REVIEW ARTICLE

Genetic and epigenetic factors influencing vitamin D status[†]

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Abstracts

The global prevalence of vitamin D deficiency appears to be increasing, and the impact of this on human health is important because of the association of vitamin D insufficiency with increased risk of osteoporosis, cardiovascular disease and some cancers. There are few studies on the genetic factors that can influence vitamin D levels. In particular, the data from twin and family-based studies have reported that circulating vitamin D concentrations are partially determined by genetic factors. Moreover, it has been shown that genetic variants (e.g., mutation) and alteration (e.g., deletion, amplification, inversion) in genes involved in the metabolism, catabolism, transport, or binding of vitamin D to it receptor, might affect vitamin D level. However, the underlying genetic determinants of plasma 25-hydroxyvitamin D3 [25(OH)D] concentrations remain to be elucidated. Furthermore, the association between epigenetic modifications such as DNA methylation and vitamin D level has now been reported in several studies. The aim of current review was to provide an overview of the possible value of loci associated to vitamin D metabolism, catabolism, and transport as well epigenetic modification and environmental factors influencing vitamin D status.

Keywords: Vitamin D; Polymorphisms; CYP2R1, Epigenetic, Genome-wide association study

Introduction

Several studies have reported a strong relationship between vitamin D status and different health outcomes (Bahrami et al., 2017; McCullough et al., 2009; Tabatabaeizadeh et al., 2017). Attention has now turned to the gene-environment interactions that could have an impact on various vitamin D-related disorders (Ahn et al., 2009; Dickinson et al., 2009). For instance, it is possible that hypovitaminosis D occurs in the presence of specific variations of genes related to vitamin D metabolism. Individuals with specific vitamin D-related genotypes may therefore require specific personalized advice to optimize their vitamin D status.

A clear understanding of the genetic factors involved in determining vitamin D status is therefore necessary to appreciate the possible gene-environment interactions of vitamin D. Several twin and family studies have reported the heritability of vitamin D to be between 23-80%. In the Framingham Offspring Study, the heritability of 25-hydroxyvitamin D3 [25(OH)D] was estimated to be 28.8 % (Shea et al., 2009). A study of 384 monozygotic and 684 dizygotic twin pairs from the Twins UK study reported the heritability of [25(OH)D], 1,25-dihydroxyvitamin D3 [1,25$(OH)_2$D], parathyroid hormone (PTH), and vitamin D binding protein to be 43 %, 65 %, 60% and 62% respectively (Hunter et al., 2001). In 100 adult pairs [Monozygotic (n=40) and dizygotic (n= 59] from the longitudinal Canadian population based study, the heritability of serum 25(OH)D levels was reported to be up to 77 % (Orton et al., 2008). Genetic factors appear to contribute to 70 % of the variation in seasonal serum 25(OH)D level among 510 adult male twins (Karohl et al., 2010). In a family study of subjects selected to study asthma, 25(OH)D and 1,25(OH)2D levels were reported to be 80 % and 30 % genetically determined, respectively (Wjst et al., 2007). It has been reported that the heritability of [25(OH)D] and [1,25(OH)2D] was 23 and 16 % in African Americans and Hispanics from the Colorado and 41 and 20 % in Hispanics from Texas, respectively (Engelman et al., 2008). This discrepancy in heritability may be attributed to

study design, methods of vitamin D measurement or heritability estimation, allele frequencies and environmental variables that were extant, such as season.

Although, there are great variations in the heritability estimation of vitamin D levels between studies, it is clear that genetic factors play an important role in determining serum vitamin D concentrations.

In this review we have summarize the impact of several gene loci that have been reported to be related to vitamin D metabolism that affect vitamin D status have been detected in linkage studies, candidate gene studies and genome-wide association studies (GWAS) as well the impact of epigenetic modification and environmental factors that are known to influence vitamin D status.

The metabolism of [25(OH)D]

The production of vitamin D3 relies on exposure to ultraviolet rays of sun (Datta et al., 2017). Sun light, acting on 7-dehydrocholesterol (7-DHC) in the epidermis of skin, causes to the vitamin D3 production. 25(OH)D generates mainly in the liver through hydroxylation by cytochrome P450 enzyme CYP2R1. A second hydroxylation occurs in the distal tubules of the kidney by the enzyme CYP27B1; then the active form 1,25(OH)2D, is made as a potent seco-steroid hormone(Eyles et al., 2005)(Figure 1). The dietary intake of vitamin D3 or the ergosterol-derived vitamin D2 can be obtained from foods or supplements. 25(OH)D is the most widely accepted marker of overall vitamin D status, reflecting vitamin D input, from either dietary or environmental sources (Holick, 2007). Also, this molecule (25(OH)D] and/or [1,25(OH)2D) is the most common reported marker of vitamin D status in epidemiological studies. Though, some studies have investigated the association between genetic variations for both 25(OH)D and 1,25(OH)2D (Engelman et al., 2008; Lauridsen et al., 2005; Wjst et al., 2006).

Candidate genes related to vitamin D status can be divided into several categories; (i) genes involved in upstream production of 25(OH)D (inflow), (ii) genes involved in downstream activation of 25(OH)D to the active ligand 25(OH)D, and hormone (outflow), (iii) carrier protein features (which binds to the 25(OH)D molecule and the active ligand 25(OH)D, (iv) receptor and related co-activating proteins (affect executive ability of the ligand-receptor), and (v) other second-order processes that effect the regulatory pathways such as calcium and/or parathyroid hormone concentrations. Recent advances in the genetics of vitamin D metabolism have emphasized the importance of several genes, but there are many aspects of vitamin D metabolism that remain unclear.

Genetic Studies of Vitamin D

Several human studies have shown genetic variants that are related to vitamin D status. These studies are categorized according to their goals, including linkage studies, studies of candidate genes of vitamin D metabolism pathway, and genome wide association studies (GWAS).

Linkage Studies

The classical strategy to analyzing the genetics of inherited disease or continuous phenotypes uses linkage analysis, which enables the identification of particular genetic intervals within a chromosome are associated with an increased disease susceptibility.

Genetic markers are usually genotyped in families, and linkage analysis establishes a locus which segregates with the disorder in a pedigree among affected subjects. The linkage degree between a trait or disease condition and genetic markers is calculated through the logarithm of odds ratio score (LOD). The threshold of significance in linkage studies has been defined as a LOD score of 3.3 (Center, 1995).

Although linkage studies have been very powerful at characterizing genes that are responsible for inherited diseases, they have also been useful for determining genes that may contribute to quantitative traits, for instance plasma vitamin D. Wjst and colleagues have shown that the level of serum 25(OH)D is under genetic control (Wjst et al., 2007). In particular they found an interesting region on chromosome 2 that contains genes with known VDREs (Wjst et al., 2007).

The Framingham Offspring Study (n=1,762 participants) had a maximum LOD score (1.16) for circulating 25(OH)D for a locus on chromosome 14, which did not reach threshold significance (Shea et al., 2009). The unsuccessful linkage studies may be due to the polygenic variation in serum vitamin D, and the measurement method of vitamin D concentrations. While linkage studies have been unsuccessful in identifying replicated genetic architecture of vitamin D metabolism, the candidate gene approach and GWAS have identified several reproducible associations with vitamin D status.

Candidate Gene Studies

Candidate gene studies evaluate whether SNPs frequency relates with a continuous trait, such as vitamin D status, most usually assessed in a group of unrelated subjects.

Importantly, associations identified using a candidate gene approach may be due to the effect of a nearby causal variant with which it is in linkage disequilibrium (LD). Thus, these studies must be confirmed in several independent populations, including those of different ethnic groups, for confirmation. Several SNPs related with serum 25(OH)D have been reported in candidate gene studies, and are shown in Table1.

25 Hydroxyvitamin D-1-α hydroxylase (*CYP27B1*)

The *CYP27B1* (cytochrome P-450) gene has been mapped to chromosome 12(at q13.1-q13.3), and consists of a 6.66 kb fragment. *CYP27B1* encodes a 1α-hydroxylase which

converts 25(OH)D to 1,25(OH)2D, the active form of vitamin D. Some *CYP27B1* variants are related to vitamin D status. The SNP rs10877012 (C/A) has been widely investigated for an association with serum 25(OH)D levels. The rs10877012 C allele was found to be associated with a lower serum 25(OH)D in 379 African American and 379 Caucasia population (Signorello et al., 2011), gestational diabetic patients (Ramos-Lopez et al., 2008) and large cohort British study participants (n=9377) (Hyppönen et al., 2009). In a Canadian multiple sclerosis patients study, two other SNPs, rs4646536 (C/T) and rs703842 (C/T) were reported to be associated with serum 25(OH)D concentrations (Table1) (Orton et al., 2008). However, these associations were not replicated in the cross-sectional Insulin Resistance Atherosclerosis Family Study among African American or Hispanic population (Engelman et al., 2008). This inconsistent data may be due to the relatively small sample sizes, or different ethnicities of the populations (Berry and Hyppönen, 2011).

Vitamin D 25-hydroxylase (*CYP2R1*)

The *CYP2R1* gene extends over 14.29 kb and is located on chromosome 11p15.2. *CYP2R1* is a hydroxylase with high affinity for vitamin D, and is responsible for the conversion of cholecaliferol to 25(OH)D in the first step in the activation pathway (Shinkyo et al., 2004). A missense mutation in exon 2 of *CYP2R1* gene, may result in vitamin D deficiency (Cheng et al., 2004). LD studies in simplex type 1 German diabetes families (609 subjects) revealed that the *CYP2R1* SNP, rs10741657 was related to serum 25(OH)D concentrations (Ramos-Lopez et al., 2007). This was repeated in unrelated subjects from a cohort study (Bu et al., 2010) and in a British populations (Cooper et al., 2011). But this association was not replicated in patients with gestational diabetes mellitus (Ramos-Lopez et al., 2008). Two other *CYP2R1* SNPs, rs12794714 and rs10766197 were found to be positively associated with serum 25(OH)D concentrations in a cohort study (Bu et al., 2010). The SNP rs10766197 is situated

in the 5' flanking region of the *CYP2R1* gene (Wjst et al., 2006). The SNP rs12794714, leads to a synonymous alteration in the *CYP2R1* exon which is associated with 25(OH)D status, and was repeated in a larger study containing 2,610 subjects (Cooper et al., 2011). In a study on 201 healthy Danish pedigrees, four SNPs in *CYP2R1* (rs1562902, rs7116978, rs10741657 and rs10766197) were significantly associated with circulating 25(OH)D levels in the studied population. Several of the SNPs were in strong LD, and the associations were driven by *CYP2R1*-rs10766197 and rs10741657. Genetic risk score analysis revealed that participants with no risk associated alleles of the *CYP2R1*-rs10766197 and rs10741657 loci, were associated with a significantly higher serum 25(OH)D compared to cases with the risk alleles (Nissen et al., 2014). Overall, there was 14.1, 20.9 and 16.5% variation in serum 25(OH)D levels between individuals having no risk alleles and having all four risk alleles in adults, children or both combined, respectively.

Vitamin D binding protein (*GC*)

The *GC* amplifies vitamin D binding protein (DBP), a group-specific component globulin (GC). *GC* is situated on chromosome 4q12-q13, spanning 63.84 kb. There are two SNPs in exon 11 of *GC*, rs7041 (G/T) and rs4588 (C/A) which cause a Glu/Asp amino acid and Thr/Lys amino acid change at codon 416 and 420, respectively(Braun et al., 1992). The association between the rs4588 SNP with serum 25(OH)D levels was identified and confirmed in many studies including a young Canadian population (Fu et al., 2008), Polish individuals (Kurylowicz et al., 2006), white women (Kurylowicz et al., 2006), Hispanics and African Americans (Engelman et al., 2008), Dutch patients with chronic obstructive pulmonary disease (COPD) (Janssens et al., 2010), Han Chinese (Lu et al., 2012), and British (Cooper et al., 2011). Individuals with the rs4588 C allele had lower serum of 25(OH)D levels (Kurylowicz et al., 2006). The effect of rs4588 on serum 25(OH)D levels has been confirmed in candidate gene studies (Ahn et al., 2009; Cooper et al., 2011; Engelman et al.,

2008; Fu et al., 2008; Janssens et al., 2010) or the haplotypes obtained from either rs4588 or rs7041(Abbas et al., 2007) Other *GC* SNPs with high LD with rs4588 was rs2282679 which is on intron 12 of *GC* gene associated with serum 25(OH)D concentrations. This association identified from a study conducted on American males (Ahn et al., 2009) and was replicated in African Americans (Signorello et al., 2011), a British population (Cooper et al., 2011), and Han Chinese population (Lu et al., 2012).

Another variant with high LD with the rs4588 SNP is the rs1155563 polymorphism. It has also been reported to be related to serum 25(OH)D concentration in several studies (Ahn et al., 2009; Hibler et al., 2012; Lu et al., 2012). Two SNPs were identified (rs16846876 (Hibler et al., 2012) and rs222020 (Bu et al., 2010)) which are not in LD with both rs4588 and rs7041, proposes that allelic heterogeneity may be exists. As well as, rs2298849 located in the first intron, has been linked with serum 25(OH)D levels in an African American population (Signorello et al., 2011) and population (n= 1494) of Chinese postmenopausal women (Xu et al., 2014). In addition to the associations in *GC* were observed in candidate gene studies, gene-level principal components analysis (PCA) has shown that *GC* gene polymorphisms were significantly associated with circulating 25(OH)D. Also, rs7041, rs222035, and rs842999 are strongly correlated ($r^2 > 0.92$) and rs1155563 is in high correlation with rs17467825 ($r^2 > 0.86$) (Hibler et al., 2012). Moreover, there were two SNPs (rs1155563 and rs17467825) in LD with rs4588 respectively (Hibler et al., 2012). So, both in SNP- and gene-level analysis associations between *GC* and [25(OH)D] confirmed. Six SNPs in the *GC* gene (rs4588, rs16846876, rs2282679, rs12512631, rs17467825 and rs842999) were significantly related with serum 25(OH)D levels in families of Danish populations. *GC*-rs4588 and rs842999 were in strong LD. Carriers without risk alleles of *GC*-rs4588 and -rs842999 had higher serum 25(OH)D levels than carriers of risk alleles (Nissen et al., 2014).

Vitamin D receptor (*VDR*)

The *VDR* gene spans 63.49 kb on the 12q12-q14 in the human genome. *VDR* has a considerable noncoding region including exons 1F–1C and exons 2–9, which codify *VDR* protein (Uitterlinden et al., 2004). The minor allele of *VDR* SNP rs2228570 (the Fok1 polymorphism, rs10735810) leads to a *VDR* protein with three amino acid longer through directly introducing a new translation start codon (Smolders et al., 2009). It influences the activity of *VDR* protein (Haussler et al., 1998), leads to a fewer transcriptional activator effectiveness and alters the functional properties of the receptor (Arai et al., 2001). The T allele of rs2228570 was related with a upper concentration of 25(OH)D in a longitudinal population-based twin study (Orton et al., 2008), in children with autism spectrum disorder (Coşkun et al., 2016) , and a cohort study including 212 patients with multiple sclerosis and 289 healthy controls (Smolders et al., 2009). Furthermore, two adjacent SNPs that reside upstream of exon 1A (rs7139166 and rs4516035), were evaluated for their relationship with 25(OH)D status. The rs7139166-rs4516035 (G–A) haplotype presented upper promoter activity, and the rs7139166-rs4516035 (C–G) haplotype was related with low concentrations of 25(OH)D (d'Alésio et al., 2005). An intronic SNP (rs10783219) was found to be related with serum 25(OH)D level (Engelman et al., 2008).

Vitamin D 24-hydroxylase (*CYP24A1*)

CYP24A1 covering 20.53 kb mapped on chromosome 20q13.2-q13.3. *CYP24A1* codifies the 1,25(OH)2D inactivation protein. An intronic SNP (rs17219315) was related to serum 25(OH)D concentrations in a family-based study (Wjst et al., 2006). In a large study conducted on 1787 healthy non-Hispanic white individuals in United States, the association of baseline levels of 25(OH)D for two SNPs (rs2209314, rs2762939) (Barry et al., 2014).

7-dehydrocholesterol reductase (*DHCR7*)

The *DHCR7* gene is 14.02 kb long and is located on chromosome 11q12-q13. *DHCR7* encodes a reductase which converts 7-DHC into cholesterol in epidermis{Waterham, 2000 #110}. The proceeding substrate of the vitamin D synthetic process is converted to vitamin D through UVB radiation found in sunlight (Figure1). Mutations in *DHCR7* result in impaired gene activity and subsequently accumulation of 7-DHC which leads to Smith-Lemli-Opitz syndrome (Tint et al., 1994). For first time an association between the *DHCR7* (rs11234027) and serum [25(OH)D] levels was identified by a GWAS of 4,501 samples and 2,221 additional individuals in the replication phase from European ancestry (Ahn et al., 2010). The other GWAS performed by the SUNLIGHT consortium in 33,996 subjects from 15 cohorts of European descent identified a novel association on *DHCR7* (rs12785878) (Wang et al., 2010). Among the 945 Uygur ethnic and 928 Kazak ethnic population *DHCR7/NADSYN1*-rs12785878 was significantly related with vitamin D deficiency(Xu et al., 2015). Also, a study that contained 2,610 individuals from a U.K. population confirmed these susceptibility genes (Cooper et al., 2011). In 2897 unrelated healthy Chinese subjects, haplotype TGGGCCC of *DHCR7/NADSYN1* rs1790349-rs7122671-rs1790329-rs11606033-rs2276360-rs1629220-rs2282618 have genetic protective properties toward a lower [25(OH)D] level(Zhang et al., 2013).

Other genes

In contrast to genes discussed above, evidence about variation in other genes has been limited. Fibroblast growth factor-23 (*FGF23*) is a circulating factor that contributes to the metabolism of phosphate and vitamin D. FGF23 reduces urinary phosphate reuptake through down-expressing a sodium-dependent phosphate co-transporter, via inhibiting the CYP27B1 enzyme, and by inducing 1,25(OH)2D inactivation (Saito et al., 2003; Shimada et al., 2001). In a study included Finish children (n=183) and adolescents (n=110 girls) aged 7–19 years, among nine *FGF23* polymorphisms were identified; three of them were common: rs3832879,

rs7955866 and rs11063112. This SNPs significantly related with plasma PTH level and urinary Pi excretion (Pekkinen et al., 2015).

The calcium-sensing receptor (*CASR*) contributes to calcium homeostasis through sensing extracellular blood calcium levels. The majority of previous studies assessed the relationship between *CASR* gene and calcium levels and few have evaluated its association with vitamin D metabolites. In several studies, no associations was observed between intracellular domain polymorphisms of *CASR* rs1801725, rs1042636, rs1801726, and 25(OH)D or 1,25(OH)2D levels (Harding et al., 2006; Jenab et al., 2009). These finding suggest that *CASR* may not play a major role in regulation of vitamin D metabolites (Hibler et al., 2012).

The CUBN gene encodes the protein cubilin which is a peripheral membrane protein amplified in different tissues such as renal proximal tubules, intestinal epithelium, thymus, and placenta (Christensen and Nielsen, 2006). The role of cubilin in the endocytic pathway of the 25(OH)D–DBP complex has been elucidated. There is evidence that the loss of a functional cubilin leads to urinary loss of the 25(OH)D and consequently reduce serum levels of 25(OH)D and 1,25(OH)2D (Nykjaer et al., 2001). Although, Ramos-Lopez and colleagues found no association between the polymorphisms of cubilin and the circulating levels of 25(OH)D or 1,25(OH)2D (Ramos-Lopez et al., 2010). Lipoprotein receptor-related protein 2(*LRP2*) encodes the proteins megalin which plays a role in renal re-absorption of 25(OH)D by receptor-mediated endocytosis. It has been shown that variation in *LRP2* are associated with non-skeletal health outcomes. An allele of *LRP2* polymorphism rs3755166 is related with increased risk of Alzheimer's disease in Chinese Hans [Odds ratio (OR) = 1.38; 95% confidence interval(CI):1.02–1.87, P= 0.04]{Wang, 2011 #111}and AA genotype associated with higher risk of Alzheimer's disease in Europeans without ApoE4 mutation (OR=1.41; 95% CI 1.10–1.90, P= 0.03).

Retinoid X receptors (RXRs), like *VDR*, is a ligand-activated transcription factor involved in cell growth, development, differentiation, and apoptosis. The binding of 1,25(OH)2D to the VDR induces allosteric conformational alterations in *RXR* that simplify the recruitment of coregulators(Haussler et al., 2008; Yee et al., 2005). Wjst *et al*., reported significant correlations between *RXRA* SNP rs3132299 and 1,25(OH)2D as well as rs877954 and 25(OH)D levels, but, different statistical methods were applied in this study and in UDCA population(Wjst et al., 2006) indicated no statistical significant association for these SNPs. In a study among prostate cancer patients no association was found between polymorphisms in *RXRA* and serum levels of 25(OH)D or 1,25(OH)2D (Ahn et al., 2009). But, Hibler and coworker demonstrated a significant positive trend for increasing serum 1,25(OH)2D levels with each extra copy of the *A* allele for rs9409929(Hibler et al., 2010).

Genome wide Association Studies

Over one million SNPs across the whole genome can be rapidly genotyped in hundreds or thousands of samples in GWAS. This approach provides a wide failed to identify and establish novel associations between loci and biological pathways. But, only the common SNPs with the minor allele frequency greater than 1 % are targeted in GWAS.

The first GWAS of genetic factors affecting serum 25(OH)D, studied 70,987 SNPs among 1,012 related subjects from the Framingham Heart Study (Benjamin et al., 2007). In this study, no SNPs achieved a significant association after adjustment for age, sex, systolic/diastolic blood pressure, body mass index, waist, high density lipoprotein cholesterol, smoking, glucose, triglyceride, diabete , hypertension treatment, lipid lowering medication therapy, hormone replacement medication therapy, asthma medication therapy, alcohol use, and prevalent cardiovascular disease. Another GWAS genotyped 309,200 SNPs among 229 Hispanic Americans also unsuccessful to identify loci significantly related with 25(OH)D levels (Engelman et al., 2010). The first GWAS, consisting 4,501 participants and 2,221

additional subjects, in the replication phase from European ancestry confirmed two candidate genes, GC (rs2282679) and CYP2R1 (rs1993116) and identified a novel association on DHCR7(rs11234027) after adjustment for age, vitamin D assay batch, study, sex, body mass index, season, dietary and supplement vitamin D intake, and region/latitude(Ahn et al., 2010). The other GWAS recruited 33,996 subjects of European descent and found CYP24A1 (rs6013897), GC (rs2282679), and CYP2R1 (rs10741657), and DHCR7 (rs12785878) with vitamin D after correction for age, sex, body mass index, and season(Wang et al., 2010). Another study in Western Australian Pregnancy Cohort found CYP2R1 (rs11023332, rs1007392) and GC (rs17467825, and rs1155563), and NPY (rs156299) after adjustment for gender, body mass index and season (Anderson et al., 2014).

A recent GWAS on 3,538 individuals from a Punjabi Sikh population identified a novel locus at chromosome 20p11.21 represented by rs2207173 between *FOXA2* and *SSTR4* to be related with serum 25(OH)D levels. Another association with a locus was suggested at rs11586313 in the regulatory region of the *IVL* gene located on chromosome 1q21.3. In addition, this study replicated known genes related with serum 25(OH)D concentrations adjusted for age, gender, body mass index and type 1 diabetes status including *GC*(rs2282679) and *CYP2R1*(rs12794714) reported in Europeans and the *DAB1*(rs6680429), reported in Hispanics(Sapkota et al., 2016).

Epigenetic Studies of Vitamin D

Epigenetic modification are heritable alterations in gene expression that are not as a result of DNA sequence change (Stefanska et al., 2012). Epigenetic alterations include post-translational modification of the amino acid tails of histones, such as acetylation, methylation, and phosphorylation of histones and abnormal expression of microRNAs. Increased methylation resulted to lower gene expression.

It has been shown that the active form of vitamin D [1,25(OH)2D] is epigenetically active; also, 1α-hydroxylase and 24-hydroxylase are under epigenetic control (Burrell et al., 2011; Novakovic et al., 2009). In a study investigating the relationship between vitamin D supplementation and DNA methylation status of candidate genes (*CYP27A1*, *CYP2R1*, *CYP27B1*, *CYP24A1*), mean methylation ratio of the *CYP2R1* gene was dependent on the level of increased serum 25(OH)D in response to supplementation. Actually, hypermethylation was most in patients with a low response to vitamin D supplementation. It may therefore lead to gene silencing of *CYP2R1* (Zhou et al., 2010).

In a small African American GWAS, a different level of methylation in *CYP2R1* and *CYP24A1* was detected between cases with severe vitamin D deficiency and vitamin D sufficient subjects (Zhu et al., 2013). Vitamin D deficient individuals had increased methylation of *CYP2R1* and decreased methylation of *CYP24A1*. This pattern of methylation may contribute to vitamin D deficiency, by reducing conversion to the active metabolite, and elevating inactivation (Zhu et al., 2013). Although, the statistical significance of these finding did not correct for multiple testing.

Zhou *et al* analyzed the methylation status of *CYP2R1*, *CYP24A1* and *CYP27B1* in post-menopausal women supplemented by vitamin D (Zhou et al., 2014). Methylation status of responders to supplementation (increase serum 25(OH)D after intervention) were compared to non-responders to supplementation (limited increase in serum 25(OH)D after intervention), Interestingly non-responders had higher methylation status of *CYP2R1* at baseline and after intervention. After supplementation both responders and non-responders were found to have lower methylation levels of *CYP24A1* (Zhou et al., 2014). These findings led to the proposition of an association between methylation status, and a response phenomenon. In the same study no differential methylation was found for *CYP27B1*(Zhou et al., 2014). Beckett and co-researcher described a negative relationship between *CYP2R1*/*CYP27B1* methylation

status and circulating 25(OH)D. Also, the association between serum 25(OH)D and *CYP24A1* without correction for vitamin D intake was significant, this suggests that alteration in *CYP24A1* methylation happens in response to serum 25(OH)D concentrations, rather than methylation status (Beckett et al., 2016).

Global DNA hypomethylation is a common epigenetic event that may result from hypomethylation of repetitive DNA elements. Loss of global methylation may lead to chromosomal instability (CI), loss of imprinting (LOI), and activation of transposable elements, consequently genome disturbances (Tapp et al., 2013). The effect of vitamin D on global methylation remains unclear. Until now, few studies have evaluated the relationship between vitamin D status and global DNA methylation and reported inconsistent results (Nair-Shalliker et al., 2014; Valencia et al., 2014). In a study performed in healthy adults of South Australia was showed the inverse association between exposure to solar UV radiation and global DNA methylation was not affect by vitamin D.Valencia *et al.* reported no significant DNA methylation differences in response to vitamin D allocation (Valencia et al., 2014). In a clinical trial performed by Hubner *et al*, reported that the administration of combined oral dose of B vitamins (folic acid, vitamin B12 and vitamin B6) and vitamin D had no effect on DNA methylation, particularly in cases without severe vitamin deficiency (Hübner et al., 2013). Tapp and coworker studied the effects of nutritional factors on age-related DNA methylation in the human rectal mucosa specimens and demonstrated a weak positive association between vitamin D and DNA methylation(Tapp et al., 2013). However limited studies investigated the association between vitamin D and DNA methylation, and this area not completely understood and future large research is need.

Environmental Studies of Vitamin D

Previous studies have identified season and several measure of sun exposure as main determinants of circulating 25(OH)D levels(de la Jara et al., 2004; Islam et al., 2006; Janssen et al., 2002; Meddeb et al., 2005). Van Der Meer *et al* reported that significant determinants of serum 25(OH)D were: consumption of fatty fish, use of vitamin D supplements, area of uncovered skin, use of tanning bed, consumption of margarine and preference for sun(Van Der Meer et al., 2008).

In another study, dietary sources of vitamin D (fatty fish and vitamin D–fortified low-fat dairy products) was associated to vitamin D status among older Swedish women during winter(Burgaz et al., 2007). In a study among Danish women association between vitamin D level, sun exposure and dietary intake was observed. Overall, individual specific-environmental factors involved in 13% of the variation in serum 25(OH)D (Burgaz et al., 2007).

Snellman *et al* in a twin study demonstrated that the average of serum 25(OH)D level was lower during the winter than summer season. Half of the variability in 25(OH)D during the summer season was attributed to genetic factors. Also, they claimed that individual-specific environmental elements explain one fourth of the difference in serum 25(OH)D independent of season (Snellman et al., 2009).

Gender, season, and physical activity were reported to influence serum 25(OH)D concentrations in rural Chinese adolescents. There was a strong association between [25(OH)D] level and male gender only. Summer season and male gender significantly reduced the risk of being in the lowest tertile of 25(OH)D (Arguelles et al., 2009).

In many regions, serum 25(OH)D levels are reported to be lower in dark skinned than fair-skinned individuals (Harris and Dawson-Hughes, 1998; Mithal et al., 2009; Nesby-O'Dell et

al., 2002). For instance, in the USA the mean serum 25(OH)D levels in all age groups of non-Hispanic whites was 1.2–1.7 times higher than in Mexican Americans and non-Hispanic blacks after adjusting for season and latitude (Looker et al., 2002). New Zealanders of European origins had the highest mean serum 25(OH)D level, compared to Pacific Islanders and Maori (Rockell et al., 2006). Cultural/behavioral factors also are important in these variations(Farrar et al., 2011).

Discussion

Several genetic polymorphisms are related to vitamin D status, and these may provide an understanding of the pathophysiology of vitamin D related diseases or drug targets. Mendelian-randomization analysis; the random assortment of genes which occurs during gamete formation, secures an equal distribution of confounding factors among different genotypes; and can be used for evaluating whether genetically affected risk factors are associated with the clinical outcomes.

The main contribution of the genetic analysis of vitamin D status has been to highlight the important control points in vitamin D metabolism. Most studies have focused on GC and CYP2R1, but the DHCR7 gene has received little attention, but may be an important determinant of vitamin D status. Another potentially clinically important aspect of understanding the genetic determinants of vitamin D is to be able to identify individuals who would benefit from supplementation. However, the underlying genetic mechanism related with to serum 25(OH)D concentrations remain poorly understood and clinical usage is not applicable. Further research is needed in order to clarify the genetic determinants of serum 25(OH)D concentrations, and to uncover the mechanisms of action responsible for these associations.

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Figure legend:

Metabolism of vitamin D.

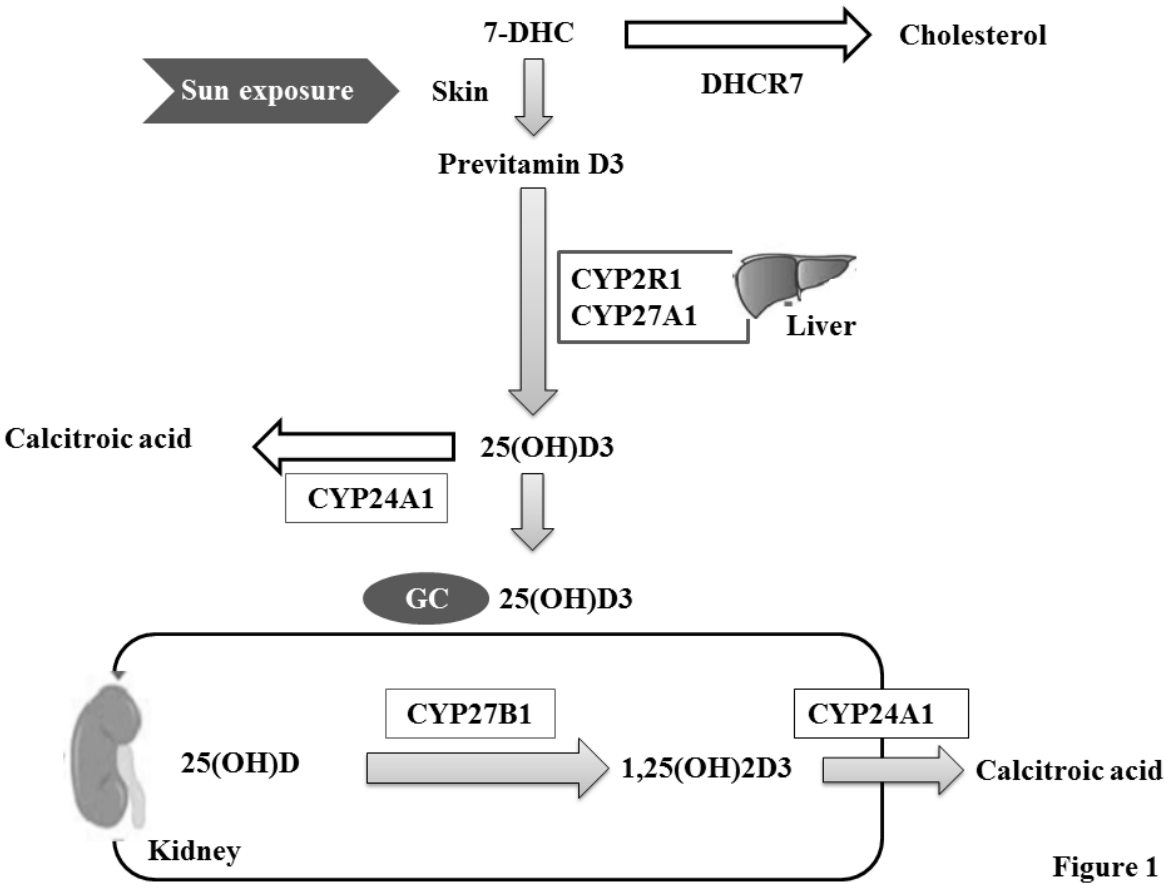

Figure 1

Table1. SNPs related with 25(OH)D level

Gene	Position	SNP	location	m/M	Beta (SE)	P value	Reverences
CYP2R1	11p15.2	rs1993116	Intron	A/G	0.25 (0.05)	2.90E- 17	(Ahn et al., 2010)
		rs12794714	Exon	A/G	-5.03 (1.24)	0.0001	(Bu et al., 2010)
		rs10766197	5' Flanking	A/G	-4.53 (1.27)	0.002	(Bu et al., 2010)
		rs10741657	5' Flanking	A/G	4.12 (1.33)	0.01	(Bu et al., 2010)
		rs7116978	Intron	T/C	67.5	<0.0001	(Barry et al., 2014)
		rs1562902	5' Flanking	C/T	68.1	0.0005	(Barry et al., 2014)
CYP27B1	12q13.1-q13.3	rs4646536	Intron	T/C	0.17 (0.071)	2.00E- 02	(Orton et al., 2008)
		rs703842	5' Flanking	T/C	0.18 (0.071)	1.50E- 02	(Orton et al., 2008)
		rs10877012	Promoter	A/C	0.02 (0.008)	1.00E- 02	(Hyppönen et al., 2009)
CYP24A1	20q13	rs6013897	3' Flanking	A/T	-0.03 (0.014)	1.60E- 02	(Cooper et al., 2011)
		rs2209314			2.67	0.03	(Barry et al., 2014)
VDR	12q13.11	rs10783219	Intron	T/A	-0.16 (0.056)	4.00E- 03	(Engelman et al., 2008)
		rs2228570	5' UTR	G/A	-0.24 (0.10)	3.00E- 03	(Orton et al., 2008)
DHCR7	11q13.4	rs11234027	5' Flanking	A/G	-0.18 (0.03)	3.40E- 09	(Ahn et al., 2010)
		rs12785878	5' Flanking	T/G	-0.04 (0.013)	9.90E- 04	(Cooper et al., 2011)
GC	4q12-q13	rs222020	Intron	C/T	5.79 (1.86)	1.00E- 03	(Bu et al., 2010)
		rs2282679	Intron	C/A	-0.11 (0.015)	8.90E- 13	(Cooper et al., 2011)
				C/A	-0.38 (0.03)	1.80E- 49	(Ahn et al., 2010)
		rs4588	Exon	C/A	0.98 (0.40)	1.00E- 02	(Kurylowicz et al., 2006)
				C/A	0.29 (0.075)	<0.001	(Engelman et al., 2008)
				A/C	-4.22 (0.93)	<0.0001	(Sinotte et al., 2009)
		rs7041	Exon	T/G	-0.18 (0.06)	3.00E- 03	(Engelman et al., 2008)
				T/G	-0.08 (0.012)	2.50E- 10	(Cooper et al., 2011)
		rs1155563	Intron	C/T	-3.37 (0.69)	<0.001	(Hibler et al., 2012)
		rs17467825	3' Flanking	NA	-3.44 (0.69)	<0.001	(Hibler et al., 2012)
		rs16846876	3' Flanking	NA	-2.95 (0.67)	0.001	(Hibler et al., 2012)
		rs12512631	NA	C/T	4.69	<0.0001	(Barry et al., 2014)
		rs1155563	intron	C/T	8.44	<0.0001	(Barry et al., 2014)

SNPs: Single nucleotide polymorphisms; CYP2R1: Cytochrome P450 Family 2 Subfamily R Member 1; CYP27B1: Cytochrome P450 Family 27 Subfamily B Member 1; CYP3A4: cytochrome P450, family 3 A Member 4; VDR: DHCR7: Vitamin D receptor; 7-dehydrocholesterol reductase; GC: Vitamin D binding protein