Evaluation Of The Correlation Between Vitamin D Receptor Polymorphism and The Respone To Treatment in Chronic Hepatitis C Patients

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Hepatogastroenterology and Tropical Medicine

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Abstract

Introduction: Immune effects of vitamin D including its cytokine regulating actions are mediated through the nuclear vitamin D receptor. Genetic polymorphisms affecting the vitamin D receptor *FokI* gene have been implicated in several immune disorders

Aim of the study: In this study we assess the effect of *FokI* gene polymorphism and vitamin D levels on response to treatment in chronic hepatitis C genotype 4.

Patients and methods: Blood samples of 50 responders and 50 non-responders to pegylated interferon and ribavirin were tested for the *FokI* gene polymorphism and serum vitamin D levels. 20 healthy controls were also tested for serum vitamin D levels

Results: Mean baseline vitamin D level was 5.9 ±3.9 and 32.5 ±12.9 ng/ml in HCV patients and controls respectively (P: <0.001). All HCV patients were deficient to vitamin D (<30ng/ml). At the end of treatment serum vitamin D levels remained unchanged in non-responders (7.2 ±2.9 vs 6.5 ±4.8ng/ml, p:0.3), while in responders serum vitamin D levels improved significantly (5.3 ±2.8 vs 65.8 ±16.2ng/ml, p: <0.001). VDR *FokI* polymorphism (ff or Ff) was detected in 45 (90%) non-responders vs only 20 (40%) responders (p:<0.001). Logistic regression revealed that higher age, alkaline phosphatase and VDR *FokI* polymorphism were associated with non-response.

Conclusion: The *FokI* VDR polymorphism is independently associated with poor response to therapy in genotype 4 HCV. Analogous to *IL28* gene polymorphisms *FokI* should be incorporated as a predictor of response in the pre-therapeutic assessment of patients.

Key words:

Hepatitis C- vitamin D- polymorphism- treatment- response

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List of abbreviations

AAP: American Academy of Pedriatrics
BSA: Body surface area
Bp: base pair
BOC: Bocepreveir
CCR10: chemokine recptor type 10
CHC: Chronic hepatitis C
CI: confidence Interval
DAA: direct acting antiviral
DBP: vitamin D binding protein
DCs: dendritic cells
F/f: presence of a restriction site in one allele of FokI polymorphism
FF: absence of a restriction site in both alleles of FokI polymorphism
ff: presence of arestriction site in both alleles of FokI polymorphism
FGF23: Fibroblast growth factor 23.
FNB: Food and nutrition board
HALT-C: hepatitis C long term treatment against cirrhosis
HCC: Hepatocellular carcinoma
HCV: hepatitis C virus
IFN: Interferon
LT: liver transplantation
M.tb: Mycobacterium tuberculous
MELD score: Model for End Stage Liver Disease
MS: multiple sclerosis
NHANES: National Health and Nutrition Examination Survey
OR: Odd's Ratio
PAMP: pathogen associated molecular pattern
PI's: protease inhibitors
PRR: pattern recognition receptors
PTH: parathyroid hormone
RANKL: Receptor activator of nuclear factor kappa β ligand
RBV: ribavirin
RDA: recommended daily allowance
SD: Standard deviation
SLE: systemic lupus erythamtosus
SNPs: single nucleotide polymorphism
SOC: standard of care
SVR: sustained virological response
TGF: Transforming growth factor
TLRs: toll like receptor
TNF: Tumour necrosis factor

TVR: telaprevir
UL: upper limit
UVB: ultraviolet B
VDR: vitamin D receptor

Introduction and aim of work

Rationale and Background:

The activated hormonal form of vitamin D, 1-25- dihydroxyvitamin D, is essential for calcium and bone homeostasis, Vitamin D deficiency is associated with many common and serious pathological conditions, including cancer, autoimmune disease, cardiovascular disease, insulin resistance, and diabetes **(Holick MF, 2004)**. Vitamin D, beyond its known role, possesses important immune functions, favouring innate immunity response and cell differentiation **(Holick MF, 2007)**.

There is also an association between vitamin D status and both cholestatic and noncholestatic chronic liver diseases **(Klein GL et al, 2002).** Patients with chronic hepatitis C have higher incidence of severe vitamin D deficiency (25-hydroxyvitamin D, 25(OH)D <10 ng/mL) compared to the normal control (25% versus 12%, P<0.0001) **(Lange CM et al, 2011)**.

In immune-competent patients, higher vitamin D levels at baseline were able to predict sustained virological response achievement following interferon and ribavirin combination therapy for chronic hepatitis C in genotype 1**(Petta S et al, 2010)**.Clinicians have increasing interest in vitamin D because it is easily modifiable and its supplementation may improve response to antiviral treatment **(Bitetto D et al, 2011).**

Most of the biological activities of activated vitamin D are mediated via a nuclear vitamin D receptor (VDR), which serves as a ligand-activated transcription factor. Polymorphisms within the VDR gene may, therefore, result in defective gene activation and consecutive impaired effector functions of vitamin D, such as calcium homeostasis, cell

differentiation, or immune regulation. In addition, polymorphisms within the promotor region of the 1alpha-hydroxylase are associated with dysregulated Immunity. A recent study detected a correlation between VDR polymorphism and SVR rate in genotype 1 despite a lack of correlation with vitamin D serum levels, the authors suggested that this polymorphism might be a more precise indicator to the defect in vitamin D metabolism and its consequences on the immunity and HCV clearance **(Lange CM et al 2011).**

Aim of the work:

Evaluating the correlation between vitamin D receptor polymorphism FokI and the response to treatment in chronic hepatitis C patients.

Chapter 1
Vitamin D

There is renewed interest in vitamin D synthesis, metabolism, and action. The two principal driving forces for heightened interest can be traced to: 1) the worsening, worldwide trend to nutritional vitamin D insufficiency **(Mithal A et al, 2009)** and 2) new knowledge regarding the nonhormonal, intracrine,and paracrine actions of 1-hydroxylated vitamin D metabolites in man **(Hewison M , 2008).**

Vitamin D is not technically a vitamin, ie, it is not an essential dietary factor; rather, it is a prohormone produced photochemically in the skin from 7-dehydrocholesterol. The molecular structure of vitamin D is closely allied to that of classic steroid hormones (eg, estradiol, cortisol, and aldosterone) in that they have the same root cyclopentanoperhydrophenanthrene ring structure **(Feldman D et al, 2005)**.

Sources of vitamin D:

The major endogenous, synthetic source of vitamin D1 for humans is the epidermis. VitaminD3 is produced in the skin by a UVB-mediated, photolytic, non enzymatic reaction that converts 7-dehydrocholesterol to previtamin D3 **(Holick MF, 2008)**.

PrevitaminD3 undergoes a subsequent nonenzymatic, thermal isomerization conversion to vitamin D3, also in the skin. From the skin, vitamin D3 finds its way into the general circulation. In the hepatic parenchyma, vitaminD3 is converted by one of several, high-capacity cytochromeP450s to 25-hydroxyvitamin D3 (25OHD3); the microsomal CYP2R1 appears to have the highest affinity for substrate vitamin **(Strushkevich N, 2008)**.

Food	IUs per serving*	Percent DV**
Cod liver oil, 1 tablespoon	1,360	340
Swordfish, cooked, 3 ounces	566	142
Salmon (sockeye), cooked, 3 ounces	447	112
Tuna fish, canned in water, drained, 3 ounces	154	39
Orange juice fortified with vitamin D, 1 cup (check product labels, as amount of added vitamin D varies)	137	34
Milk, nonfat, reduced fat, and whole, vitamin D-fortified, 1 cup	115-124	29-31
Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces (more heavily fortified yogurts provide more of the DV)	80	20
Margarine, fortified, 1 tablespoon	60	15
Sardines, canned in oil, drained, 2 sardines	46	12
Liver, beef, cooked, 3 ounces	42	11
Egg, 1 large (vitamin D is found in yolk)	41	10
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 0.75-1 cup (more heavily fortified cereals might provide more of the DV)	40	10
Cheese, Swiss, 1 ounce	6	2

* IUs = International Units.
** DV = Daily Value.

Table 1.1 Vitamin D dietary sources. Adapted from U.S. Department of Agriculture, Agricultural Research Service, 2011.

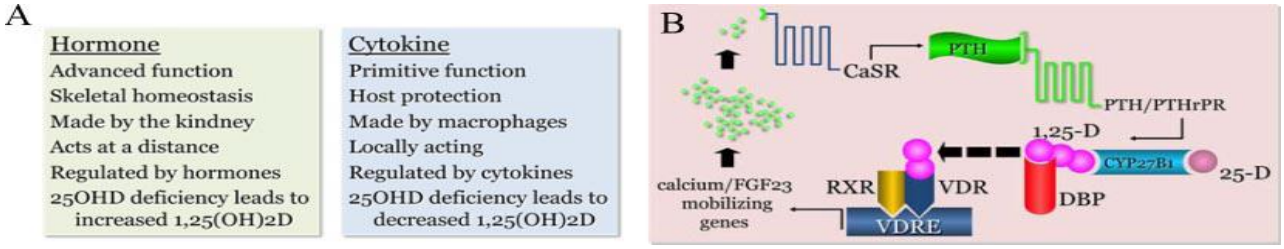
Functions and metabolism of vitamin D:

25OHD is the most plentiful and stable metabolite of vitamin D in human serum, qualities determined by the heightened affinity by which 25OHD is bound by the serum vitamin D binding protein and other members of the albumin superfamily of proteins found in the blood **(Chun RF et al, 2008)**.

As such, the 25OHD level in the serum is the best indicator of vitamin D entering the host, either by cutaneous synthesis or by ingestion in the diet **(Chun RF et al, 2008)**.

In cross-sectional studies, especially those performed in populations living at relatively elevated latitudes in North America, Europe, and Asia, serum levels of the 25OHD metabolite are maximal some 30–60 d after peak sunlight exposure in the summer months. 25OHD is a prohormone or immediate precursor metabolite to the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D) 1,25(OH)2D

is the product of a single enzyme, the mitochondrial CYP27B1 hydroxylase, and it serves as a highaffinity ligand for the vitamin D receptor (VDR) in target tissues where it acts to modulate expression of vitamin D-directed genes. 1,25(OH)2D circulates in the serum at concentrations that are roughly 0.1% that of the prohormone 25OHD **(Adams JS et al, 2008)**.



1.1 Summary of distinctions between the two, phylogentically discrete functions of vitamin D. (Adams and Hewison, 2010)

The previous figure defines the evolutionarily distinct, but preserved, functions of vitamin D. The more evolutionarily advanced function of vitaminDis that of a hormone. This function is reserved for species bearing an endoskeleton where the 1,25(OH)2D hormone serves as a circulating regulator of both mineral and skeletal homeostasis in the host. The only recognized source of the hormone in man is the CYP27B1-hydroxylase; this enzyme is confined principally but not entirely to the proximal tubular epithelial cell of the kidney. 1,25(OH)2D synthesis in the kidney is regulated by other hormones. It is stimulated primarily by PTH and inhibited by circulating fibroblast growth factor 23 (FGF23) made by osteocytes. **(Quarles LD, 2008)**

The more evolutionarily primitive function of vitamin D is that of a cytokine generated to protect the inside environment of the host (single cell organisms to man) from microbial invaders in the external environment **(Adams JS et al, 2008)**.

The 1,25(OH)2D cytokine is synthesized primarily by monocyte-macrophages and acts in an intracrine mode via interaction with the VDR to modulate the innate immune response to invading microbial agents **(Liu PT et al, 2006).**When produced in sufficient quantities, 1,25(OH)2D can escape the confines of the monocyte-macrophage to interact with and control the cytokine profiles of activated, VDR-expressing T- and B-lymphocytes in the local, inflammatory microenvironment **(Adams JS et al, 2008).**

A key distinction between the 1,25(OH)2D hormone and cytokine systems is that an inadequate supply of substrate 25OHD will stimulate the renal CYP27B1-hydroxylase to maintain or increase production of the active, 1,25(OH)2D metabolite via secondary hyperparathyroidism, whereas a deficiency of substrate for the extrarenal CYP27B1-hydroxylase leads to a decrease in product 1,25(OH)2D **(Adams JS et al, 2008)**.

Research has shown, for example, that vitamin D3 is one of the primary biological regulators of calcium homeostasis. Vitamin D3's important biological effects occur only as a consequence of its metabolism into a family of daughter metabolites, including the key kidney-produced metabolite 1,25-dihydroxyvitamin D3 [1,25(OH)2D3]. Researchers consider 1,25(OH)2D3 to be a steroid hormone and believe that it functions the same way as other steroid hormones—by interacting with its cognate vitamin D receptor (VDR) **(Feldman D et al, 2005)**.

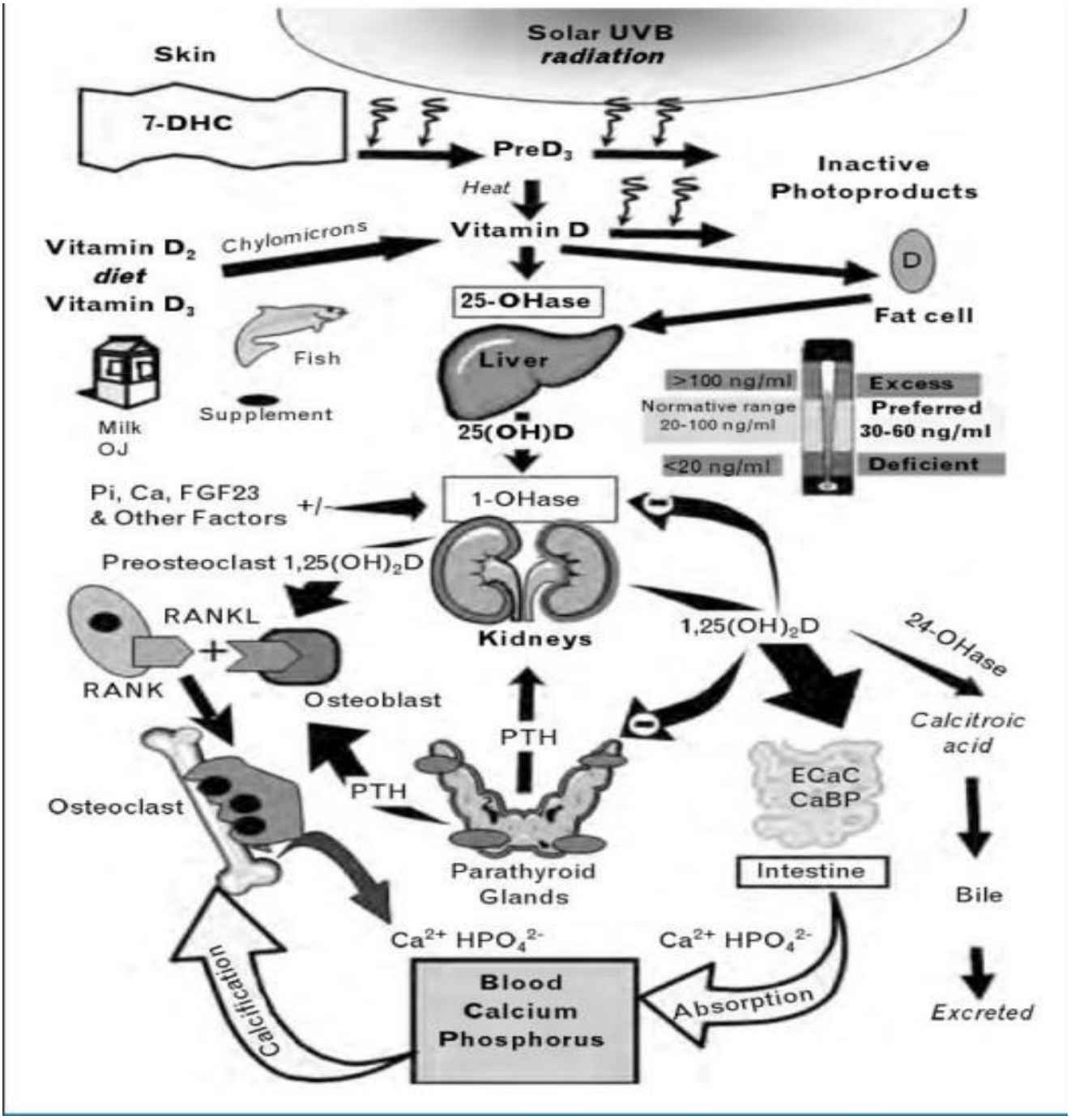


Figure 1.2 Summary of the metabolic pathway of vitamin D synthesis and activation and its relation to calicium and phosphorous homeostasis and bone density.

Adapted from Curr Opin Gastroenterol. 2012;28(2):139-150. © 2012 Lippincott Williams & Wilkins