In Focus

Familial tumoral calcinosis: a valuable vehicle for discovery

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While electrolytes such as sodium and potassium exist in different anatomic and functional aqueous compartments, calcium and phosphate lead double lives in soluble and solid phases. There are substantial external and internal fluxes of calcium and phosphate to maintain a metabolically active apatite-based endoskeleton. Once absorbed from the intestine, one has to maintain calcium and phosphate in solution during its lifetime in the body outside the skeleton. The normal turnover and maintenance of external balance mandate excretion of a finite amount of calcium and phosphate in a small volume of urine dictated by water conservation. Numerous mechanisms are functional to ensure that calcium and phosphate crystalizes in bone and nowhere else, as the consequences of extra-osseous calcification are undoubtedly dire [1].

This is a formidable homeostatic challenge on the organism, but the selection pressure is of such enormity that we have already evolved multiple mechanisms replete with precision and redundancy to prevent ectopic calcification, including matching of tandem fluxes and tightly controlled concentrations well-coordinated across organs and numerous anti-calcification activities, which were established since the dawn of the endoskeleton in vertebrae evolution. It is easy to fathom that disruption of this network can lead to external (total body excess) and internal (ectopic calcium phosphate deposit) imbalances. Chronic kidney disease (CKD), believed by some to qualify as a 'metabolic disaster', furnishes such an example.

CKD has reached epidemic proportions globally, and the leading cause of death is cardiovascular [2, 3]. The pathophysiology of uremic cardiovascular disease is complex and poorly

understood, but it is likely intimately linked to mineral disturbances. The entity CKD-bone and mineral disease (MBD) really encompasses most complications in uremia including uremic cardiovascular pathology. Understanding CKD-MBD is tantamount to understanding CKD complications. Attempts to unravel the pathophysiology of CKD-MBD is daunting due to the sheer complexity of the condition, the immense heterogeneity among individuals and the fact that most pathogenic intermediates are not measured.

Rare monogenic diseases with identifiable single etiologic origins are useful because one is confident that the myriad of pathophysiologic changes, no matter how diverse and seemingly hopeless to link, can all be eventually traced either directly or indirectly to that one distinct lesion. The investigative power cannot be overemphasized. Mendelian monogenic hypertensive diseases are rare compared with primary hypertension, but every one of them has provided insights into the complex trait of primary hypertension [4]. In the mineral field, monogenic disorders have likewise enlightened us on our understanding of complex traits; these include mutations in parathyroid hormone (PTH), its cognate receptor PTHR, PTHR signaling molecules, vitamin D 1- α -hydroxylase, vitamin D receptor, phosphate transporters, low-density lipoprotein receptor-related protein 5 and of course fibroblast growth factor (FGF)23.

Fibroblast growth factor-23 is a bone-derived peptide hormone whose facade evolved in front of our eyes at an impressive pace to near 'celebrity' status since its discovery in 2000 as the causative gene for autosomal dominant hypophosphatemic rickets (ADHR) [5]. This was followed shortly by the discovery that it is the factor responsible for the acquired ectopic endocrine disease tumor-induced osteomalacia [6, 7], and the prime pathogenic effector, although not the candidate gene, in X-linked hypophosphatemic rickets [8]. In addition to

its role as the phosphaturic hormone that responds to a sustained phosphate load, FGF23 physiology has ramified into regulation of vitamin D, PTH [9] and even calcium [10]; lately, FGF23 has established an unprecedented link to iron metabolism [11]. In the world of clinical nephrology, since the description of elevated FGF23 in end-stage renal disease a decade ago [12], the literature has exploded with epidemiologic associations supplemented with animal and cell culture models conjuring up imageries of this 'evil toxic hormone', and spawning proprietary pursuits of therapeutic blockers and neutralizing antibodies. However, the pathogenic role of FGF23 in CKD is still unresolved; testimonial to this statement is that not a year can lapse before an editorial with some inquisitive title reminds us of the uncertain significance of high FGF23 in CKD; an illustrative handful is shown [13–18]. Associative findings have extended beyond CKD to the general population proposing a role for FGF23 in cardiovascular disease at large [19, 20]. FGF23 has certainly been 'busy' in the limelight in both the basic and clinical literature. But, now as much as ever, we are in desperate need of enlightenment. Perhaps the monogenic diseases can be of some assistance.

The mirror image of ADHR (FGF23 excess) is 'familial Tumoral Calcinosis' (FGF23 deficiency). The clinical features of familial tumoral calcinosis (FTC) were known for a long time [21, 22], and in 2004, the first causative gene was identified as GALNT3 (UDP-N-acetyl- α -D-galactosamine: polypeptide-N-acetyl-galactosaminyl transferase 3) [23]. Presently, a practically picture-perfect paradigm is posed of a loss-of-function genetic endocrine disease with distribution of mutations sequentially poised at the enzyme that process the hormone (~20 mutations), the hormone *per se* (~10 mutations) and one of its co-receptor (1 mutation) all culminating in loss of FGF23 bioactivity [24–26]. From this database, one can draw several key conclusions. Without FGF23, the kidney, replete with all other phosphaturic hormones and intrinsic phosphate sensing, is incapacitated to excrete phosphate adequately, i.e. the redundancy is insufficient. The phosphate retention is unequivocally detrimental to many organs. The clinical manifestations are extremely protean both in characteristics and in severity despite the common element of FGF23 deficiency. Outcome can be dire, and no definitive therapy is available.

The kindred with compound heterozygous FGF23 mutations reported by Shah and coworkers [27] is noteworthy in several aspects. In addition to the known soft tissue calcifications, the subjects have extensive calcification of a large number of vessels. This has been described in FTC, but the authors secured tissue for pathology and found that the medium size arteries have the medial calcification of Monckeberg without intimal involvement or atherosclerosis but with some evidence of osteogenic transformation, in a manner similar but not identical to uremic vasculopathy. This highlights that this lesion can occur in the absence of FGF23 in humans akin to the FGF23 knock-out mouse [28]. High FGF23 may worsen the situation (with yet to be defined mechanisms even if true) in uremic vasculopathy, but it is certainly not required for vascular calcification. Phosphotoxicity seems to be the main driver, but understandably, it is not identical to uremia.

The serum phosphate levels of the affected members were reported to be 5.8–7.6 mg/dL (1.85–2.43 mM). These values may not be that alarming to many clinicians who are conditioned by a large fraction of their time spent managing the severe hyperphosphatemia of CKD. Serum phosphate is a rather poor indicator of phosphate load and phosphotoxicity, so mild hyperphosphatemia may mean severe phosphotoxicity [29]. The ravages of phosphotoxicity in FTC are unequivocal. Aggressive restriction of dietary phosphate, especially added inorganic phosphate, cannot be overemphasized, and binders should be instituted early and in adequate doses vigilantly.

The affected individuals were compound heterozygotes with a >5-Mb deletion of the FGF23 gene inherited from the mother, and a point mutation Q67K in the FGF23 inherited from the father. This resulted in undetectable full-length FGF23 and cumulated C-term fragments (the authors kindly harmonized the units from the two assays for the readers to compare). The biologic consequence of the Q67K mutant was modeled but not tested. The mutation is past the divergent N-terminus in the β 1 strand of the β -trefoil core and is likely an unstable and degradation-prone rather than a catalytically inactive protein although non-functional Q67K FGF23 cannot be ruled out. The zero plasma full-length FGF23 is compatible with protein instability. Since C-terminal fragments can act as competitive antagonists [30], an important question is whether these fragments also function as inhibitors *in vivo* and contribute to the low FGF23 bioactivity in FTC. This competitive blockade hypothesis can be tested.

The highly variable clinical manifestations of FTC are unlikely to be explained solely by environmental factors and suggest genetic modifiers at work. Clinicians have observed that members in the same family bearing the same mutation can have widely diverse presentations. The authors here embarked on an important step to exome-sequence the subjects. The rationale is obvious but the single hurdle (of yore) was cost, which is dropping perennially to within the fiscal capability of most investigators. There were three candidates—Wnt5 is haploinsufficient in the three affected individuals from deletion from the maternal lineage. Osteoprotegerin and secreted frizzled-related protein (sFRP-1) both carried heterozygous missense mutations. These are candidates with links to mineral metabolism that can be speculated to be disease modifiers. We were not given data on whether haploinsufficiency is functionally important or whether the missense mutations in osteoprotegerin and sFRP-1 affect their expression or function. Despite this caveat, this is still nonetheless an important effort in the right direction utilizing available affordable technology to probe at an important problem.

It is very unlikely that rare diseases will have a sizeable cohort in the literature although registries are designed to achieve that, and one cannot insist on prescribing therapy based only on 'evidence' from prospective randomized placebo-controlled trials. Case reports remain a viable and important means of dissemination of data to invigorate progress. This is the duty of not just researchers but all practitioners. Sir William Osler vigorously encouraged his trainees to always 'observe, record, tabulate, and communicate.' In a busy practice, it is tempting and in fact pragmatic not to pursue beyond standard of care, but this will seriously hinder discovery and

progress. The practitioners are the ones in the 'field' who capture these cases and expand from mere provision of care to discovery and then apply the knowledge back to patient care. Bravo to the clinicians who bring these patients to investigation.

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CONFLICT OF INTEREST STATEMENT

None declared.

(See related article by Shah *et al.* Severe vascular calcification and tumoral calcinosis in a family with hyperphosphatemia: a fibroblast growth factor 23 mutation identified by exome sequencing. *Nephrol Dial Transplant* 2014; 29: 2235–2243.)

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