Genetic Hypercalciuria

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Hypercalciuria is an important, identifiable, and reversible risk factor in stone formation. The foremost and most fundamental step in dissecting the genetics of hypercalciuria is understanding its pathophysiology. Hypercalciuria is a complex trait. This article outlines the various factors that compromise the attempt to dissect the genetics of hypercalciuria, summarizes the clinical and experimental monogenic causes of hypercalciuria, and outlines the initial results from attempts in studying polygenic hypercalciuria. Finally, the problem is set in perspective of the current database, technologic advances and limitations are highlighted, and prospects of further advances in the field are speculated upon.

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Pathophysiologic Considerations

Biomineralization: Crystallization of Calcium Salts as a Physiologic Event

“Calcium stone formation” is mineral crystallization in body tissue or fluid. The deposition of inorganic minerals, crystalline or noncrystalline, around biomolecules is universal in biology, where inorganic crystals are harnessed to become an integral part of organic tissue to provide hardness and strength. These inorganic substances are capable of reversible interaction with biomolecules so that the crystalline structures can be remodeled for physiologic needs. Williams (9) discussed the unique ability of calcium to interact with biomolecules from the point of view of its concentration, binding strength, rate constants, and molecular structure. Calcium salts have highly adaptable coordination geometry that greatly facilitates binding of calcium, in its solid state or solution, to the irregular geometry of proteins.

The remarkable material properties of bones and teeth result from the activities of proteins that function at the organic–inorganic interface (10). Proteins have evolved domains that specifically interact with calcium crystals. The aspartate-phosphoserine-phosphoserine-glutamate-glutamate (DpSpSEE) motif described in the saliva protein statherin is also found in other calcium–interacting proteins, such as osteopontin (11, 12). Unlike the EF hand that binds ionic calcium, this DpSpSEE motif binds solid-phase calcium phosphate crystals and is conserved down to invertebrates such as crustaceans. This motif is expressed in a protein at the foot pad that allows the mussel to attach to calcareous substratum (13).

More than 200 yr ago, Hunter (14) articulated about the similarity between stone formation and calcification. He pointed out the equivalence of enamel, eggshell, gallstones, and kidney stones. Thus, mineralization can be arbitrarily divided into physiologic and pathologic. The partition is not always distinct but can be distinguished on the basis of favorable versus
undesired consequences to the organism. Physiologic crystallization includes formation of exoskeleton, pearl, endoskeleton, and dentition, whereas pathophysiologic crystallization includes pyrophosphate arthropathy, pigmented gallstones, vascular calcification, and urolithiasis. Pathologic calcium crystallization is a physiologic process in the wrong place and the wrong time.

**Multiorgan Approach to Hypercalciuria**

Because isolated hypercalciuria *per se* is not detrimental, a clinician’s interest in hypercalciuria concerns the complications that include mainly nephrolithiasis and nephrocalcinosis and perhaps also less morbid conditions such as hematuria (15) and possibly polyuric enuresis in children (16). The pathophysiology of calcium nephrolithiasis *versus* nephrocalcinosis is incompletely understood, but the reader is referred to a recent review by Sayer *et al.* (17). It is not the purpose of this review to tackle the debate about the relative importance of hypercalciuria *versus* hyperoxaluria in calcium oxalate stones, but a recent study clearly demonstrates that urinary calcium and oxalate are equally important for stone formation (18).

A majority of patients with this condition have been categorized as “idiopathic hypercalciuria.” The term is decades old and seemed to have secured an indelible place in medical idiom. Although linguistically correct without doubt, it carries an implicit presumption that this is a single condition that is inevitably incorrect and furthermore takes on certain overtones of futility in unraveling the underlying cause. Although the use of this term is convenient in common clinical lingo, one must caution the absolute and unquestioned acceptance of this term as a “diagnosis.” Calcium homeostasis involves multiple organs by predominantly endocrine mechanisms and fluxes through three target organs in concert effect calcium homeostasis. Any analysis of hypercalciuria should at least take the three organs into consideration. Pak *et al.* pioneered this attempt back in 1975 by introducing a tripartite classification of absorptive, resorptive, and renal hypercalciuria (19). From a pathophysiologic and genetic point of view, this is a very important classification.

Figure 1 depicts three scenarios in which in each, a primary disturbance occurs in one of the three calcium homeostatic organs. Figure 1A shows an example of pure renal leak. Although a lot of causes of hypercalciuria such as acid load–induced hypercalciuria has a component of renal leak, multiple mechanisms often come into play. Pure renal hypercalciuria can be seen in mice with deletion of the TRPV5 calcium channel gene (20). Compensatory intestinal hyperabsorption and bone resorption maintains serum calcium at normal levels (20). It is understandable how the usual battery of clinical tests at the outpatient setting may not recognize the hypercalciuria as a renal leak. One has to possess some or all of the following data: (1) Serum parathyroid hormone and 1,25-(OH)2 vitamin D are higher than expected; (2) hypercalciuria is inappropriate for the slightly elevated parathyroid hormone, normal serum calcium, and normal filtered calcium; (3) persistent hypercalciuria is present even during fasting; and (4) increased bone resorption markers and/or reduced bone mineral density. This battery of information may not be feasible in clinical practice with the time, infrastructure, and financial constraints. However, it is absolutely imperative that one not abandon the quest for a complete definition of pathophysiology and strive to uncover a proximal (closer to the underlying lesion) phenotype.

Figure 1, B and C, illustrates two additional scenarios with increased bone resorption and increased gut absorption as primary disturbances that cause hypercalciuria. Although the un-
underlying causes are diverse and the physiologic profiles are completely different, all three situations culminate in hypercalciuria. Some have argued that because it is so difficult to separate and characterize the defects in these organs, one should forego that and simply aggregate everything into a pot of heterogeneous conditions. We submit the counter argument that unless one continuously strives to dissect and classify on the basis of pathophysiologic characteristics, it will be hopeless to advance our understanding.

**Dissecting the Genetics of Hypercalciuria**

Approximately half of the patients who are labeled as having idiopathic hypercalciuria have a positive family history of kidney stones (21). Familial idiopathic hypercalciuria has been described as an autosomal dominant trait in the earlier literature. This was a de facto conclusion based on the observation that it did not match a recessive pattern, and the male-to-male inheritance ruled out gender-linked transmission (22–26). This is clearly an oversimplification of the genetics of familial hypercalciuria. Not all genetic diseases can be dealt with in a Mendelian approach. Some unavoidable ambiguity in both genotyping and phenotyping notwithstanding, it is usually possible to discern whether one has clinical autosomal dominant polycystic kidney disease and whether one has mutations in polycystin-1 or -2. Such definitive assignments are not possible for hypercalciuria because of a number of immanent impediments. Hypercalciuria is a complex trait that cannot be slotted into classical Mendelian categories. The inherent difficulties of dissecting the genetics of hypercalciuria are schematically summarized in Figure 2. As the central tenet to all genetic approaches involves some form of genotype-phenotype correlation, phenotypic ambivalence can deliver a cataclysmic blow to the effort. Figure 2A depicts the breakdown of the classical Mendelian perfectly co-segregating “one gene locus—one phenotype” paradigm. Each of the following characteristics of hypercalciuria imparts equivocation in the genotype-phenotype correlation (Figure 2B).

**Continuous Variable**

Whereas the presence or absence of a calcareous calculus in the urinary tract can theoretically and practically be classified as a “yes-no” event, the more proximal and more important phenotype, hypercalciuria, cannot assume such binary status. Akin to parameters such as BP and body mass, urinary calcium excretion is a continuous variable. Although there is little difficulty in assigning disease status at the extreme ends of the spectrum, there is really no sharp distinction of margins. “Disease” in this case refers to the bearing of increased statistical risk of nephrolithiasis and/or nephrocalcinosis. Hypercalciuria is an example par excellence of a quantitative trait (Figure 2). Further compounding the lack of discrete boundary is that the same amount of calcium in a 24-h urine sample can have completely different implications depending on the urine volume; pH; concentration of all of the calcium-interacting anions such as citrate, phosphate, and oxalate; and concentration of promoters and inhibitors of crystallization. Not to mention that each one of these other factors is also a continuous variable.

Although there is no good way of creating a sharp distinction when there is none, one can partially circumvent the problem by analyzing relative supersaturation of calcium oxalate and phosphate in addition to the amount of calcium in clinical samples.

**Secondary and Compensatory Effects**

As discussed above, hypercalciuria can be accompanied by an extensive panoply of effects that are downstream from or as compensatory responses to the primary disturbance. This renders the use of hypercalciuria singularly as a phenotypic variable very risky. As shown in Figure 1, three completely different syndromes all share the feature of hypercalciuria. Unless one commits extra effort to dissect out and identify the primary versus secondary and compensatory changes, using one parameter as “phenotype” will severely undermine the genotype-phenotype correlation.

**Phenocopy**

There are a host of nongenetic factors that can affect gut calcium absorption, bone calcium release, and renal calcium handling. The cartoon in Figure 2A displays some dietary (rep-
resenting completely nongenetic) factors that can influence urinary calcium excretion (27). Other than a dietary history, which is semiquantitative at best, it is very difficult to rule out increased dietary calcium intake as a cause for hypercalciuria. The cartoon also shows that high dietary protein (source of acid) and sodium can also cause “physiologic hypercalciuria” (27,28). In these instances, one can at least estimate from the 24-h urine sample the impact of salt (urinary Na⁺/H⁺) and acid (urinary NH₄⁺/H⁺ and SO₄²⁻) on urinary calcium. As it is difficult to collect and analyze 24-h urine under the luxury of dietary control in the setting of a clinical practice, metabolic studies in the setting of a clinical research center are indispensable. In addition to dietary factors, there are multiple other nongenetic factors that affect intestinal calcium absorption and bone formation/resorption (not covered in Figure 2A). The only safeguard against phenocopic artifacts is sound knowledge and data in pathophysiology.

### Polygenic Influence

Adopting an exaggerated view for the sake of emphasizing a point, one can make the extreme statement that there is no such condition as a pure monogenic disease. Consider some quintessential monogenic human diseases. Two different individuals may have the identical mutation in cystic fibrosis transmembrane regulator (CFTR) and yet the clinical severity in cystic fibrosis can differ vastly (29). Similar can be said for the same mutation in β-globin causing very mild or very severe sickle cell anemia (30). There is no doubt that modifier genes are at work. Consider the idealized situation in the laboratory, where one specifically deletes one and only one gene in a murine

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**Table 1. Monogenic diseases that lead to hypercalciuria: Intestine as the primary defect**

<table>
<thead>
<tr>
<th>Disease</th>
<th>OMIM</th>
<th>Gene/Gene Product</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital lactase deficiency</td>
<td>223000</td>
<td>Unknown, autosomal recessive, locus linked to 2q21</td>
<td>Hypercalciuria, nephrocalcinosis, hypercalcemia (33)</td>
</tr>
<tr>
<td>Congenital sucrase-isomaltase deficiency</td>
<td>222900</td>
<td>SI/sucrase-isomaltase</td>
<td>Hypercalciuria. Nephrocalcinosis, Hypercalcemia (34,35)</td>
</tr>
<tr>
<td>Glucose/galactose malabsorption</td>
<td>606824</td>
<td>SLC5A1/sodium-glucose co-transporter</td>
<td>Hypercalciuria, renal calculi, nephrocalcinosis hypercalcemia, glucosuria (36–38)</td>
</tr>
<tr>
<td>Blue diaper syndrome</td>
<td>211000</td>
<td>Unknown, autosomal recessive or X-linked, possibly SLC16A10/T-type amino acid transporter (39)</td>
<td>Hypercalciuria, nephrocalcinosis, hypercalcemia, indicanuria (40)</td>
</tr>
<tr>
<td>Hypophosphatemia and absorptive hypercalciuria (type 3)</td>
<td>182309</td>
<td>NPT2/sodium-phosphate co-transporter (41)</td>
<td>Phosphate wasting, hypercalciuria, renal calculi (41)</td>
</tr>
<tr>
<td>Hereditary hypophosphatemic rickets with hypercalciuria</td>
<td>241530</td>
<td>Gene unknown, NPT2 locus excluded (42,43), autosomal recessive</td>
<td>Phosphate wasting, hypercalciuria (44)</td>
</tr>
<tr>
<td>Williams-Beuren syndrome (not monogenic)</td>
<td>194050</td>
<td>7q11.23 deletion, ELN/elastin, LIMK1/LIM domain kinase 1, RFC2/replication factor C, subunit 2</td>
<td>Hypercalciuria (45–47)</td>
</tr>
<tr>
<td>Hyperabsorptive hypercalciuria (not monogenic)</td>
<td>607258</td>
<td>4q33-qter deletion</td>
<td>Hypercalciuria, nephrocalcinosis (48,49)</td>
</tr>
<tr>
<td>Down’s syndrome (not monogenic)</td>
<td>190685</td>
<td>Trisomy 21</td>
<td>Hypercalciuria (50–56)</td>
</tr>
</tbody>
</table>

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model with a completely homogeneous (as far as one can tell) genetic background. This is probably the closest example that one can fathom of a pure monogenic disorder. It is already well known that the same gene knockout in the background of different strains can have completely different effects. As the investigator delights with the emergence of the good news that 80% of the null mice survives and hence can be studied, one should wonder about the effect of the gene deletion on the 20% of the mortal ones. Penetrance of a singular gene may be small or even negligible, but multiple genes in concert can have a significant impact on the phenotype. Envision five hypothetical genes that have an impact on urinary calcium excretion. Each of these genes is polymorphic in Homo sapiens with multiple fully functional alleles that have small quantitative differences in function. For instance, gene product 1 influences intestinal calcium absorption, gene product 2 modifies the ability of dietary acid to release calcium from bone, gene product 3 controls paracellular calcium permeability in the thick ascending limb, gene product 4 influences calcium absorption in the distal convoluted tubule, and gene product 5 alters the sensitivity of the renal calcium sensing receptor to plasma calcium concentration. If each of these gene products contributes to a trivial 0.5 mmol/d (20 mg) increase in urinary calcium excretion, then inheriting all five predisposing alleles can theoretically raise urinary calcium by 2.5 mmol/d (100 mg).

Loci Heterogeneity

Similar reasoning along the lines of polygenic influence is loci heterogeneity. Different underlying genetic lesions can start the pathophysiologic cascade in vastly different manners, but by the time the lesion reaches the whole organism (clinical) level, they may have converged on an end organ in a very restricted manner. Renal cysts in humans can result from mutations in ADPKD1, ADPKD2, or ARPKD (31). In animals, there is an enormous collection of monogenic mutations that converge into a final common phenotype of renal cyst formation (32). As depicted in Figure 2B, two individuals with identical phenotypes may actually have no overlap whatsoever in their underlying disease-causing genetic loci. Thus, launching a genetic search from hypercalciuria as a starting point may lead an investigator to multiple and confusing destinations. However, these different loci will likely have a different pathogenic pathway en route to hypercalciuria and will manifest with different proximal phenotypes.

The complex nature of hypercalciuria is addressed in more detail below. Before one embarks on this formidable exercise,
Table 3. Monogenic diseases that lead to hypercalciuria: Kidney as the primary defect

<table>
<thead>
<tr>
<th>Disease</th>
<th>OMIM</th>
<th>Gene/Gene Product</th>
<th>Phenotype</th>
<th>Renal</th>
<th>Gastrointestinal</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dent’s disease (X-linked recessive nephrolithiasis, X-linked hypophosphatemic rickets, low molecular weight proteinuria)</td>
<td>300009</td>
<td>CLC5/chloride channel 5</td>
<td>Hypercalciuria, hyperphosphaturia, tubular proteinuria, Fanconi syndrome, nephrocalcinosis, renal calculi (70,71)</td>
<td></td>
<td></td>
<td>Rickets</td>
</tr>
<tr>
<td>Lowe oculocerebrorenal syndrome</td>
<td>309000</td>
<td>OCRL/inositol polyphosphate 5 phosphatase</td>
<td>Hypercalciuria, aminoaciduria, phosphaturia, Fanconi syndrome, nephrocalcinosis, renal calculi (72,73)</td>
<td></td>
<td></td>
<td>Vitamin D-resistant rickets</td>
</tr>
<tr>
<td>Bilateral macular coloboma with hypercalciuria</td>
<td>248190</td>
<td>Unknown probably autosomal recessive</td>
<td>Hypercalciuria (74)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td>277900</td>
<td>ATP7B/copper-transporting ATP-ase</td>
<td>Hypercalciuria, renal calculi, hyperphosphaturia, partial Fanconi syndrome, proteinuria, distal tubular acidosis (75–81)</td>
<td>Liver disease</td>
<td></td>
<td>Osteoporosis (82)</td>
</tr>
<tr>
<td>Tyrosinemia type 1</td>
<td>276700</td>
<td>FAH/fumarylacetacetase</td>
<td>Hypercalciuria, Fanconi syndrome, nephrocalcinosis, renal calculi (83)</td>
<td></td>
<td></td>
<td>Hepatomegaly, liver disease</td>
</tr>
<tr>
<td>Glycogen storage disease type 1a</td>
<td>232200</td>
<td>G6PC/glucose-6-phosphatase</td>
<td>Hypercalciuria, Fanconi syndrome, hypocitraturia, nephrocalcinosis, proteinuria (84,85)</td>
<td></td>
<td></td>
<td>Hepatomegaly, poor growth, hypoglycemia</td>
</tr>
<tr>
<td>Familial hypomagnesemia with hypercalciuria and nephrocalcinosis</td>
<td>248250</td>
<td>PCLN-1/paracellin-1 or claudin 16 (87)</td>
<td>Hypercalciuria, magnesium wasting, nephrocalcinosis (88–90); heterozygotes more prone to renal calculi (91)</td>
<td></td>
<td></td>
<td>Osteopenia, fracture</td>
</tr>
<tr>
<td>Bartter syndrome type 1</td>
<td>601678</td>
<td>NKCC2/sodium-potassium-chloride co-transporter</td>
<td>Hypercalciuria, nephrocalcinosis, metabolic alkalosis (92)</td>
<td></td>
<td></td>
<td>Osteopenia (98)</td>
</tr>
<tr>
<td>type 2</td>
<td>241200</td>
<td>ROMK/renal outer-medullary potassium channel</td>
<td>Hypercalciuria, nephrocalcinosis, metabolic alkalosis (93)</td>
<td></td>
<td></td>
<td>Osteopenia</td>
</tr>
<tr>
<td>type 3</td>
<td>607364</td>
<td>CLC-Ka-b chloride channels</td>
<td>Less severe hypercalciuria, no nephrocalcinosis (94)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type 4</td>
<td>602522</td>
<td>Barttin</td>
<td>Congenital deafness (95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type 5</td>
<td>601199</td>
<td>CaSR/calcium-sensing receptor</td>
<td>Overlap between autosomal dominant hypocalcemia and Bartter syndrome (96,97)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
some consideration will be given to monogenic causes of hypercalciuria both in human diseases and in animal models.

**Hypercalciuria as a Monogenic Trait**

Monogenic diseases often conjure up a sense of disinterest (disgust to some) in many practitioners because of their exotic nature and commonly perceived detachment from the everyday reality of medical practice. This is a misconception but a perfectly understandable one. As one peruses a typical catalogue of this nature (e.g., tables in this article), one notices that half of the names of diseases are not recognizable and the other half are not pronounceable, all of which will probably never show up in our clinic. With such pretext, how does one justify devoting the majority of his or her time and effort on such a minority of medical illnesses? The answer lies in the relatively “clean” nature of the primary lesion. These diseases allow one to build upon two irrefutable facts: The mutation of a single gene and an unmistakable phenotype well known and characterized by clinicians as a delineated syndrome. These two facts allow investigators to plant two pillars on solid grounds and work out the elusive black boxes in between.

A number of monogenic causes of hypercalciuria have been identified, and there is no simple classification. Tables 1 through 5 present a summary of these diseases in terms of their effects on the three principle organs of calcium homeostasis. We took the liberty to include experimental monogenic diseases (Table 5) because these man-made monogenic disease models are rapidly becoming more common than naturally occurring monogenic diseases. Tables 1 through 4 classify these disorders into whether the primary lesion primarily ails the gastrointestinal tract, kidneys, bone, or other organs. Regardless of the site of the primary lesion, multiple organs are always involved.

Detailed discussion of each of these conditions is beyond the objective of this review. Some highlights are presented. Gastrointestinal monogenic diseases associated with hypercalciuria are uncommon (Table 1) (33–58). There is a group of monogenic diseases in which intestinal calcium hyperabsorption has been

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**Table 3. Continued**

<table>
<thead>
<tr>
<th>Disease</th>
<th>OMIM</th>
<th>Gene/Gene Product</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal dominant hypocalcemia</td>
<td>601198</td>
<td>CaSR/calcium-sensing receptor (activating mutations)</td>
<td>Hypercalciuria (99,100)</td>
</tr>
<tr>
<td>Familial isolated hypoparathyroidism</td>
<td>146200</td>
<td>CaSR/calcium-sensing receptor (activating mutations)</td>
<td>Hypercalciuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PTH/parathyroid hormone (102–104)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCMB/glial cells missing B (105)</td>
<td></td>
</tr>
<tr>
<td>Familial hypertensive hyperkalemia or</td>
<td>145260</td>
<td>WNK4/protein kinase lysine deficient 4</td>
<td>Hypercalciuria, mild</td>
</tr>
<tr>
<td>pseudohypoaldosteronism type 2</td>
<td></td>
<td>(106)</td>
<td>metabolic acidosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WNK1/protein kinase, lysine deficient 1</td>
<td>Normocalciuria (107)</td>
</tr>
<tr>
<td>Renal tubular acidosis</td>
<td>267300</td>
<td>ATP6V1B1/V-ATPase, β subunit (108)</td>
<td>Nephrocalcinosis, hypercalciuria</td>
</tr>
<tr>
<td>distal AR, with deafness</td>
<td></td>
<td></td>
<td>Hyperabsorption</td>
</tr>
<tr>
<td>distal AR, normal hearing</td>
<td>602722</td>
<td>ATP6V0A4/V-ATPase, α subunit (109)</td>
<td>Nephrocalcinosis, hypercalciuria</td>
</tr>
<tr>
<td>distal AD</td>
<td>179800</td>
<td>SLC4A1/anion exchanger (110,111)</td>
<td>Nephrocalcinosis, renal stones (110)</td>
</tr>
<tr>
<td>mixed RTA</td>
<td>259730</td>
<td>C4II/cartonic anhydrase II (112)</td>
<td>Nephrocalcinosis, urolithiasis (112–114)</td>
</tr>
<tr>
<td>Liddle’s syndrome</td>
<td>177200</td>
<td>SCNN1B and SCNN1G/epithelial sodium channel, β and γ</td>
<td>Hypercalciuria and nephrocalcinosis in two</td>
</tr>
<tr>
<td></td>
<td></td>
<td>subunits</td>
<td>cases (115)</td>
</tr>
</tbody>
</table>
described (Table 1) but the mechanism is undetermined (33–40). A second group of monogenic diseases have vitamin D-mediated intestinal hyperabsorption including primary renal diseases such as renal phosphate wasting (41–44). Finally, there is a group of chromosomal deletion syndromes (not monogenic) in which intestinal hyperabsorption has been described but the mechanisms are unknown (45–58). Monogenic diseases that affect primarily the bone leads to hypercalciuria largely via increased calcium release into the circulating pool (Table 2) (59–69). These can be due to conditions related to structural defects in bone such as osteogenesis imperfecta (59–61), part of a hyperparathyroid state (62–65), or ricket-like bone lesion (66–69).

Primary renal monogenic lesions are summarized in Table 3. Several treatises of a similar nature have been published (5–8), and we do not delve into this topic any further except to highlight a few notions. There is a large group of monogenic proximal tubulopathies with variable features of the Fanconi syndrome that, when full-blown, represent general proximal tubule dysfunction (70–86). The mechanism of hypercalciuria is not understood in these diseases, but this is an excellent example of loci heterogeneity. We elected to classify primary renal phosphate wasting from mutations of the Na-phosphate co-transporter NaPi-IIa in Table 1 because of the compensatory increase in vitamin D. Thick ascending limb tubulopathies involve reduced paracellular calcium permeability (87–91), reduction of driving force (92–98), and the erroneous resetting of physiologic control for calcium absorption (99–105). The distal tubulopathies also represent a heterogeneous group of primary hypercalcurias (106–113). Despite the near identical effects on Na\(^+\), K\(^+\), and H\(^+\) homeostasis by WNK1 and WNK4 mutations, patients with WNK4\(^{G596E}\) mutation have hypercalciuria and low bone mineral density (107), while patients who carry the WNK1 intronic deletion are not reported to have hypercalciuria. The way by which WNK kinase mutations lead to hypercalciuria is not yet clear. The renal tubular acidoses (108–112) present a complex picture of the multiorgan origin of hypercalciuria. In addition to gut and bone compensation, acidaemia per se can increase bone resorption. Finally, a group of hypercalciuric diseases (116–121) with a wealth of mechanisms are awaiting to be explored (Table 4).

Animal models of monogenic diseases are valuable. The genetically altered animals listed in Table 5 have hypercalciuria from targeted deletion of known disease-causing genes (122–127) and genes coding for important calcium homeostatic proteins (20,128–134), and some have hypercalciuria of serendipity (135,136). Animal models using CIC5 knockdown (122) or knockout (123,124) have captured some but not all of the salient features of Dent’s disease. This most likely reflects polygenic modifiers in action. Even in a classical monogenic disorder, there may be some loci heterogeneity as evident by the fact that some patients with classic features of Dent’s do not harbor mutations in CIC5 (137). The variable phenotype of calbindin-28K deletion is another testimony of polygenic modifiers (130–133). An intact animal with a known deliberate gene deletion and a phenotype that bears resemblance to human disease is highly useful for working out the intermediate steps in the pathogenesis.

### Hypercalciuria as a Complex Trait

Calcium nephrolithiasis is a very distal (many steps downstream from the primary lesion) phenotype that can result from

### Table 4. Monogenic diseases that lead to hypercalciuria: Unknown mechanisms

<table>
<thead>
<tr>
<th>Disease</th>
<th>OMIM</th>
<th>Gene/Gene Product</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Renal</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>219700</td>
<td>CFTR/cystic fibrosis transmembrane conductance regulator</td>
<td>Microscopic nephrocalcinosis (116); hypercalciuria (117)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calcium absorption not defined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Decreased bone mineral density, osteomalacia (119)</td>
</tr>
<tr>
<td>β-Thalassemia</td>
<td>141900</td>
<td>HBB/β-globin</td>
<td>Hypercalciuria, proximal tubulopathy (118)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calcium absorption not defined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Decreased bone mineral density, osteomalacia (119)</td>
</tr>
<tr>
<td>Beckwith-Wiedemann syndrome</td>
<td>130650</td>
<td>p57(KIP2)/cyclin-dependent kinase inhibitor 1C NSD1/nuclear receptor binding SET domain protein 1 H19/H19 gene</td>
<td>Hypercalciuria, nephrocalcinosis (120)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calcium absorption not defined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Osteopenia (121)</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>261600</td>
<td>PKU1/phenylalanine hydroxylase</td>
<td>Hypercalciuria (121)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calcium absorption not defined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Osteopenia (121)</td>
</tr>
</tbody>
</table>
Table 5. Animal models of monogenic hypercalciuria

<table>
<thead>
<tr>
<th>Gene</th>
<th>Animal Model</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLC5</td>
<td>Ribosomal knockdown (122) Mouse knockout (123)</td>
<td>Hypercalciuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normocalciuria, hyperphosphaturia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not described</td>
</tr>
<tr>
<td></td>
<td>Mouse knockout (124)</td>
<td>Hypercalciuria, hyperphosphaturia, proteinuria</td>
</tr>
<tr>
<td>NPT2</td>
<td>Mouse knockout (125)</td>
<td>Hypercalciuria, hyperphosphaturia, renal calcifications</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher alkaline phosphatase, retarded secondary ossification</td>
</tr>
<tr>
<td>NHERF-1</td>
<td>Mouse knockout (128)</td>
<td>Male: Normal overall renal function, hypercalciuria, hyperphosphaturia, hypermagnesuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female: Bone fracture, 25–30% reduced bone mineral density, 40% reduced bone mineral content, low serum alkaline phosphatase</td>
</tr>
<tr>
<td>TRPV5</td>
<td>Mouse knockout (20)</td>
<td>Hypercalciuria, hyperphosphaturia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced trabecular and cortical thickness of bones</td>
</tr>
<tr>
<td>VDR</td>
<td>Mouse knockout (128,129)</td>
<td>Hypercalciuria on high-calcium, high-lactose diet</td>
</tr>
<tr>
<td>Calbindin-D28k</td>
<td>Mouse knockout</td>
<td>Normocalciuria, normocalcemia (130) Hypercalciuria (131,132) Normocalciuria (132)</td>
</tr>
<tr>
<td>Calbindin-D28k and VDR</td>
<td>Mouse double knockout (134)</td>
<td>Hypercalciuria, hyperphosphaturia</td>
</tr>
<tr>
<td>NKCC2</td>
<td>Mouse knockout (126)</td>
<td>Hypercalciuria, polyuria, hydronephrosis, proteinuria</td>
</tr>
<tr>
<td>Caveolin-1</td>
<td>Mouse knockout (135)</td>
<td>Hypercalciuria in male, bladder stone</td>
</tr>
<tr>
<td>AKr1b1</td>
<td>Mouse knockout (136)</td>
<td>Hypercalciuria, hypercalcemia, hypermagnesemia, increased urine volume and decreased urine osmolarity</td>
</tr>
<tr>
<td>CaSR</td>
<td>Not knockout; isopropyl methane sulfonate mutagenesis, CaSR activating mutation (127)</td>
<td>Ectopic calcifications, no hypercalciuria by calcium/creatinine ratio</td>
</tr>
</tbody>
</table>
a variety of causes. Even if one takes a step more proximal from calcium stones to hypercalciuria, one is still confronted with a phenotype governed by multiple organs with numerous genetic and environmental determinants. There is no doubt that hypercalciuria is a complex trait. After the display of the difficulties in studying a trait as complex as hypercalciuria, one wonders whether there is any hope in ever unraveling the genetics in this conditions. The approaches outlined by the classic review by Lander and Schork (138) a decade ago are still in active use today, and the reader is referred there for an excellent detailed discussion. A more recent update can be found in the review by Risch and Merikangas (139).

One can fathom ways to bypass or counteract the complexity and enable some degree of informative genotype-phenotype correlation. One can confine the phenotype to the most severe end of the calciuria continuum, restrict it to a subgroup of fasting hypercalciuria or hypercalciuria accompanied by heightened intestinal absorption and reduced bone density, or include only hypercalciuric patients with a strong family history. All of these efforts are directed to capture a more proximal phenotype and hopefully more homogeneous genetics. This underscores once again the critical importance of good metabolic evaluation of patients and that hypercalciuria per se is not an adequate phenotype for genetic studies. Another approach is to use single large kindreds. The reason is that the degree of polygenic influence and loci heterogeneity in the general population is much reduced in a single pedigree as long as the number of individuals offers adequate power for the analysis. Allele-sharing method between sibling pairs is founded on the rationale that even though a trait is polygenic, the inheritance of a given gene (albeit amid numerous modifiers) is Mendelian (138–140). In this section, we highlight a few issues and review some existing data that are germane to hypercalciuria.

**Association**

The easiest method that has been widely used in a lot of complex traits is a population-based association study (Figure 3). This is a case-control design that examines the frequency of a particular allele in affected versus unaffected individuals. Unfortunately, this is also the weakest design in terms of generating definitive information. The ambiguity of affected versus unaffected has already been discussed. The polygenic nature and loci heterogeneity further worsens the problem. In addition, the phenomenon of population admixture renders this method ridden with artifacts (138). It is highly likely that two populations differ in aspects other than the disease of interest; hence, any given allele will have a good chance of having different frequency in two different populations (Figure 3).

Positive studies are common with the association approach, and the caveats are summarized in Figure 3. If the different alleles of the gene of interest have defined differences in function and that difference can account for some phenotypic differences, then an association will carry more meaning. If the alleles are simply polymorphisms, the one has to be extra cautious in interpreting the outcome. After more than a decade of copious positive case-control studies of angiotensinogen and angiotensin-converting enzyme gene polymorphisms in more than a dozen clinical conditions, we are hardly closer to the truth. Having stated the caveats and limitations with this technique, there is no doubt that association studies are readily and easily performed.

The wealth of information derived from monogenic human diseases and rodents with single gene deletions provides a valuable database for candidate genes to be screened in humans. Genes along the vitamin D axis were examined. Small studies have shown association of restriction site polymorphisms with one form of phenotypic parameter or another (141–143). However, several other studies have found no difference in the phenotype of vitamin D receptor expression, induction, allelic frequencies, coding region sequence between controls, and well characterized hyperabsorptive hypercalciuric stone formers (144,145). The closest positive outcome has been the identification of potential susceptibility locus in the vicinity of the vitamin D receptor gene in 47 French-Canadian kindreds using sib-pair analysis (146). One must exercise utmost caution and rigor before accepting such data as proof of the hypothesis.

Other candidate genes were also pursued. Apart from its known implication in Bartter syndrome and autosomal dominant hypocalcemia, the calcium sensing receptor (CaSR) is a candidate gene for multigenic hypercalciuria and bone resorption. One study showed associative correlation between a single amino acid change (unknown whether it is a polymorphism or a mutation) and the phenotype (147); there were no point mutations in seven families with idiopathic hypercalciuria.
In 55 French-Canadian pedigrees, no association was found between CaSR locus and idiopathic hypercalciuria and calcium nephrolithiasis (149). Direct sequencing of the ClC-5 chloride channel was negative in a case-control study (150). Thus far, association studies with candidate genes have not been particularly informative in genetic hypercalciuria.

**Linkage**

Linkage is a powerful alternative and complement to association studies. As opposed to association, linkage detects genotype-phenotype co-segregation within families and has been the workhorse for disease-locus discovery in monogenic diseases (151,152). The Hôpital Maisonneuve-Rosemont group from Montreal has used nonparametric (i.e., a model-free test to avoid a priori assumption of a particular inheritance pattern) linkage analysis in large numbers of French-Canadian concordant affected sib-pairs on a large number of candidate genes including the vitamin D receptor (146); 1-a-hydroxylase (153); the CaSR (148); and crystallization modifiers such as osteopontin, Tamm-Horsfall protein, and osteocalcin-related gene (154).

Instead of candidate genes, Reed et al. (155) performed a linkage analysis of three large kindreds with absorptive hypercalciuria using nonparametric testing. This was the first successful genome-wide search in this complex trait. One large kindred contributed to the particularly high logarithm of odds score of >12 for the region in 1q23.4 to 24, which contains several known genes and a number of putative genes. After several known genes were sequenced and no sequence differences were found in affected versus unaffected individuals, a hypothetical protein with adenyl cyclase motifs that spans 104.5 kB with 33 exons (Gen Bank AL035122) was identified (156). During the course of the work, a homologous rat gene coding for a soluble adenyl cyclase (sAC) was cloned by Buck et al. (157). The human ortholog of sAC is polymorphic with 18 base substitutions (156). When this gene was examined outside the original kindreds, five sequence variations occurred with increased frequency in the hypercalciuria population compared with normal volunteers, and the number of base substitutions seems to correlate with lower spinal bone density and intestinal calcium absorption (156,158), two cardinal features of the absorptive calcium syndrome. The pathophysiologic relationship between the human ortholog of sAC and intestinal calcium absorption remains to be determined. The efforts on human familial hypercalciuric nephrolithiasis has yielded some encouraging but far-from-groundbreaking information.

**Animal Models**

A large number of rodent models of complex traits have been generated in the past several decades (159,160), and they are catalogued at the Rat Genome Database (http://www.rgd.mcw.edu). These models are instrumental for both geneticists and physiologists. The best animal model of polygenic hypercalciuria to date is the genetic hypercalciuria stone-forming rats (GHS rats) created by Bushinsky et al. (161) more than one and a half decades ago. That one can breed the physiologic extreme end of calciuria in “normal” rats to a hypercalciuric state with complex pathophysiology lends credence to the polygenic determinants of calcium excretion. Although the physiology differs somewhat from those described in humans, the GHS rat definitely shows multiorgan pathophysiology with intestinal hyperabsorption, increased bone calcium mobilization, and primary renal leak, a “full house” coverage of Figure 1. The well-characterized pathophysiology has been summarized in several recent reviews (6,162,163).

There are two reasons that the study of these animals is more utilitarian than humans in studying complex traits. In addition to a valuable source of pathophysiology, a major reason for having such strains is identification of disease susceptibility loci using breeding experiments. A region of the chromosome (perhaps containing one gene) that determines the magnitude of a continuous trait is called a quantitative trait locus (QTL). With the a priori assumption that the trait is genetic, one crosses two inbred strains (hypercalciuric and normocalciuric) to produce recombinant strains followed by backcrosses with one of the original strains until one can link the genetic loci (QTL) being delivered, to the phenotypic trait (hypercalciuria). One works on the implicit hypothesis that the QTL confers the hypercalciuria. The loci identification will be followed by mapping of the genes. Hoopes et al. (164) reported on several QTL that are linked to the hypercalciuria in the GHS rats. Although none of the QTL are in regions that harbor the usual set of suspect candidate genes, these are indeed encouraging results, and the hurdle to surmount now is to proceed from locus to gene.

Although traditional QTL approaches are powerful, a recent advance in this field that may revolutionize the dissection of rodent polygenic traits is the development of chromosome substitution strains (165,166). In classical QTL studies, one crosses two inbred strains with differing phenotypes to create hybrids that then are intercrossed and/or backcrossed eventually to generate recombinant inbred or cogenic strains that carry portions of the genome of the original parental disease-carrying strain in the genetic background of the nonaffected parental strain (Figure 4). Because these strains are hypercalciuric, linkage can be performed to identify loci that segregate with the QTL of interest. With knowledge of the loci, finer structural mapping can be performed to eventually hone in on the genes. In the chromosomal substitution approach, instead of pieces of DNA randomly scattered through the genome, a panel in which entire chromosomes from one strain are replaced with the corresponding chromosome from another is created (165,166) (Figure 4) in defined and uniform genetic background. The latter approach is more challenging because the panel of strains has to be generated and is limited by large DNA regions (entire chromosome versus the typical 20 cm of QTL). However, no crosses (usually a colossal number for QTL) need to be performed and genotyping is not required because the segregation of the genome is known from the way the panel is generated.

Genetic manipulations in rodents are usually performed to examine the whole organism phenotype of the disruption of a gene product or to create a model of known monogenic diseases for detailed invasive phenotypic characterization that is not possible in humans. With the ever-expanding list of artifi-
cial rodent strains with single gene deletions with hypercalciuria (Table 5), another approach will be to take the phenotype of a knockout mouse and search large databases such as stone registry to search for humans who “resemble” the mouse with deletion of gene X and sequence gene X in those individuals.

In summary, hypercalciuria is an important risk factor for nephrolithiasis, and its genetic component is a complex trait that defies simple methods of human genetics. A combination of approaches are required to advance this field, including detailed proximal phenotype definition and database management and sharing on a large scale, continued efforts in studying monogenic causes of hypercalciuria in human and genetically altered rodents, and application of standard and new polygenic approaches in both human and rodent models of hypercalciuria.

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