

# Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease. A review of evidence

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## SUMMARY

The Vitamin D endocrine system regulates multiple aspects of calcium metabolism and cellular differentiation and replication in the immune system, endocrine pancreas, liver, skeletal muscles and adipocytes. It plays an important role in glucose homeostasis, notably, in the mechanism of insulin release. Actions of vitamin D are mediated by the binding of 1, 25-(OH)2D3 to a specific cytosolic/nuclear vitamin D receptor (VDR), a member of the steroid/thyroid hormone receptor superfamily. Several frequent polymorphisms are found in the *VDR* gene and were reported to be associated with a variety of physiological and pathological phenotypes in many populations. In this paper, we will review the evidences suggesting associations of allelic variations in the *VDR* gene and phenotypes related to body weight, glucose homeostasis, diabetes and its vascular complications.

**Key-words:** Vitamin D · Vitamin D receptor (VDR) · Vitamin D binding protein (DBP) · Type 1 diabetes · Type 2 diabetes · Obesity · Insulin secretion · Insulin sensitivity · Cardiovascular disease.

**Reis AF, Hauache OM, Velho G. Vitamin D endocrine system and the genetic susceptibility to diabetes, obesity and vascular disease. A review of evidence**  
*Diabetes Metab* 2005;31:318-325

## RÉSUMÉ

**Vitamine D et prédisposition génétique au diabète, à l'obésité et aux maladies artérielles.**  
**Une revue de la littérature récente**

Le système hormonal de la vitamine D joue de multiples rôles physiologiques. En dehors de ses cibles classiques impliquées dans l'homéostasie calcique, la forme active de l'hormone, 1, 25-(OH)2D3, aurait une action biologique dans nombreux tissus, organes ou systèmes, parmi lesquels on peut citer le système immunitaire, le pancréas endocrine, le foie, les muscles et les adipocytes. La vitamine D joue un rôle important dans la sécrétion physiologique d'insuline et dans le maintien d'une tolérance normale au glucose. La forme active de l'hormone, 1, 25-(OH)2D3, se lie à un récepteur intracellulaire spécifique (VDR), qui fait partie de la super-famille des récepteurs nucléaires des hormones stéroïdes. Plusieurs variants, fréquents dans la population générale, ont été identifiés dans le gène *VDR*, et des associations avec différents traits phénotypiques ont été rapportées dans de nombreuses populations. Dans cette revue, nous résumons et discutons les résultats plus significatifs de la littérature concernant les associations alléliques du gène *VDR* avec les variations du poids corporel, l'homéostasie glucidique, les diabètes sucrés et leurs complications vasculaires.

**Mots-clés :** Vitamine D · Récepteur de la vitamine D (VDR) · Protéine de liaison de la vitamine D (DBP) · Insulino sécrétion · Sensibilité à l'insuline · Diabète de type 1 · Diabète de type 2 · Obésité · Maladie cardiovasculaire.

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Received: February 2nd 2005; revised: June 6th 2005

The vitamin D endocrine system regulates multiple aspects of calcium metabolism and cellular differentiation and replication in many target organs in addition to those directly involved in calcium homeostasis (bones, intestinal tract, kidneys and parathyroid gland). These include the immune system, endocrine pancreas, liver, skeletal muscles and adipocytes [1-3]. It is now clear that the vitamin D endocrine system plays an important role in glucose homeostasis and, notably, in the mechanisms of insulin release. In animal models and in humans, vitamin D deficiency is associated with impaired insulin secretion, which is normalised by vitamin D administration [4-6]. Correlations between serum concentrations of vitamin D metabolites, plasma glucose and insulin secretion were observed in humans [5, 7]. The molecular pathways by which 1, 25-dihydroxyvitamin D<sub>3</sub> [1, 25-(OH)<sub>2</sub>D<sub>3</sub>] regulates insulin synthesis are not precisely defined [8]. However, it was shown that vitamin D promotes general activation of protein synthesis in pancreatic  $\eta$ -cells [9], modulates the glycolytic pathway [10], enhances Ca<sup>++</sup> influx into  $\eta$ -cells [11] and stimulates the conversion of proinsulin to insulin [12].

The circulating metabolites of vitamin D bind with high affinity to the vitamin D-binding protein (DBP) [13], a single-chain serum glycoprotein. DBP is encoded by the *Gc* gene, a member of a multigene cluster that includes albumin and  $\zeta$ -fetoprotein genes, located at chromosome 4q11-q13 [13]. Studies in knocked-out mice demonstrated the important role DBP plays in maintaining stable serum stores of vitamin D metabolites and modulating their bioavailability, activation and end-organ responsiveness [14]. Sequence variations in the *Gc* gene give rise to three major electrophoretic variants of DBP [15]. These variants differ by amino acid sequence as well as by attached polysaccharide structures. They also differ by their binding affinity for vitamin D and its metabolites [16]. Several studies in non-Caucasian populations have suggested associations of DBP phenotypes or related genotypes with type 2 diabetes mellitus or with glucose or insulin levels [17-23]. However, these results were not confirmed in larger cohorts of American or French Caucasian subjects [24, 25].

The actions of the vitamin D endocrine system are mediated both by genomic and non genomic pathways [26]. The former are activated by the binding of 1, 25-(OH)<sub>2</sub>D<sub>3</sub> to a specific cytosolic/nuclear vitamin D receptor (VDR), a member of the steroid/thyroid hormone receptor superfamily [5]. Non genomic pathways are activated via a putative membrane vitamin D receptor (mVDR) and might be responsible for rapid effects of vitamin D [27]. The *VDR* gene is located on chromosome 12q12-q14 in humans. Rare loss of function mutations in its coding regions are associated in homozygous carriers with an autosomal recessive form of familial vitamin D-resistant rickets [28]. Several frequent polymorphisms are also found in the *VDR* gene

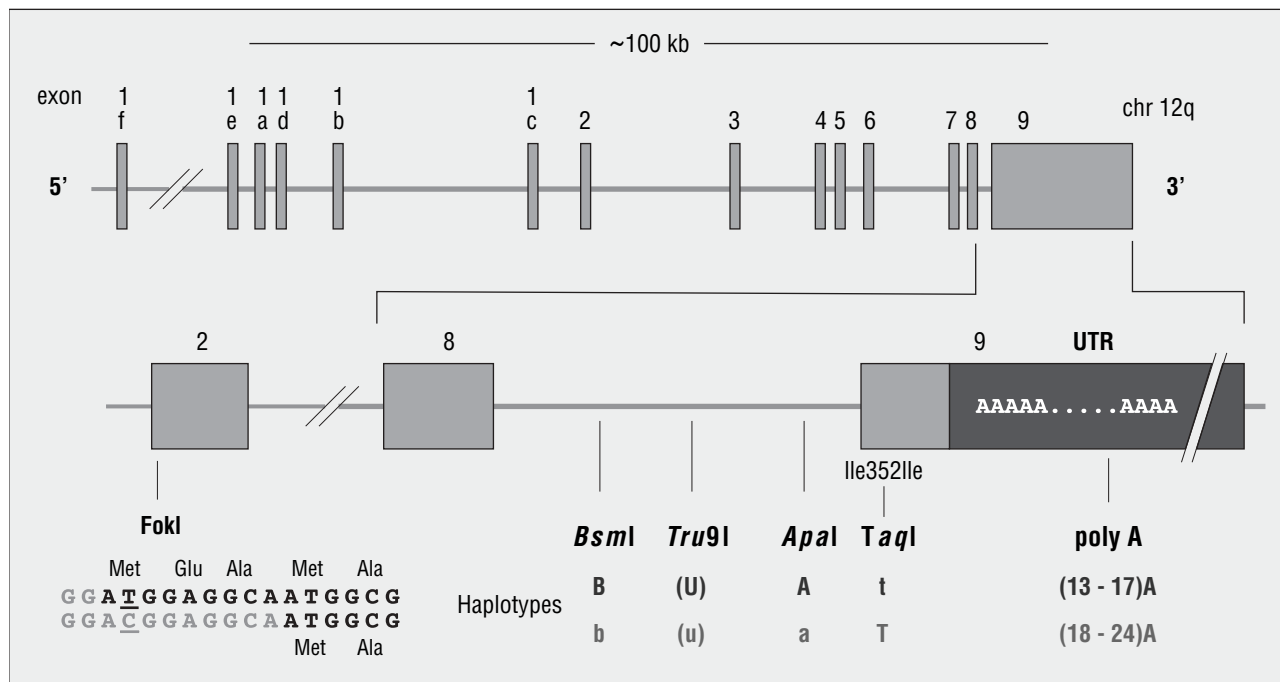
(*fig 1*) [29], and were reported to be associated with a variety of physiological and pathological phenotypes in many populations. These include variations in circulating levels of 1, 25-(OH)<sub>2</sub>D<sub>3</sub> [30], variations in bone mineral density [31, 32], modulation of intrauterine and early postnatal growth [33, 34] and adult height [34]. Other studies showed associations with variations in body weight [35], insulin sensitivity [36], insulin secretion in response to glucose [37, 38], susceptibility to type 1 [39] or type 2 diabetes [36] and severity of coronary artery disease [40]. However, not all associations were consistently found in all populations.

In this paper, we will review the evidences suggesting associations of allelic variations in the *VDR* gene and phenotypes related to body weight, glucose homeostasis, diabetes and its vascular complications.

### *VDR* allelic variation and susceptibility to type 1 diabetes

VDR is expressed in many cell types of the immune system [41] and immune defects are observed in VDR knock-out mice [42]. These observations are consistent with a physiological role for vitamin D in the modulation of immune responses. An increasing body of data in mice and humans suggest that vitamin D could modulate the pathophysiological process leading to autoimmune diabetes [43]. Thus, studies in NOD mice showed increased diabetes incidence in animals with vitamin D deficiency in the early weeks of life [44]. Conversely, oral administration of vitamin D or vitamin D analogues was shown to prevent diabetes onset in the animals as long as treatment was maintained [45]. Epidemiological data in humans showed a threefold increase in the incidence of type 1 diabetes when vitamin D deficiency was present in the first months of life [46].

These observations led investigators to look for possible associations of VDR polymorphisms with type 1 diabetes. A first study was performed in Southern Indian families with type 1 diabetes and in this population the "b" allele of the *BsmI* variant or "b"-allele containing haplotypes were shown to be preferentially transmitted by parents to affected offspring [39]. Several populations with different genetic backgrounds have been studied since [47-53]. *VDR* polymorphisms were found to be associated with risk for type 1 diabetes in many but not all of these investigations, but the statistical power of some studies was probably low. Different risk alleles, genotypes or haplotypes were found in different populations (data summarised in *table 1*). One possible explanation for these contrasting results could be the presence of variable degrees of linkage disequilibrium between the variants and the putative functional mutation or mutations in the different populations. The map of single nucleotide polymorphisms (SNP) of the *VDR* gene region was developed [54], and a recent study analysed associations of 98 *VDR* SNPs in up to 3,763 type 1 diabetic families from the UK, Finland, Norway, Romania, and USA



**Figure 1**  
 Exon-intron structure of the VDR gene and position of frequently studied VDR polymorphisms [29]. The five SNPs in exon 2 (*FokI*), intron 8 (*BsmI*, *Tru9I*, *ApaI*), and exon 9 (ATT->ATC, Ile352Ile; *TaqI*) have been defined, historically, by the associated restriction enzyme. Genotypes are designated conventionally by the first letter of the name of the enzyme, except for *Tru9I*, designated using “U” or “u” to avoid confusion with *TaqI* genotypes. A capital letter indicates the absence of the cut site, whereas a lower-case letter indicates its presence. The sequence variation at the *FokI* polymorphism (T > C) modifies the translation initiation start site (ATG) resulting in a protein shortened by three amino acids. Haplotypes defined by *BsmI*, *ApaI* and *TaqI* variants and frequently found in Caucasians are in linkage disequilibrium with alleles of the poly-A sequence in the 3'-untranslated region (3'-UTR).

[55]. Only weak evidence of association (P = 0.02-0.05) was observed between four novel SNPs and type 1 diabetes. Moreover, when the SNPs were tested in an independent set of 1,587 patients and 1,827 control subjects from the UK, no evidence of association was found.

It's not possible to exclude that the positive results that were published might be due to spurious false-positive associations. However, environmental factors influence the circulating levels of active vitamin D forms and thus may modulate genotype-related risk. It is noteworthy that

**Table I**  
 VDR gene variants and type 1 diabetes.

Population origin	At risk genotype	Reference
Southern India	b allele bAT haplotype	McDermott et al. [39]
Germany	Bat haplotype	Pani et al. [47]
Taiwan	B allele	Chang et al. [48]
Germany	TT genotype	Fassbender et al. [49]
Hungary (gender-specific association in girls)	combined presence of b, a and u alleles	Gyorffy et al. [50]
Dalmatia (South Croatia)	BB/AA/tt combined genotypes	Skrabic et al. [51]
Japan	B allele	Motohashi et al. [52]
Finland	no association	Turpeinen et al. [53]
UK, Finland, Norway, Romania, USA	no association with 98 SNPs	Nejentsev et al. [55]

Genotypes at four SNPs in intron 8 (*BsmI*, *Tru9I*, *ApaI*) and exon 9 (ATT->ATC, Ile352Ile; *TaqI*) of the VDR gene. Genotypes were designated conventionally by the first letter of the name of the enzyme, except for *Tru9I*, which was designated using “U” or “u”. A capital letter indicates the absence of the cut site, whereas a lower-case letter indicates its presence.

the north-south gradient and seasonal variations observed in the incidence of Type 1 diabetes have been attributed, in part, to variations in the effectiveness of ultraviolet light-mediated conversion of provitamin D into vitamin D. More detailed analyses need to be performed, taking into account, for instance, the circulating levels of vitamin D, but also a stratification by HLA genotypes. Moreover, the impact of the polymorphisms on VDR mRNA and protein levels in the immune system will need to be properly assessed in these populations [38]. Taken together, the available data on vitamin D and type 1 diabetes suggest that this hormonal system might contribute to the environmental determinants that lead to the autoimmune destruction of  $\eta$ -cells and diabetes. However, its role in the genetic determinants of type 1 diabetes, mediated by VDR allelic variation, is still debatable.

### **VDR allelic variation and modulation of insulin secretion and action. Implications in the susceptibility to type 2 diabetes and obesity**

The role of the vitamin D endocrine system in the susceptibility to type 2 diabetes is still unsettled. Results of a recent epidemiological study suggest that high levels of vitamin D are associated with increased insulin sensitivity and with a decreased risk of type 2 diabetes in Caucasians [56]. Other studies addressed the possible role of VDR and of VDR allelic variations in the mechanisms of glucose homeostasis. It is well established that pancreatic  $\eta$ -cells express VDR [3]. Studies in mice expressing functionally inactive mutant VDR showed that disruption of the VDR signaling pathway is associated with a pronounced impairment in oral glucose tolerance and insulin secretory capacity, together with a reduction in pancreatic insulin mRNA levels in normally fed animals [8]. The pancreas morphology was normal and the insulin secretion defect was independent of alterations in mineral homeostasis.

Physiological studies in humans suggest that allelic variations in the VDR gene modulate  $\eta$ -cell function. It was first reported in a cohort of nondiabetic Bangladeshi subjects, recruited from a population considered at risk for type 2 diabetes, that *ApaI*, *BsmI* and *TaqI* SNPs influence insulin secretion in response to an oral glucose tolerance test [37]. In that study, the *aa*, *bb* or *TT* genotypes were associated with decreased insulin levels, half as much as those of *AA*, *BB* or *tt* genotypes, with intermediate levels observed in heterozygous subjects. These results were confirmed in an extended study of the same population [38], where it was also observed that the VDR genotype is a significant determinant of VDR mRNA and protein levels in peripheral blood mononuclear cells. Moreover, insulin secretion in response to an oral glucose load was significantly correlated with VDR mRNA in these cells. Although no direct measurements were performed in  $\eta$ -cells, these results provided an indirect functional support to the allelic associations of VDR gene and insulin secretion.

Other studies have suggested associations of the *FokI* [57] or *BsmI* [36] variants with insulin sensitivity estimated by the HOMA method (based on fasting glucose and insulin levels), but not with insulin secretion, in nondiabetic Caucasian subjects. However, these results are yet to be confirmed by measurements of insulin sensitivity with reference methods, such as the euglycaemic hyperinsulinemic glucose clamp. Contrasting results were observed regarding allelic associations with hyperglycaemia and type 2 diabetes. In a large community-based study of 1545 Caucasian adults [36], fasting plasma glucose and prevalence of glucose intolerance were significantly higher in nondiabetic subjects with the *aa* genotype of *ApaI* as compared with those with the *AA* genotype, but only a trend towards an association of this variant with type 2 diabetes was observed. It is noteworthy that the *aa* genotype was associated with decreased HOMA insulin sensitivity in that study. Another large study was performed in a German cohort of 1539 young aircrew men recruited during routine medical qualification for flying duty [58]. An interaction between physical activity and VDR genotype in the modulation of fasting plasma glucose was observed in that study. In subjects with low physical activity, *BB* genotype carriers had significantly higher levels of fasting glucose and higher prevalence of fasting plasma glucose > 5.55 mmol/l than carriers of other *BsmI* genotypes. These effects were not observed in subjects with high physical activity.

We have found no association between type 2 diabetes and *ApaI*, *BsmI*, *Tru9I* and *TaqI* SNPs in a cohort of French Caucasian subjects [35]. A smaller study in a Polish cohort led to similar negative results [59]. However, we have observed associations with the susceptibility to obesity in the subset of our cohort with an early diagnosis of diabetes, i.e. before the age of 45 years (1<sup>st</sup> tertile of the distribution of age of diagnosis of diabetes in that cohort) [35]. The presence of the *TT* genotype of *TaqI* SNP or of the *bb* genotype of the *BsmI* SNP accounted for a difference of about 9 kg of body weight or 4 kg/m<sup>2</sup> of BMI, and for a ~30% increase in the prevalence of obesity as compared to the presence of other genotypes. Associations of VDR genotypes with body-weight related phenotypes were observed in other [60, 61] but not all studies [37]. An association of the *bb* genotype of *BsmI* SNP with increased bone mineral density and with increased body weight was observed in healthy, non obese, premenopausal American Caucasian women [60]. In that study, the association with body weight was shown to be independent from variations in bone mineral density. In a group of Swedish premenopausal healthy women the *BB* genotype of the *BsmI* SNP and the shorter alleles of the *poly-A* repeat were associated with high muscle strength and with increased fat mass and body weight [61].

The pathophysiological mechanisms of these associations remain unexplained. A direct effect of vitamin D on adipocyte differentiation and metabolism is a possible

mechanism as VDR is expressed in preadipocytes [62]. It has been shown *in vitro* that vitamin D inhibits UCP2 (uncoupling protein 2) expression in adipocytes [63] and the differentiation of preadipocytes [64, 65], and stimulates the terminal differentiation of adipocytes [66] and the synthesis and secretion of lipoprotein lipase [67]. It is noteworthy that inverse associations of BMI with circulating levels of 25-hydroxyvitamin D were observed in Caucasian subjects [68, 69], and that *VDR* polymorphisms were found to influence circulating levels of vitamin D [30]. However, one might speculate that the associations of *VDR* polymorphisms with fat mass and obesity might also be related to allelic modulation of insulin secretion.

### *VDR* and vascular disease

Vitamin D has antiproliferative, antiangiogenic and antioxidant properties [70]. Thus, it could have a protective role against chronic degenerative disorders such as cardiovascular disease. Epidemiological studies observed an inverse relationship between circulating levels of 25-hydroxyvitamin D and the risk for cardiovascular disease [71] and for peripheral arterial disease [72]. A few studies evaluated associations of *VDR* polymorphisms with cardiovascular disease. A study of 41 Dutch subjects with coronary artery disease assessed by coronary angiography reported an association of the *BsmI* variant with the severity of coronary disease [40]. In that study, patients with severe coronary stenosis were more likely to have the *bb* genotype. Another study analysed 293 German subjects considered at risk for coronary artery disease because of angina pectoris and known hypercholesterolemia, and who underwent diagnostic coronary angiography [73]. The prevalence of coronary artery disease was significantly increased in *BB* as compared to *bb* carriers, with intermediate prevalences in heterozygous subjects. However, a much larger study in a cohort of 3441 German subjects failed to confirm these results [74]. Interestingly, vitamin D is a negative regulator of the renin-angiotensin system [75], and it was suggested that the inverse relationship between vitamin D levels and the risk for cardiovascular disease could be explained, in part, by an effect on blood pressure [76]. This hypothesis still needs to be tested. Other cardiovascular phenotypes have been explored, and an association of the *B* allele of the *BsmI* variant with calcific aortic valve stenosis have been reported [77].

Regarding the microvascular complications of diabetes, an inverse relationship between the severity of retinopathy and serum 1, 25(OH)<sub>2</sub>D<sub>3</sub> concentrations was observed in subjects with type 2 diabetes [78]. An association between the *TaqI* polymorphism and risk for severe retinopathy was observed in a cohort of French type 1 diabetic patients [79]. In that study, *TT* genotype was associated with high risk and the *Tt* genotype with low risk for severe retinopathy.

## Conclusions

Despite more than ten years of intensive investigation, evidence is suggestive but not conclusive regarding the role of allelic variations in the *VDR* gene in the polygenic susceptibility to a number of complex diseases and traits, such as insulin secretion, diabetes, obesity or cardiovascular disease. Almost one hundred polymorphisms have been now described across the *VDR* gene region [54], but they have not been systematically analysed in large populations and their effects on *VDR* function are undetermined. In most investigations, only a few polymorphisms have been studied, and most of these polymorphisms were anonymous SNPs with unknown functional effects. It is postulated that the observed associations of these SNPs with complex traits are due to linkage disequilibrium with a functional variant in the *VDR* gene. The promoter region regulates the production of *VDR* mRNA, while the 3'-untranslated region of the *VDR* gene is involved in the regulation of stability or degradation of mRNA. Functional variants have been detected in both regions [29] and their interaction could regulate mRNA availability for translation into *VDR* protein. For instance, the most frequent haplotypes defined by *BsmI*, *ApaI* and *TaqI* variants in Caucasians, *BAt* and *baT*, are in linkage disequilibrium with short and long alleles, respectively, of the *poly-A* sequence in the 3'-untranslated region (fig 1). In some studies, these haplotypes were associated with variable gene transcription, mRNA stability, and *VDR* protein levels [38].

More studies are needed to conciliate the contrasting results observed in clinical studies of different populations, notably the observation of different risk alleles, genotypes or haplotypes in different populations, and to clarify the mechanisms underlying the associations of the *VDR* variants with various phenotypes that have been tested. These studies will need to be based on extended *VDR* haplotypes that take into account the promoter and the 3'-untranslated region. The interaction with circulating levels of vitamin D will also need to be considered.

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### Glossary and notes

**Allele:** One of the variant forms of a gene at a particular locus, or location, on a chromosome. Different alleles might be associated with or be responsible for different functional properties of the encoded protein.

**Association study:** Association or population studies test whether a genetic marker occurs more frequently in cases than in controls defined by a given phenotype or trait. If an association is observed, the marker itself is either in the susceptibility locus responsible for the trait, or close to (in linkage disequilibrium with) the susceptibility locus. However, frequently, associations only represent type I errors (false positive results). Results of population-based case-control studies are very sensitive to population stratification bias (cases and controls with different genetic background), which is a serious limitation of these tests. Nevertheless, case-control studies remain useful, as it has been proposed that susceptibility genes with small effects on complex polygenic traits might only be detectable by large association studies.

**Genotype:** 1) The genes that an organism possesses. 2) The alleles of a particular locus (gene, genetic marker).

**Haplotype:** A set of closely linked genetic markers present on one chromosome, which tend to be inherited together. Some haplotypes may be in linkage disequilibrium (see definition below).

**Linkage disequilibrium:** Association of specific alleles at different loci. Linkage disequilibrium is defined as a condition in which the haplotype frequencies in a population deviate from the values they would have if the alleles at the loci defining the haplotype were combined at random.

**Phenotype:** 1) The observable attributes of an organism. 2) The detectable manifestations of a specific genotype. 3) A variable and detectable trait (clinical, biological) in the context of a genetic study.

**Polymorphism:** A common variation among individuals in the sequence of DNA.

**Restriction enzyme:** Enzymes that recognize a specific sequence of DNA and cut the DNA at that site. Genetic variations at the site where a restriction enzyme cuts DNA are known as RFLPs (restriction fragments length polymorphisms). Such variations affect the size of the resulting fragments. RFLPs can be used as markers for genetic studies.

**Single nucleotide polymorphism (SNP):** Common variation of 1 base-pair that occurs in human DNA at a frequency of once every 1,000 bases. These variations can be used as markers for genetic studies. The large majority of the SNPs are silent, that is, do not have functional significance. Conversely, SNPs in regulatory or coding regions of a gene might modify protein structure and/or function.

**Vitamin D synthesis:** Vitamin D<sub>3</sub> (cholecalciferol) is scarce in the typical western diet and is mainly generated in the skin when light energy is absorbed by a precursor molecules, 7-dehydrocholesterol. Cholecalciferol has little biological activity and needs to be metabolised to the hormonally-active form. In a first step within the liver, cholecalciferol is hydroxylated to 25-hydroxycholecalciferol by the enzyme 25-hydroxylase. This step is loosely regulated, and blood levels of 25-hydroxycholecalciferol reflect the amount of vitamin D produced in the skin or ingested. In a second step within the kidney, 25-hydroxycholecalciferol is further hydroxylated by the enzyme 1-alpha-hydroxylase to 1, 25-dihydroxycholecalciferol, the biologically active form of vitamin D<sub>3</sub>. The enzyme activity is tightly regulated and serves as the major control point in the production of the active hormone. Parathyroid hormone and low blood levels of phosphate are the major inducers of 1-alpha-hydroxylase.