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EDITORIAL COMMENTARY

Hypercalciuria revisited: one or many conditions?

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Abstract Idiopathic hypercalciuria is a defect occurring in 5–10% of the general population and most commonly detected in patients with calcium kidney stones or osteoporosis. Although high-penetrance autosomal dominant inheritance cannot be ruled out, hypercalciuria is probably a polygenic phenomenon. Findings obtained in monogenic disorders characterized by renal calcium stones, and/or hypercalciuria, and/or nephrocalcinosis, have suggested a number of genes as candidate genes in the pathogenesis of idiopathic hypercalciuria, i.e. soluble adenylate cyclase, calcium sensing receptor, vitamin D receptor and 1-alpha hydroxylase, sodium-phosphate co-transporter-2, claudin-16, chloride channel 5, etc. All the genetic findings obtained so far do not support the idea of different types of idiopathic hypercalciuria, i.e. absorptive, renal, and resorptive. On the contrary, they support clinical observations, which suggest idiopathic hypercalciuria as a single disorder characterized by altered calcium transport in the intestine, kidney and bone, due to various different combinations of multiple genetic and dietary players.

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The conventional definition of hypercalciuria in adults (calcium excretion exceeding 7.5 mmol/24 h in men or 6.25 mmol/24 h in women) was proposed by Hodgkinson and Pyrah, who studied 258 healthy individuals and 344 stone formers in Leeds, UK in 1958 [1]. To unify its definition in men, women and children, hypercalciuria was also defined as a 24 h calcium excretion greater than 0.1 mmol/kg body weight (4 mg/kg b.w.) [2], or 0.6 mmol/mmol creatinine (0.21 mg/mg creatinine) in morning urine [3]. Criticisms have been raised against these definitions. The cut-off is arbitrary, since the distribution of calciuria in the population is gaussian, and this has been shown in children too [4]. All these ranges have often been accused of being too restrictive, but they were chosen, partly, as a function of the risk of kidney stone formation, which significantly increased above their upper limits [1]; notwithstanding, many of the so-called hypercalciuric subjects never develop stones during their life. As calcium excretion varies in relation to nutrients and age, it is very difficult to establish a normal range for single individuals from findings in the general population. Calcium excretion declines from infancy to teenage, then rises from the third to the sixth decades of life, dropping again after 55–60 years in both genders [2, 5]. It also varies according to family characteristics, and a familial distribution of calcium excretion and hypercalciuria has been observed [6].

Based essentially on Pak's original classification [7], hypercalciuria has been classified by Bataille et al. [8] as a complex set of six different pathophysiological categories, i.e. absorptive tp. I, tp. II, tp. III, renal, fasting and dietary calcium-dependent, each category relying on pathophysiological assumptions and clinical observations. A

more synthetic classification has since been proposed by Pak [9], comprising only three categories, i.e. hypercalciuria with hypercalcemia, hypercalciuria with high urinary sodium excretion, and absorptive and renal hypercalciuria, thus suggesting that some of the former categories do not make sense—in clinical terms at least—and that hypercalciuria is less heterogeneous than was previously thought. It is particularly worth noting that Pak places absorptive and renal hypercalciurias in the same category.

At least five clinical arguments support the conviction that idiopathic hypercalciuria is always one and the same disorder.

(1) Coe et al. [10] reported that, in idiopathic hypercalciuric patients on a low-calcium diet, calcium balance constitutes a continuum (not discrete classes), from those in a positive balance, which suggests an element of increased intestinal calcium absorption, to those in a negative balance, suggestive of different mechanisms of hypercalciuria.
(2) In children with fasting hypercalciuria the lack of an increase in bone turnover implies that renal and absorptive hypercalciuria may not be two distinct entities [11].
(3) In studies on families of children with hypercalciuria, Nicolaidou et al. [12] and Harangi and Mehes [13] found that renal and absorptive hypercalciuria may coexist in the same family, an argument in favor of a single form of idiopathic hypercalciuria. Lerolle et al. confirmed the simultaneous finding of different forms of hypercalciuria in the same family [14].
(4) It is quite common in clinical practice to find that the repeating of calcium loading tests after an interval of several years may lead to a different diagnosis from the one established by earlier tests. For instance, Aladjem et al. observed that more than 50% of children initially diagnosed as having absorptive or renal hypercalciuria had different test results after an interval of 3–7 years [15].
(5) Different hypercalciuria phenotypes may be induced by the administration of 1,25-dihydroxy-vitamin D3 [1,25-(OH)2D3)], depending on dietary calcium intake [16]: if it is normal, administration of the vitamin increases intestinal calcium absorption and produces an increase in urinary calcium excretion (absorptive hypercalciuria); if calcium intake is restricted, however, calcitriol administration leads to increased bone turnover and higher urinary calcium levels (resorptive hypercalciuria).

Studies on the inheritance of hypercalciuria also support the theory that idiopathic hypercalciuria is a single disorder. It is generally accepted that urinary calcium excretion is governed by genetic factors. Stone formers have a relative risk of having abnormal calciuria 9.18-times more than do controls [17], which is far higher than the risk of having abnormal calcium intake (< 2) [18], which suggests that the genetic risk of hypercalciuria is far higher than the diet-related risk. As the trait recurred in each generation and was not gender related, they suggested an autosomal dominant transmission of hypercalciuria. The same pattern was identified in four other studies. Pak et al., in four generations of a family with absorptive hypercalciuria [19], observed an autosomal dominant pattern. Harangi and Mehes suggested that renal hypercalciuria is inherited as an autosomal dominant trait, while absorptive hypercalciuria is more likely to have dietary causes [13]. Nicolaidou et al. studied 40 children with hypercalciuria and found that 47.5% of them had at least one hypercalciuric first-degree relative, while 52.5% were sporadic cases due possibly to a de novo mutation or other causes [12]. Lerolle et al. also supported the idea of an autosomal dominant transmission of the trait [14].

Those studies considered idiopathic hypercalciuria as a monogenic trait with a Mendelian transmission, but as many as 55–60% of probands look like sporadic (single) cases within a given family [6, 12, 20], and if hypercalciuria really is a monogenic, autosomal dominant disorder, it is highly unlikely that a genetic mutation can have occurred in one in two hypercalciuric stone formers: this would demand a higher de novo mutation rate than has ever been observed in humans. The exclusion of probands from the analysis reduces the proportion of hypercalciuric siblings to as few as 10%, a far cry from the proportion expected in the case of an autosomal dominant inheritance [21], and more suggestive of a polygenic inheritance.

As a matter of fact, we now know that multiple genes and gene interaction with environmental factors determine such quantitative traits as calcium excretion, the values of which are distributed along a continuous scale, with hypercalciuria occurring when urinary calcium exceeds a conventional threshold. Indeed, while just one or a few genes sustain phenotypes distributed in discrete classes, only multiple genes can sustain a continuous distribution of phenotypes that cannot be divided into single phenotypic classes [17, 22].

In a study on 63 parents and their offspring, the overall additive effect of multiple genes (heritability) was 47% [23], as mirrored by the percentage variance in calcium excretion. Hunter et al. found a similar calcium excretion heritability rate (52%) in a large sample of twins [24].

The mode of calcium excretion inheritance is not known. Using a complex segregation analysis on 221 nuclear families, Loredo-Osti et al. tested four genetic models: major gene models (co-dominant, recessive or dominant), an additive polygenic model, a mixed major/polygenic model and a null model. The co-dominant major gene

model emerged as the most likely, heritability being 58% with a polygenic component of 11% [25]. Thus, consistent data from different groups and geographical areas support the conviction that at least 50% of the variance in calciuria is governed by a number of genes or a true polygenic effect [23–25].

After a decade of research, no single gene has been identified with a sufficient degree of certainty as the sole cause of idiopathic hypercalciuria, and studies to confirm previous findings quite frequently have given contradictory results, as typically observed in association studies on complex traits [22].

Here again, therefore, the above-mentioned genetic observations support the theory that there is only one form of idiopathic hypercalciuria.

According to available reports, the vitamin D receptor (*VDR*), soluble adenylate cyclase (*sAC*), calcium sensing receptor (*CaSR*) [26] and claudin-16 (*CLDN16*) genes [27] expressed in the intestine, kidney and bone may contribute to calcium excretion and idiopathic hypercalciuria. They are the candidate genes that have attracted most of the investigators' interest, but many other genes may also be involved. In addition, calcium excretion level depends, of course, on the dietary intake of calcium and is also influenced by nutrients, such as sodium, by animal protein intake, and by vitamin D storage (which is dependent on intake, environmental factors and renal function) [28–30]. Idiopathic hypercalciuria thus appears to us as a single disorder (because the agents involved—be they genes, nutrients or calcium-handling organs—are the same), in which the proportional influences of the activity of different gene products in calcium-handling organs and of the interaction between environmental and genetic determinants may vary considerably in different individuals and in different conditions. If we take this view, the "classical" pathogenic classifications of hypercalciuria are not sufficient to explain the cell and tissue changes that lead to a stable increase in calcium excretion; at best, they merely identify some prevailing mechanisms.

If we reason along those lines, experimental evidence should also be reconsidered. It is generally assumed that the genetic hypercalciuric stone-forming (GHS) rat model developed by inbreeding in Bushinsky's laboratory [31] supports a role for the VDR in enterocytes in human hypercalciuria, and the review by Srivastava and Alon, recently published in *Pediatric Nephrology* [32], supports such an assumption. As concerns this model, however, it may be worth considering the example of hypertension. There are numerous rat models of arterial hypertension obtained by inbreeding [e.g. the Milan hypertensive rat (MHR), the spontaneously hypertensive rat (SHR)] that differ in terms of the gene or mechanism responsible for their hypertension. If we extrapolate the findings in these models to primary hypertension in humans, they may—at best—suggest a potential pathogenic role for certain candidate genes or mechanisms in human hypertension and their possible contribution, in combination with environmental factors and other genes, to the complex condition known as primary hypertension. The same can probably be said of hypercalciuria and the GHS rat. The point to make is that, in models of disease obtained by inbreeding, the selected population of animals is genetically extremely homogeneous, and this reduces the genetic background noise due to minor genes. The end result is a strain of animals selected to have only one or a few genes responsible for a given phenotype (be it hypercalciuria, hypertension, obesity, diabetes, or any other condition). A significant linkage was found in the GHS model between hypercalciuria and a region of chromosome 1 at D1Rat169, though some linkage to regions of chromosomes 4, 7, 10, and 14 also emerged [33]. Crossed with other wild rats, these genetically selected animals will produce offspring subject to the laws of classic Mendelian inheritance, so models like the GHS selected by Bushinsky are not models of complex disease, they are virtually models of Mendelian inherited, i.e. monogenic, disease. Equally important is the fact that, unlike complex diseases in which different environmental conditions have an important role in the outward manifestations of the disease, in the case of inbred models, the animal stabling conditions are often extremely standardized, with little room for variability, and this makes them even less capable of representing the conditions of complex disease. The most the GHS model can tell us, therefore, in pathogenic terms, is that a VDR-dependent mechanism contributed to the pathogenesis of the complex disease going by the name of hypercalciuria in the wild rats from which the model was started. There is also the far from negligible fact that the gene encoding VDR is not localized in the regions linked with hypercalciuria in such a model [33].

In the light of the above considerations, evidence obtained thus far supports the idea that idiopathic hypercalciuria is a complex/polygenic disorder, so it seems to be unfounded, from the genetic perspective at least, to assume that it can fit into different pathophysiological classes according to different trigger events in its onset. What is the implication of such a conclusion? For therapeutic purposes, according to the information that is actually available, it means there is no need to tailor the treatment according to the clinical characterization of idiopathic hypercalciuric patients. Srivastava and Alon come to the same conclusion, albeit via a different route [32]. A more crucial issue concerns the fallout on genetic research on idiopathic hypercalciuria: studies on the role of genes in the pathogenesis of human disease suffer from problems relating to the proper classification of

disease [34]—the better the phenotypic characterization, the greater the homogeneity of the case population and the higher the chances of recognizing any association between potentially responsible genes and the disease in question. However, if there is only one form of idiopathic hypercalciuria, there is no need for us to classify patients according to complex, time-consuming and variable pathophysiological definitions.

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