

## Rickets: Twists and turns in the Gordian knot

M. K. Garg, Sanjay Kalra<sup>1</sup>

Department of Endocrinology, Command Hospital, Southern Command, Pune, Maharashtra, <sup>1</sup>Department of Endocrinology, Bharti Hospital and B.R.I.D.E., Karnal, Haryana, India

Traditional scientific wisdom teaches that rickets, so plentiful in India, is caused by vitamin D deficiency and cured by vitamin D replacement. Rickets also occurs in the presence of vitamin D sufficiency, as in hypophosphatemic and hypocalcemic rickets; this, however, is not the topic of this editorial. It is still not understood why some children develop rickets, while others do not, even in the face of severe Vitamin D deficiency. This editorial tries to probe the recent twists and turns that researchers have taken while trying to unravel this Gordian knot.

### NOT AS SIMPLE AS CALCIUM INTAKE

The role of calcium deficiency on the background of vitamin D deficiency as the causation of rickets has been highlighted in a recent article by Aggarwal, *et al.*<sup>[1]</sup> They compared 67 cases of rickets with 68 healthy controls. Among cases, serum 25-hydroxy vitamin D [25(OH) D] levels were comparable, but calcium intake, total and from dairy intake was significantly lower as compared to controls. They concluded that rickets develops when low dietary calcium intake coexists with a low or borderline vitamin D nutrition status. However, they have not highlighted another interesting finding: Cases of rickets mount robust parathyroid hormone (PTH) response as compared to controls with same vitamin D levels. This was again explained by calcium deficiency which exacerbated PTH response in the presence of vitamin D deficiency. A similar observation has been made by an Indian study among elderly population, in which more than half of population did not mount PTH response in spite of severe vitamin D

deficiency [25(OH) D <10 ng/ml].<sup>[2]</sup> A recently published study also substantiated this observation by analyzing lab results of more than 300,000 pairs of 25(OH) D and PTH levels in a population. They also found that >50% of patients do not mount PTH response.<sup>[3]</sup> Similar observation in two different populations with different calcium intake suggests that there are other mechanisms/factors responsible for PTH response rather than calcium intake. It is also likely same mechanisms/factors may also contribute to development of rickets in patients with vitamin D deficiency rather than dietary calcium deficiency.

### BIOCHEMISTRY REVISITED

Endogenous vitamin D is synthesized in epidermis. Exogenously, vitamin D can be derived from vegetable and animal sources. Vitamin D is produced in the skin by a UVB-mediated, photolytic, non-enzymatic reaction that converts 7-dehydrocholesterol to pre-vitamin D<sub>3</sub>. Pre-vitamin D<sub>3</sub> undergoes a subsequent non-enzymatic, thermal isomerization conversion to vitamin D<sub>3</sub> in the skin. Vitamin D<sub>3</sub> enters circulation and gets bound to vitamin D binding protein and is taken up by liver. In the hepatic parenchyma, vitamin D<sub>3</sub> is converted by one of several cytochrome P450 (CYP2R1, CYP2D11, CYP2D25) to 25(OH) D. 25(OH) D is the most plentiful and stable metabolite of vitamin D in human serum. This 25(OH) D is filtered in renal glomerulus and internalized in proximal convoluted tubules with the help of megalin and converted to 1,25 dihydroxy vitamin D [1,25(OH)<sub>2</sub>D] by mitochondrial 1- $\alpha$ hydroxylase (CYP27B1). This reaction requires NADPH and ferredoxin for electron transfer during hydroxylation reaction. 1,25(OH)<sub>2</sub>D serves as a high affinity ligand for the vitamin D receptor (VDR) in target tissues where it acts to modulate expression of vitamin D-directed genes. VDR is present in most tissues and cells in body including parathyroid cells. 1,25(OH)<sub>2</sub>D is metabolized by 24-hydroxylase (CYP24A1) enzyme to relatively inactive metabolites like 24,25 dihydroxy vitamin

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Corresponding Author: Col. M. K. Garg, Department of Endocrinology, Command Hospital, Southern Command, Pune, Maharashtra, India.  
E-mail: mkgargs@gmail.com

D and calcitriol acid.<sup>[4]</sup> Calcium sensing receptors are also present on parathyroid cells, which modulate PTH secretion by binding with divalent cations.

## CALCIUM ABSORPTION

Any mechanism which affects the responsiveness of parathyroid cells will modify PTH secretory response. Activated vitamin D modifies PTH secretory response. Obviously, the same mechanism will also be operative in other VDR responsive cells. Hence, we speculate that patients who mount PTH response will have a less effective protective mechanism against vitamin D deficiency and will negatively affect calcium absorption from gut. On the contrary, those with a protective mechanism will suppress the PTH response and have more efficient absorption of dietary calcium. This suggests that it is the calcium absorptive mechanism, rather than dietary calcium intake, which predisposes an individual with vitamin D deficiency to develop rickets and PTH response.

## GORDIAN KNOT: GENETIC POLYMORPHISM

What could be the underlying mechanism(s) of this absent PTH response to vitamin D deficiency? Answer to this can explain above enigma and it may lie in genetic polymorphism. There are several genes involved in vitamin D metabolism namely GC (group component), DHCR7 (7-dehydrocholesterol reductase), NADSYN1 (nicotinamide adenine dinucleotide synthetase), CYP2R1, CYP27B1, CYP24A1, and VDR gene. 25(OH) D has high heritability (28-80%)<sup>[5]</sup> but others disagree with this.<sup>[6]</sup>

## THE GC AND CYP2R1 GENES

Single-nucleotide polymorphisms (SNPs) in the gene GC (rs2282679, rs4588, and rs2282679) and a non-synonymous SNP (rs7041 and rs1155563), SNP in NADSYN1/DHCR7 (rs3829251 and rs1790349), and SNP in CYP2R1 (rs2060793) have been associated with lower 25(OH) D concentrations in European and Chinese ancestry.<sup>[7,8]</sup> Similar findings have been reported in Canadian adults of east Asian, but not south Asian, origin.<sup>[9]</sup> A systemic review of various genomic studies suggested that the optimal concentrations of 25(OH) D may vary according to genotype.<sup>[10]</sup> SNPs involved in above genetic loci may affect 25(OH) D levels but do not affect 1,25(OH)<sub>2</sub>D<sup>[11]</sup> and are unlikely to affect its action. Moreover, animal models with knock out loci did not develop rickets and PTH response even in the presence

of low 25(OH) D concentration.<sup>[4]</sup> Hence, these SNPs will not be able to untangle the Gordian knot of rickets pathophysiology.

## THE CYP27B1 AND CYP24A1 GENES

Some polymorphisms of CYP27B1 (1- $\alpha$ hydroxylase enzyme) may affect the efficiency of process of generating active 1,25(OH)<sub>2</sub>D. CYP27B1-1260 promoter polymorphism (rs10877012) has a substantial impact on 1,25-(OH)<sub>2</sub>D serum levels, with higher levels noted with AA variant than CC variant.<sup>[12]</sup> Other studies have also reported low levels of 1,25(OH)<sub>2</sub>D with the same SNP.<sup>[10]</sup> More evidence comes from a study of prostatic carcinoma, where one CYP27B1 tag SNP (rs3782130) and two CYP24A1 tag SNPs (rs927650 and rs2762939) were associated with adverse outcome,<sup>[11]</sup> indicating low efficiency of 1,25(OH)<sub>2</sub>D on tissue effect.

Another enzyme, CYP24A1, also plays an important role in metabolism of 25(OH) D and 1,25(OH)<sub>2</sub>D. A SNP of CYP24A1 (rs6013897) was associated with low level of 25(OH) D<sup>[13]</sup> indicating that increased activity of this enzyme can decrease active vitamin D. A study among South Asians showed increased activity of 24 hydroxylase enzyme in cultured in skin fibroblast and its association with lower serum 25OHD.<sup>[14]</sup> It can be hypothesized that in subjects with less efficient CYP27B1 and relatively efficient CYP24A1 system, there will less generation of active vitamin D in the face of vitamin D deficiency or SNPs described in THE above paragraph. Decreased level of active vitamin D will have decreased physiological effects. A negative 1,25(OH)<sub>2</sub>D response element is present on the promoter region of the PTH gene. Hence, decreased levels of active vitamin D will upregulate PTH gene expression and increase PTH level, simultaneously decreasing calcium absorption and explaining the rachitic Gordian Knot.

## INFLAMMATION

Can an internal factor other than those mentioned above be involved in the vitamin D-PTH-calcium axis? Nuclear factor kappa-B has been implicated in downregulation of CYP27B1 gene expression.<sup>[15]</sup> Indian subjects have higher levels of inflammatory markers compared to Caucasians and Europeans.<sup>[16]</sup> Hence, it can be speculated that those with underlying inflammatory conditions will be more predisposed to rickets with similar 25(OH) D levels. Study of inflammatory markers in the subjects with and without rickets may reveal this pathogenetic mechanism.

## VDR GENE POLYMORPHISM

VDR gene polymorphism has been reported to be a determinant of bone formation and intestinal calcium absorption. Most frequently studied are three adjacent restriction fragment length polymorphisms (RFLPs) for BsmI, ApaI, and TaqI at the 3' end of the VDR gene and one RFLP in the start codon of VDR gene—FokI. A meta-analysis revealed that the most common haplotype for the VDR gene, regardless of ethnicity, is baT, followed by BAa and bAT in Caucasians and bAT and BaT in Asians.<sup>[17]</sup> An Indian study reported Ft, TT, and Aa as the most common polymorphism of VDR, and FTA followed by fta as common haplotype.<sup>[18]</sup> Many studies have assessed variable number of RFLPs and its effect on bone mineral density, calcium absorption, and PTH levels. Studies from India and Turkey reported no association of any of VDR polymorphism (BsmI and FokI from India; and ApaI and TaqI from Turkey) in patients with osteomalacia or rickets,<sup>[19,20]</sup> while a study from China found that FF genotype was significantly associated with vitamin D deficiency rickets.<sup>[21]</sup> Lorentzon, *et al.* reported that ApaI genotype Aa was found to be related to higher level of PTH in healthy Caucasian girls.<sup>[22]</sup> Another study revealed a significant association of VDR FokI with PTH levels.<sup>[23]</sup> Marco, *et al.* reported that BB genotype (BsmI) had less severe secondary hyperparathyroidism in predialysis patients.<sup>[24]</sup>

However, these polymorphisms occur in the nonfunctional region of VDR and may indicate linkage disequilibrium with other truly functional polymorphisms elsewhere in the VDR gene.<sup>[25]</sup> Recently, a study reported functional haplotype alleles of the 5' 1a/1e, 1b promoter region and of the 3' untranslated region was associated with 15% lower mRNA level of VDR expression and this could impact the vitamin D signaling efficiency.<sup>[26]</sup> Three VDR tag SNPs (rs3782905, rs7299460, and rs11168314) and one SNP in VDR (rs10735810)<sup>[11]</sup> were associated with adverse outcome in patients with prostatic carcinoma indicating low efficiency of these receptor polymorphism.<sup>[14]</sup> These studies indicate that VDR polymorphisms play an important role in bone and parathyroid gland behavior, leading to differential response due to a likely tissue-specific effect of the VDR response to calcitriol.<sup>[27]</sup> Hence, in subjects with VDR polymorphisms which are less efficient, this will adversely impact osteoblastic cells and parathyroid cells, and may untie the Gordian knot.

## CALCIUM SENSING RECEPTOR

Finally, polymorphism of calcium sensing receptor which is activated at higher calcium levels will have exaggerated PTH

response at lower level of calcium. The 986S polymorphism of the CASR has recently been associated with higher serum ionized calcium levels.<sup>[28]</sup> but three other CaSR coding region polymorphisms (Ala986Ser, Arg990Gly, and Gln1011Glu) have no major influence on indices of calcium homeostasis in this female population.<sup>[29]</sup> More studies are required in this area evaluating not only interaction of calcium and CaSR but other nutritional divalent cations like magnesium, which may be contributing to the Gordian knot.

## CONCLUSION

Rickets/osteomalacia can be termed a life style disease. All humans had ample exposure to sunlight in aboriginal days. With evolution and modernization, the population started wearing clothes, moved into indoor dwellings, air conditioned offices, and cars. Hence, there started a trend of less and less sun exposure and synthesis of vitamin D. Nature is a great healer. Possibly there occurred genetic changes to overcome this vitamin D deficiency by improving efficiency of the vitamin D–PTH–calcium system. This is reflected in genetic polymorphism. However, individuals without these protective mechanisms are predisposed to clinical disease, and will develop rickets and osteomalacia when challenged by Vitamin D deficiency. More research is needed, however, before we have clear answers to the question raised at the beginning of this editorial. And this will open the Gordian knot of differential response to 25(OH) D levels in given individuals.

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