the null hypothesis and supports the conclusion that the second criterion is satisfied, i.e., data are compatible with the hypothesis that a pair of codominant alleles influence urinary calcium excretion and are at equilibrium in the population. Our estimate of the frequency of high excretors in the general population (~10%) is also comparable to literature estimates of 7-11% as the frequency of hypercalciurics (Hodgkinson and Pyrah, 1958).

Thus far we have assayed 24-hr urines from only 8 families to test the third criterion, segregation. Fig. 2 presents the results and all pedigrees except family G are compatible with the proposed hypothesis. The four matings between two intermediate parents have produced 3 low, 5 intermediate and 3 high excretors, fitting the 1:2:1 ratio very well. Similarly, the two matings between low excretor parents have produced all low excretor offspring as expected. Family G is exceptional in that both daughters
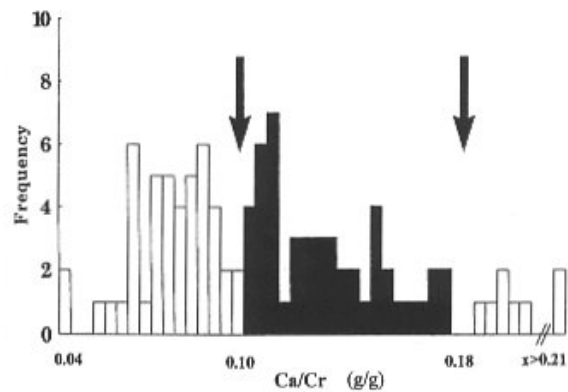
Figure 1. Distribution of mean urinary calcium excretion in g Ca/g creatinine for 101 subjects who collected three 24-hr specimens. Arrows indicate presumed antimodes separating low, intermediate and high (hypercalciuric) groups.

should be intermediates if the parents are correctly classified. Based on loading tests, the younger daughter is a high excretor, but she has fasting hypercalciuria and we believe that another mode of inheritance may be involved in that type of hypercalciuria. Her mother is an absorptive hypercalciuric. Additional family studies have been held in abeyance until testing methods minimizing the likelihood of misclassifications have been developed. Burtis *et al.* (1994) studied calcium excretion among adult stone formers on self-selected and on a defined diet. They found that subjects can be misclassified in both directions, i.e., persons classified as hypercalciuric on a self-selected diet can test normocalciuric on a defined diet and vice versa. Coefficients of variation within individuals for calcium excretion have been reduced when we have used diets in which calcium, sodium, magnesium, protein, and oxalate have been controlled and more recently even greater reductions with formula diets (unpublished data).

Previous Genetic Studies of Stones and Calcium Excretion

Though the familial occurrence of calcium oxalate stone disease has been long known, only anecdotal reports antedate the pioneering study of Gram (1932). He studied his own extended family and concluded that oxalate stone disease resulted from an autosomal dominant gene. However, he noted that one had to presume some family members were bearers of the gene without developing stones since some with multiple stone-forming offspring were free of stones throughout long lives. Gram and most others were not aware of the fact that pedigrees of common polygenic

Table 1. Test (ANOV) to determine whether differences in urinary calcium excretion between individuals exceed differences within individuals based on mean mg Ca/g Cr for 101 normal subjects who collected three 24-hr urines.

Source	DF	Sum of Squares	Mean Squares	F	Prob.
Between Individuals	100	562,255	5622.5	6.00	< 0.0001
Within Individuals	2	3249	1624.7	1.73	0.18
Residual	200	187,315	936.6		
Total	302	752,819			

Between/within F = 3.46, p <0.05

Table 2. (**A**) Estimation of gene frequencies from observed numbers of low, intermediate and high excretors among 101 healthy subjects and (**B**) Calculation of expected number of low, intermediate and high excretors from gene frequency estimates of p (Ca^L) = 0.6782 and q (Ca^H) = 0.3218 and comparison with observed numbers (Hardy-Weinberg test).

A

Excretor Class	Number of Observations	Number of Alleles Ca^L	Ca^H	
Low	44	88	0	$p(Ca^L)=137/(137+65)= 0.6782$
Intermediate	49	49	49	
High	8	0	16	$q(Ca^H)=65/(137+65)=0.3218$
Total	101	137	65	

B

Excretor Phenotype	Excretor Genotype	Freq.	Exp. Prop.	Exp. No.	Obs. No.	χ^2	Prob.
Low	$Ca^L Ca^L$	p^2	0.4599	46.4	44	0.124	
Intermediate	$Ca^L Ca^H$	$2pq$	0.4365	44.1	49	0.544	
High	$Ca^H Ca^H$	q^2	0.1036	10.5	8	0.595	
Totals		1	1.0000	101.0	101	1.263	> 0.20

disorders can readily mimic monogenic inheritance (Edwards, 1960).

The next large-scale study was that of McGeown (1960) who studied the first degree relatives of calcium stone formers and controls. She found a significant excess of stone formers among the relatives of her probands but did not propose a mode of inheritance. Subsequently two medical students and the present senior author studied the first degree relatives of 106 calcium oxalate stone formers and corresponding control relatives of the spouses of the probands (Resnick *et al*., 1968). In addition to finding a significant excess of stone formers among the relatives of probands, we found evidence that susceptibility was mediated by a polygenic system rather than by dominant inheritance.

In a significant effort to identify a genetic component in oxalate stone disease, Coe *et al*. (1979) studied 73 relatives of 9 probands with hypercalciuria. A single 24-hr
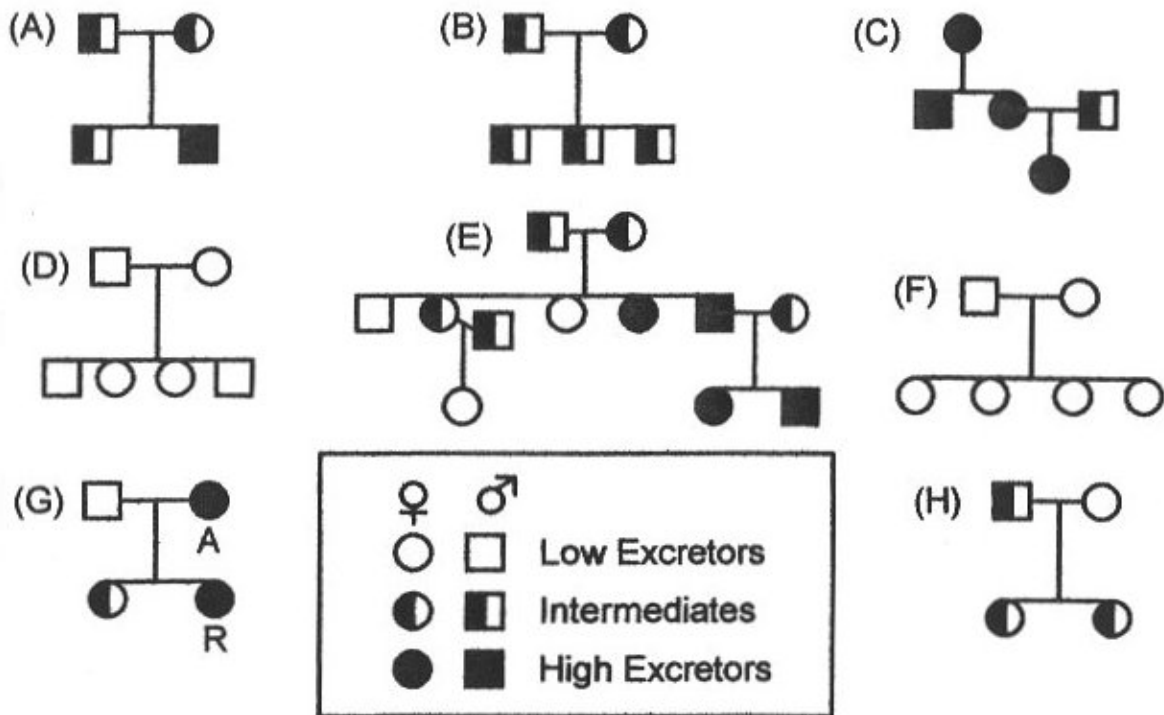
Figure 2. Pedigrees of 8 families whose members collected three 24-hr urine specimens. Symbols designate low, intermediate and high urinary calcium excretors based on mean urinary calcium excretion (g Ca/g Cr) for each subject and on the antimodes of Fig. 1.

urine specimen was obtained from 67 relatives, the remaining 6 providing several specimens. From the fact that 3-generation transmission was observed in several of their families and the patterns of transmission observed, Coe *et al.* concluded that their findings strongly suggested autosomal dominant transmission. They did not comment on the exceptions in family 5 wherein two pairs of normocalciuric parents had hypercalciuric offspring. When we collated the offspring of their matings wherein one parent was hypercalciuric, there was a non-significant deficiency of hypercalciuric offspring. In a less extensive study, Mehes and Szelid (1980) studied the parents and sibs of 10 pediatric hypercalciuric probands and also concluded that hypercalciuria was regulated by an autosomal dominant gene. As with Coe *et al.* (1979), they found two families wherein both parents were normocalciuric and only 2 of 10 siblings were hypercalciuric instead of the 5 expected, a departure approaching significance ($\chi^2 = 3.6$, p <0.059). Pooling probabilities from the two studies (Fisher, 1970), the deficiency of hypercalciuric siblings was significant ($\chi^2 = 11.4$, p = 0.023).

The two types of exceptions seen in these two studies are compatible with the codominant hypothesis. Regarding normocalciuric parents having hypercalciuric offspring, that would be expected when the parents are both intermediates. Such parents are expected to have low, intermediate and high (hypercalciuric) offspring in a 1:2:1 ratio. The deficiency of hypercalciuric siblings would arise in families wherein one parent was hypercalciuric and the other parent was a low excretor. These families would produce no hypercalciuric, only intermediate excretors. On the other hand, when a hypercalciuric parent has an intermediate excetor spouse, half the children would be hypercalciuric. Hence, in a group of families comprised of an admixture of these two types, one would expect a deficiency of hypercalciuric offspring. Thus normocalciuric parents having hypercalciuric offspring and the deficiency of hypercalciuric offspring occurring in both studies are reconciled by the codominant hypothesis.

In a more recent study, Nicolaidou *et al.* (1996) also concluded that hypercalciuria was produced by an autosomal dominant gene. They studied the parents and sibs of 40 pediatric hypercalciurics patients. They had an extreme deficiency of hypercalciuric sibs, only 2/49 being hypercalciuric. Both parents were normocalciuric in 21 families and the authors interpreted these sporadic

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Table 3. Observed numbers of matings where neither, one or both parents were hypercalciuric among those producing hypercalciuric offspring as seen by Nicolaidou *et al.* (1996) compared with expected numbers based on gene frequencies of Goodman *et al.*, $p(Ca^L) = 0.68$, $q(Ca^H) = 0.32$ (1995).

Parental Hypercalciuria		Obs	Exp.	Calc.	Exp.	χ^2
Phenotype	Genotype	No.	Prop.	Prop.	No.	
Neither	Ca^LCa^H x Ca^LCa^H	21	p^2	0.4624	18.5	0.338
One	Ca^LCa^H x Ca^HCa^H	15	$2pq$	0.4352	17.4	0.331
Both	Ca^HCa^H x Ca^HCa^H	4	q^2	0.1024	4.1	0.002
-						
Totals		40	1	1.0000	40.0	0.671*

* P > 0.3

hypercalciuric offspring as representing another subtype of hypercalciuria or as possibly new mutations. To entertain a mutational hypothesis would require a mutation rate higher than any known in humans. Again, their observations are compatible with the codominant hypothesis and provide an opportunity to test that hypothesis using the parental matings observed. Since these families were selected through the hypercalciuric child in each family, their parents represent a sample of the three types of matings producing hypercalciuric offspring in the general population, intermediate x intermediate, intermediate x high and high x high. The proportions of these matings expected, in Hardy-Weinberg terms, are p^2, $2pq$ and q^2 respectively (Elandt-Johnson, 1971). Using our estimates of the frequencies of $p = 0.68$ and $q = 0.32$, it can be seen in Table 3 that the observed and expected number of matings of the three types agree quite well. This fit not only supports the codominant hypothesis but also suggests that the frequencies of the calcium excretion alleles are not markedly different in Greece from those for U.S. Caucasians.

Discussion of the Hypothesis and its Implications

Some readers will recognize that our sample of 101 normal subjects is small for demonstrating that the previously recognized normocalciuric group is comprised of two subgroups even though we collected multiple 24-hr urine specimens. However, we interrupted collecting from additional subjects until we could examine our data and the resulting distribution. Once it became apparent that we would not obtain complete separation of excretor subgroups, presumably largely because of dietary variations, we began examining dietary control in an effort to produce discrete separations between subgroups. Some decrease in variability was seen when calcium, sodium, magnesium, protein, oxalate were controlled (unpublished results) but it soon became evident that control of dietary variation was best obtained using formula diets. Hence, it did not seem wise to collect additional data from subjects on self-selected diets, but to publish results to date since they provide considerable support for our hypothesis and open a new avenue for further research by ourselves and others.

Urinary calcium excretion data collected from our 101 volunteers have been shown to satisfy three major criteria for a single locus component of a polygenic system, between individual variability exceeding that within individuals, a Hardy-Weinberg equilibrium test and segregation analysis in a limited sample. The codominant hypothesis proposed received additional support from matings collected independently by Nicolaidou *et al.* (1996). The data also conform to polygenic component expectancies in that they exhibit additivity (Holmes *et al.*, 1994). Since all three proposed genotypes are present in both the general population and among stone formers, the higher excretion allele is neither a sufficient nor a necessary cause of oxalate stone disease as is true for polygenic components. Additional genetic evidence that it is a causal element in oxalate stone disease is provided by the dosage effect seen in relative risks. These risks are estimated presently at 2.00 for a single dose and 5.74 for a double dose (Goodman *et al.*, 1995). Biochemical knowledge that calcium is a principal constituent of oxalate stones and of the urine in which they form is equally important in establishing causality. A so-called marker gene could exhibit comparable transmission

and a higher frequency among stone formers but would not be expected to exhibit a dosage effect. Hence, establishing causality in polygenic complexes depends as greatly on biochemical or physiological knowledge as it does on genetic inference.

The value of recognizing the existence of three excretor groups for future research on calcium intake, excretion, and balance is that such studies can be more powerful when the genetic noise included when one studies admixtures of intermediate and low excretors can be eliminated. The variance of a genetically heterogeneous group will generally exceed that of a homogeneous group; hence elimination of that heterogeneity will mean smaller samples will yield answers comparable or superior to those of larger, more heterogeneous samples.

Identifying the individual genetic components in a polygenic complex and studying the risk of stone disease contributed by each component is important for both research and clinical purposes. Our results suggest that not only urinary calcium but also the amount of oxalate excreted in urine is under substantial genetic influence contributing to differences between individuals (unpublished results). This conclusion was reached using the approach documented above for calcium as a paradigm. We believe that citrate excretion is also significantly influenced by genetic factors, particularly because the inter-individual variability significantly exceeds the intra-individual variability and by the association of hypocitraturia with oxalate stone disease (Goodman *et al.*, 1995). However, we have been unable as yet to define conditions that reduce intra-individual variability in citrate excretion to a level that permits confident identification of subgroups in the normocitraturic population.

It is possible that the excretion of inhibitors or promoters of calcium oxalate crystallization is genetically influenced as has been suggested for Tamm-Horsfall protein (Hess *et al.*, 1991). However, apart from citrate, neither the excretion of the amount of a particular inhibitor/promoter nor its form (e.g., γ-carboxylation) has been shown to be clearly associated with an increased risk of stone disease. Our current estimate is that leading genes at 3 loci are almost certainly involved in the development of oxalate stone disease. However when contributions from a 4th locus at comparable frequencies to those of the first 3 are included in the computer model, very little room remains for the effects of environmental factors which are almost surely significant causal elements. Very high frequency alleles for susceptibility could fit and complications which might be revealed by further studies might alter the presently perceived limits on the number of participating loci. We have not included the locus associated with fasting hypercalciuria because this trait may well be a recessive without phenotypic effects in the heterozygous state (Holmes

et al., 1994).

The relative risks we have assessed thus far suggest that intermediate calcium excretors (Holmes *et al.*, 1994) and intermediate oxalate excretors (unpublished results) are also at increased risk of forming stones and, thus, should be considered for therapeutic intervention. *A priori*, effective therapy for those with a single dose of the susceptible gene should be more readily attained than for those with a double dose. When genotyping for the loci involved with oxalate and citrate excretion has been established, prediction of risk for offspring or other relatives of stone formers will become more precise. Once susceptibles are accurately identifiable, more effective therapies should follow and prevention of the first stone may become a reasonable goal.

It is important to realize that one need not successfully treat all of the genetic components creating susceptibility in the individual patient. Many sibs of stone formers who never form stones can be shown to have only one or two fewer doses of susceptible alleles than their stone-forming sibs. It can be shown that about half of all susceptible genes in the population of males occur in men who will never form stones because they have too few doses (two or fewer).

The strategy employed above for dissecting out the individual components of a polygenic system is a return to first principles. The codominant hypothesis selected is one favored by classic studies of polygenic inheri-tance. As students of oxalate stone disease we are advantaged by the ready availability of urine and by the fact that it is the interaction between urinary constituents which leads to stone disease. However, it seems probable that study of other accessible liquids and solids including blood and its formed elements, gastric juice, cerebrospinal fluid and feces could reveal the subtle differences expected to be associated with other common disorders were one prepared to accept the absence of high correlations and the presence of the same phenotypes but differing proportions of them among healthy as well as diseased populations. Thus we believe that the codominant pair of alleles we have detected can serve as a paradigm for the study of other loci involved in calcium oxalate stone disease and for other multifactorial disorders as well.

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Appendix A. Characteristics distinguishing monogenic from polygenic disease states. Certain attributes are corollaries of more fundamental differences.

Monogenic

Polygenic

1. Gene or gene pair generally sufficient and necessary cause of disease

Individual genes neither sufficient nor necessary causes

2. High correlation between presence of gene and occurrence of disease

Low correlation between presence of gene and occurrence of disease

3. Genes of large clinical effects; not rarely may produce fatal or seriously handicapping condition

Genes individually produce no clinical signs nor symptoms in single *or* double doses

4. At phenotypic level, most genes are dominant or recessive

Genes generally exhibit codomininance reflecting additive effects; may mimic dominant or recessive inheritance

5. Genes may have large metabolic effects, e.g., genotypes *TT*, *Tt* and *tt* may yoield enzyme activities of 100, 50 and 0%, respectively

Small metabolic differences between resistant and susceptibe alleles, e.g., 10% differences in fractional excretion between susceptible and resistant genes

6. Recurrence risks generally the same for all families, e.g., 50% for dominant and 25% for recessive inheritance

Recurrence risks vary widely between families ranging from near zero to 100 percent. May mimic monogenic inheritance when near 25 or 50%

7. Gene frequencies uncommon to rare, e.g., recessive phenylketonurea, 1/60 carriers, 1/14,000 has double dose. Dominants generally < 1/5,000

Susceptible genes for common diseases very common, e.g., about 1/3 of all alleles in population at locus regulating calcium excretion are suceptible genes

8. Genes are defective pathological old or new mutant alleles

Individual genes are normal polymorphic (frequency of 2 or more alleles > 2%) alleles and generally is not known whether susceptible or resistant is more recent mutant

9. Double dose of recessive and single dose of dominant gene rarely found in normal persons unless late-manifesting genes

Single and double dose of susceptible alleles must be found in normals, som of whom will never develop the associated disorder (lack other genes for susceptibility, environmental factors or both)

10. Phenotype generally little influenced by environmental factors unless therapy is available as is phenylketonurea or when genes create sensititvity to exogenous agents such as sunlight in xeroderma pigmentosum

Phenotype often heavilty influenced by environment, one fact making accurate genotyping from phenotype difficult

Appendix B: Glossary

alleles	genes found at the same location on a given chromosomal pair; may be the same or different; if more than 2 occur in the population are called "multiple alleles"
autosomal	referring to any chromosome other than the sex chromosomes.
codominant	describes a pair of alleles both of which manifest themselves in the heterozygous phenotype. A person with the ABO blood type AB has received one I^A and one I^B allele which are both manifest in the phenotype.
genotype	the genetic constitution of an individual at a single locus or at a group of loci.
Hardy-Weinberg equilibrium	the condition described by the Hardy-Weinberg law which states that if the frequency of one gene is p and of its allele is q, then the frequency at equilibrium of the three genotypes possible are given by the equation: $p^2 + 2pq + q^2 = 1$
monogenic	refers to modes of inheritance involving a single locus, i.e., autosomal dominant, recessive and X-linked recessive inheritance
multifactorial	refers to disorders produced by more than a single factor whether those factors are environmental or genetic. Most common diseases result from combination of a polygenic complex which creates susceptibility and environmental factors which make the susceptibility manifest in frank disease
phenotype	a trait or attribute of the individual as observed, measured or determined in some way. May reflect the effects of genes, environmental factors or both
polygenes	the individual genic components in polygenic inheritance. Classically described as genes of small additive effects, but need not be additive at phenotypic level
polygenic	describes a group of two or more gene loci the alleles of which influence a single trait or disease
segregation	the separation of the gene pairs present in a parental somatic and primary germ cells such that a gamete and thereby any offspring receives only one member of that gene pair

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Discussion with Reviewers

S.G. Scheinman: My only significant reservation about this paper is the discussion of "polygenic inheritance" on the one hand, and "a codominant pair of alleles influencing calcium excretion" on the other. If the authors consider hypercalciuria to be one set of alleles among other unrelated abnormalities each of which contribute to oxalate stones, then hypercalciuria itself can be determined by dominant, recessive or codominant alleles, an issue that can be addressed by proper analysis of data. However, if hypercalciuria itself is a "polygenic" trait, it may result from contributions from alleles at multiple genetic loci, and thus may not fit even a codominant model.

Authors: The authors definitely believe that a codominant pair of normal alleles has a primary influence on calcium excretion. The 3 studies invoking autosomal dominance for hypercalciuria were all incompatible in the same two regards and similar considerations also make recessive inheritance unlikely. However, the transmission of hypercalciuria mimics dominant inheritance in some families and recessive in others. Regarding the possibility that hypercalciuria is itself a polygenic trait, the possibility seems remote in the light of the perceived antimodes. Typically, a polygenic trait tends to exhibit a normal or quasi-normal distribution.

L.C. Cao: According to the definition of the term polygene (that is "Any of a particular group of genes that determine a phenotype in which the effects of individual genes are not discernable. Called also cumulative gene"), polygenic inheritance is quite different from monogenic. It is interesting in that you present here a model with simplified 3-loci and two codominant alleles at each locus for idiopathic oxalate stone disease. My questions are: (1) Do you think the presented codominant hypothesis needs to be proved by molecular genetics? (2) How can we define the number of genes involved in calcium oxalate nephrolithiasis and the number of susceptible alleles occurring at each locus?

Authors: Regarding question (1), recall that the existence and mode of inheritance of thousands of genes had been determined through biochemical, segregational and gene frequency analyses prior to the advent of molecular genetics and have been in almost all cases confirmed by molecular biology though some remarkable complexities have been revealed that were not anticipated. Confirmation of our hypothesis by molecular genetics would be most useful since once probes were available, one could accurately genotype patients from a single blood specimen without the necessity for several days of rigorous dietary control. Our new study includes collection of blood specimens for the molecular identification of the alleles involved. Regarding how we

can estimate the number of loci involved, this is possible from application of the 3-locus Hardy-Weinberg model. This model calculates the proportion of susceptible persons in the population from the gene frequencies for each locus entered. This datum can be compared with available estimates of the frequency of stone formers in the population derived from lifetime risks, 23.5% (Resnick *et al.*, 1968) and with observed prevalence data, for example, 18% among elderly Swedish males (Ljunghall, 1978). The estimated proportions of susceptibles from the model must always exceed the latter two estimates of the number of stone formers because some susceptibles will not have completed exposure to risk, others will have died of other causes before manifesting their susceptibility and still others will never express their diathesis because they have not been exposed to sufficient precipitating environmental factors. When four loci with the same gene frequencies as that for hypercalciuria are inserted in the model, the proportion of susceptibles from the model agrees with an observed prevalence among 60 year old Swedish males and is smaller than the estimate from data of Resnick *et al*. These observed estimes leave no room for the influence of environmental factors whereas the increase in the frequency of oxalate stone disease in the past several decades as mentioned by Dr. Nakagawa below tells us that environmental factors do play a significant role. Regarding our choice of only two alleles, systems with 3 relatively common alleles produce 6 different genotypes and if the phenotype is quantitatively determined, large sample data would be expected to yield a quasi-normal distribution as is true for erythrocyte acid phosphatase activities, no antimodes being detectable (Harris, 1970).

Y. Nakagawa: You tried to dissect out genes for hypercalciuria as a causal element of kidney stone disease. This issue is not as simple as observed in other hereditary diseases. Recent reports indicated that stone disease in Japan has been increasing annually for the past 40 years and the authors contend that this increase is due to acquired environmental factors (Koide *et al.*, 1986). In my experience genetic factors for a promoter and/or an inhibitor of crystallization might be more important than high calcium excretion.

Authors: It is possible that a fourth leading gene locus may be involved in oxalate stone disease and a locus regulating differences in amount or type of inhibitor and/or promoter surely seems a logical candidate. However, to qualify, such candidates must exhibit increased relative risks, satisfy several genetic criteria as did our calcium data, most likely exhibit dosage effects, and be causally related to stone disease at the biochchemico-physiological level. To our knowledge, none of the proteins polymorphisms detected thus far have satisfied all of these criteria. Modifying genes of lesser effects than the leading genes almost certainly exist.

L.C. Cao: Three abnormal excretions (Ca, Ox and Cit) as phenotypes were proposed in this study. Do you think it is possible to have three independent genes that separately lead to the different phenotypes (Ca, Ox and Cit)? Considering the giant system of the human body, do you think that additional genetic factors as mentioned by Dr Nakagawa could be involved in oxalate stone disease?

Authors: We think that three or four independent loci provide the leading or principal genetic causal elements in oxalate stone disease. Other uncommon or rare genes may contribute such as heterozygosity for one of the cystinuria genes and modifying genes of lesser effects may contribute but the sum of the products of all the genetic and environmental factors (all susceptible persons in the population) cannot be smaller than the observed frequency of stone formers, and that is why we believe that the limits for leading genes are as few as 3-4 loci. It is possible that the genetic components are not entirely independent at the physiological level; e.g., high intestinal absorption of calcium may increase the amount of free oxalate in the gut, increasing absorption of oxalate. However, we found no correlation between mean g Ca/g Cr and mean g Ox/g Cr.

L.C. Cao: Though many genes relating to other types of human urolithiasis have been cloned, mutations identified and a causal role established between genotype and phenotype, some of these genes are still obscure. For instance, the X-linked recessive hypercalciuria genes have been mapped to Xp11.22, and you also mentioned vitamin D receptor (VDR) alleles relating to hypercalciuria and bone mass. How do you think the CLCN5 gene that has been identified is associated with renal chloride transport (Lloyd *et al*., 1996)? This gene was found recently to relate to calcium urolithiasis, but to date there is no clinical evidence showing the linkage between renal chloride transport and hypercalciuria in patients with oxalate stone disease.

Authors: It is possible that polymorphism at the CLCN5 locus may be associated with differences in calcium excretion. Presently, too little is known about its expression in tissues including the kidney, its cellular function and its relationship to calcium excretion. Our impression is that if it is related, it will be to fasting hypercalciuria in which the effect is apparently at the renal level.

L.C. Cao: Do you believe your model may really stimulate the progress of genetic studies on urolithiasis or do you think the approach using molecular biology for disclosing the etiology of idiopathic oxalate urolithiasis may be more important than your current effort?

Authors: We believe both approaches are exceedingly useful but both have strengths and weaknesses. The molecular approach will ultimately provide the more precise genotyping but will, with present knowledge, be less useful for the development of diet or drug therapies than the biochemico-physiological approach. Further, one cannot anticipate using the same therapy at the genic level for the common *normal* polymorphic alleles in multifactorial disorders as is so easily envisioned but difficult to accomplish for monogenic disorders wherein we know the genes are defective. The rigid criteria established for genic therapy in monogenic disorders tells us that geneticists and molecular biologists recognize the potential dangers in altering human DNA. Further, it is already apparent that knowledge of the location and sequence of a gene does not lead to a quick fix for the disorder regulated by that gene. Recall that the amino acid sequence for sickle hemoglobin has been known for over 5 decades and that knowledge has had essentially no impact on therapy for sickle cell anemia.

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