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Role of vitamin D in the pathogenesis of type 2 diabetes mellitus

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Vitamin D deficiency has been shown to alter insulin synthesis and secretion in both humans and animal models. It has been reported that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes mellitus. Vitamin D replenishment improves glycaemia and insulin secretion in patients with type 2 diabetes with established hypovitaminosis D, thereby suggesting a role for vitamin D in the pathogenesis of type 2 diabetes mellitus. The presence of vitamin D receptors (VDR) and vitamin D–binding proteins (DBP) in pancreatic tissue and the relationship between certain allelic variations in the VDR and DBP genes with glucose tolerance and insulin secretion have further supported this hypothesis. The mechanism of action of vitamin D in type 2 diabetes is thought to be mediated not only through regulation of plasma calcium levels, which regulate insulin synthesis and secretion, but also through a direct action on pancreatic β-cell function. Therefore, owing to its increasing relevance, this review focuses on the role of vitamin D in the pathogenesis of type 2 diabetes mellitus.

Keywords: pathogenesis, type 2 diabetes mellitus, vitamin D, vitamin D–binding protein, vitamin D receptor
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Introduction

The suggested relationship between type 1 diabetes mellitus and vitamin D deficiency has been extensively reported [1,2]. Vitamin D treatment has been shown to improve, and even prevent, type 1 diabetes mellitus in both human [3,4] and animal models [5–7]. These effects have been mainly attributed to the immunomodulatory actions of vitamin D [2]. However, less is known on the association between vitamin D and type 2 diabetes mellitus. Vitamin D deficiency causes reduced insulin secretion in rats and humans, and its replenishment improves β-cell function and glucose tolerance [8–11]. Moreover, certain allelic variations in the vitamin D receptor (VDR) and vitamin D–binding protein (DBP) might influence glucose tolerance and insulin secretion [12–14], thus contributing to the genetic risk for type 2 diabetes. As vitamin D modulates insulin receptor gene expression and insulin secretion, it is an interesting environmental candidate for type 2 diabetes mellitus pathogenesis and development [14]. In fact, circumstantial evidence supporting this hypothesis already exists. This review will focus on the role of vitamin D as a genetic and environmental factor in type 2 diabetes mellitus, in particular, its suggested protective role and the possible mechanisms by which this protection may occur.

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Vitamin D Metabolism and Biological Function

Vitamin D increases the gut uptake of ingested calcium and phosphorus and improves calcium reabsorption by the kidney, thus resulting in the elevation of both mineral elements in plasma [15]. As a consequence, the major biological actions of this vitamin include maintenance of mineral homeostasis and regulation of bone remodelling [16,17].

Only a small amount (30%) of vitamin D can be obtained from the diet, since few foods contain it naturally [18]. As a result, overall vitamin D requirements are covered from the sunlight-induced photochemical conversion of 7-dehydrocholesterol [15,18,19] (figure 1). Industrialization has reduced exposure to sunlight, thus increasing our dependence on dietary sources of vitamin D. Whatever the source of vitamin D, it must be hydroxylated twice to produce the biologically active form of vitamin D, $1,25(OH)_2D_3$ [15] (figure 1). The first hydroxylation process takes place in the liver and forms 25-hydroxyvitamin D_3 ($25(OH)D_3$), while the second hydroxylation step, which produces the final active metabolite, occurs predominantly in the kidney [15,17]. These reactions are brought about by 25-hydroxylase in the liver and 1α-hydroxylase in the kidney, all of which belong to the cytochrome P450–dependent steroid hydroxylases [2]. Serum $25(OH)D_3$ level is a better indicator of vitamin D status than $1,25(OH)_2D_3$, since the former has a slower rate of clearance than the latter [16,17]. Moreover, secondary hyperparathyroidism may result in increased serum $1,25(OH)_2D_3$ levels and is thus an unreliable measurement in such conditions [17]. Hereafter, the term 'vitamin D' will be used exclusively to refer to the active form of vitamin D, $1,25(OH)_2D_3$, while other forms will be named specifically.

Vitamin D deficiency may cause the bone-deforming disease rickets during development, while in adults, it may cause osteomalacia and disturbed muscle metabolism owing to impairment in calcium balance [17,18]. These effects have also been observed in patients with type 2 diabetes, who may exhibit abnormalities in mineral and vitamin D metabolism that can eventually produce osteopenia [20,21]. This review, however, will not

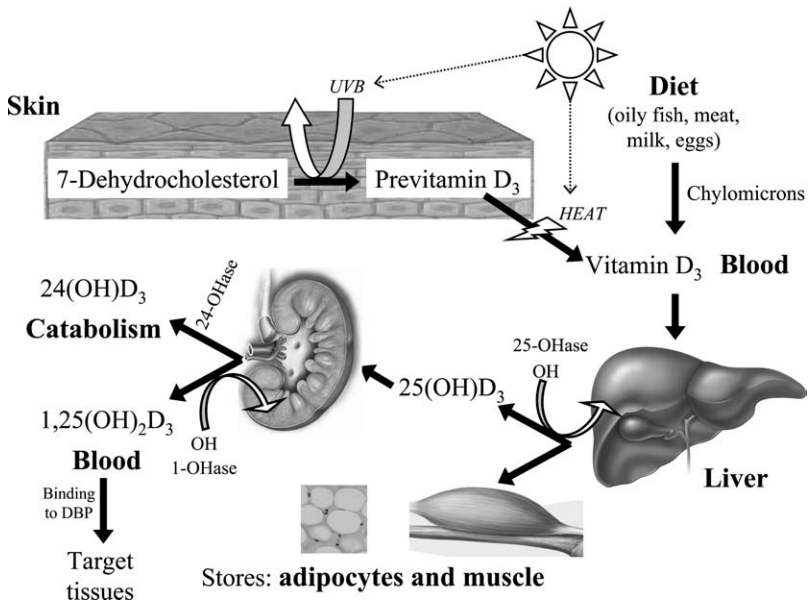

Fig. 1 Vitamin D_3 may be obtained directly from the diet or by means of the sunlight-induced photochemical conversion of 7-dehydrocholesterol to previtamin D_3. Previtamin D_3 is thermodynamically unstable and undergoes thermally induced conversion to Vitamin D_3. Whatever the source, vitamin D_3 must be hydroxylated twice to produce the biologically active form. Thus, the first hydroxylation process takes place in the liver and forms 25-hydroxyvitamin D_3 ($25(OH)D_3$) and is catalysed by vitamin D-25-hydroxylase (25-OHase). The second hydroxylation step, which produces the final active metabolite of vitamin D_3 ($1,25(OH)_2D_3$), is mediated by 25-hydroxyvitamin D_3 1α-hydroxylase (1-OHase) and occurs predominantly in the kidney. Then, $1,25(OH)_2D_3$ is released into the circulation where it binds to vitamin D–binding protein (DBP) until it reaches its target tissue by means of the vitamin D receptors (VDR). Vitamin D 24-hydroxylase (24-OHase) is the enzyme which catalyses the catabolism of the hormone in the kidney.

deal with the issue of vitamin D and bone disease in type 2 diabetes mellitus.

Apart from the above-mentioned tissues, many other vitamin D targets have been reported, such as heart, stomach, liver, brain, skin, pancreatic islets (β cells), thyroid, parathyroid and adrenal glands and immune cells [22] (figure 2). Remarkably, some of these tissues and cell types, including brain, activated lymphocytes (T and B cells), macrophages and skin, contain not only the nuclear VDR but also the enzymes required for its synthesis, thereby suggesting alternative non-classical actions [23,24].

Vitamin D and β-cell Function

There is ample evidence suggesting a role for vitamin D in insulin secretion, which includes the presence of the VDR in β cells and the vitamin D–dependent calcium-binding proteins (DBP) in pancreatic tissue [25–27]. It has been shown in both *in vitro* and *in vivo* models that vitamin D itself is essential for normal insulin release in response to glucose and for maintenance of glucose tolerance. For instance, in diet-induced vitamin D–deficient rats, there is glucose tolerance impairment which, together with hyporesponsiveness to exogenous insulin, produces altered insulin sensitivity [12,28–30]. Moreover, vitamin D deficiency results in decreased pancreatic insulin secretion, without altering glucagon secretion [31,32]. Importantly, vitamin D repletion in the early stages of experimental dietary vitamin D deficiency [9,33] or in subjects with vitamin D deficiency leads to a partial improvement in glucose tolerance and correction of insulin secretion in response to glucose [24,32,34]. In streptozotocin-induced diabetic rats, plasma calcium levels, DBP, circulating vitamin D and bone mass are reduced [35–37]. These defects have been attributed to altered vitamin D metabolism owing to an inhibitory effect of insulin deficiency on the activity of the renal $25(OH)D_3$ 1α-hydroxylase [37,38]. In an effort to further understand the role of vitamin D in β-cell function,

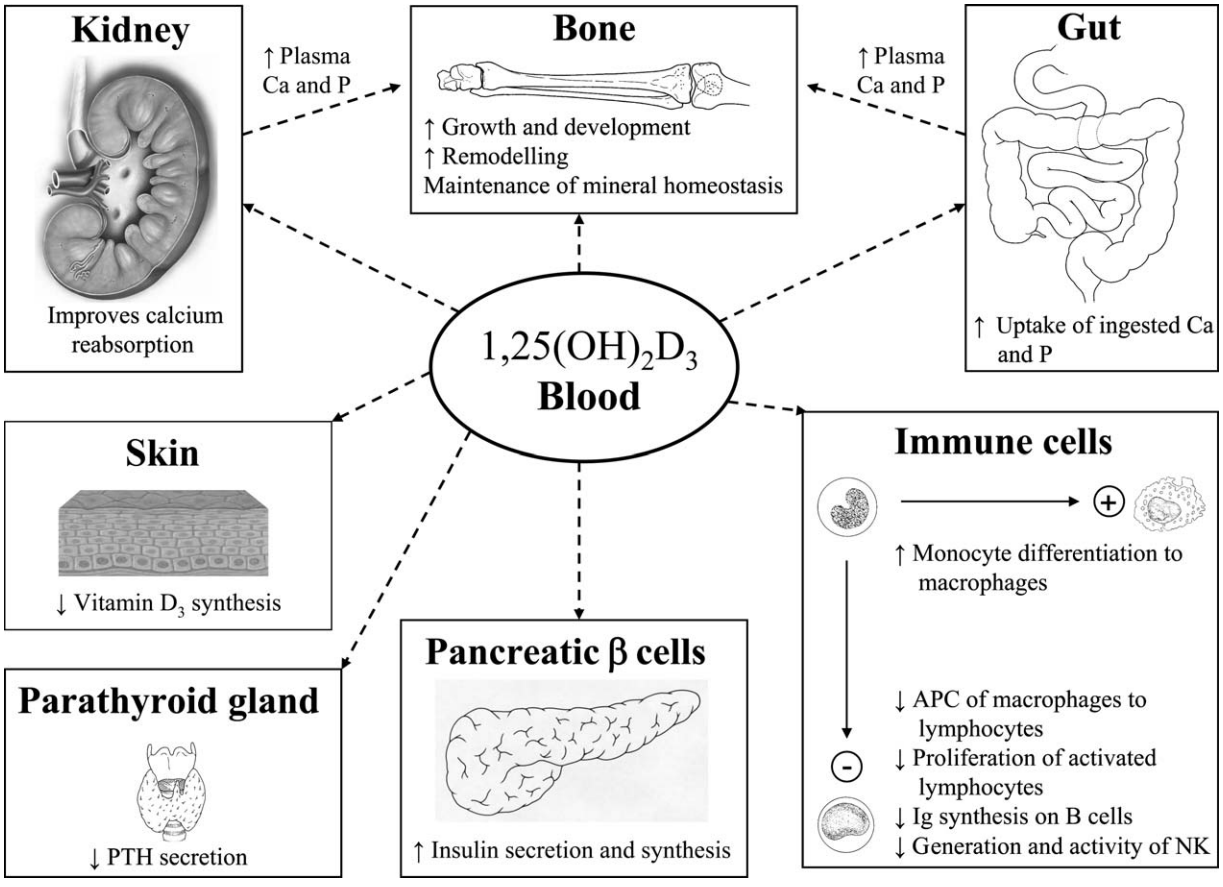

Fig. 2 Major targets and actions of vitamin D_3 on peripheral tissues. APC, antigen-presenting capacity; Ig, immunoglobulin; NK, natural killer cells; PTH, parathyroid hormone.

transgenic VDR knockout mice have been generated. However, in this model, while one group reported impaired glucose tolerance [39], others found no effect on glucose tolerance [40]. These contradictory results have been attributed to the different genetic background of the strains used to generate the transgenic mice [2].

In human beings, vitamin D supplementation improves stimulated insulin secretion in response to an oral glucose load in patients with mild (normal fasting serum glucose) type 2 diabetes mellitus, in non-diabetic healthy subjects and in subjects with vitamin D deficiency but not in patients with established type 2 diabetes mellitus [24,28,29,32]. This is accompanied by a significant increase in serum calcium levels and a reduction in serum free fatty acid levels [28]. Also, restoration of vitamin D levels has been shown to ameliorate glucose tolerance in patients with vitamin D deficiency [10] and improve insulin response in women with type 2 diabetes [41,42]. However, conflicting results have also been obtained in other populations, since it has been reported that vitamin D replacement in Asian population with vitamin D deficiency and type 2 diabetes mellitus resulted in an increase in insulin resistance and worsening of glycaemic control [43].

The effects of vitamin D on insulin secretion may follow several pathways. Evidence exists that vitamin D influences β-cell insulin secretion through a rise in intracellular calcium concentration via non-selective voltage-dependent calcium channels [44]. As a consequence, a major mechanism of action of vitamin D on insulin secretion and synthesis is likely to involve the β-cell calcium-dependent endopeptidases, which produce the cleavage that facilitates the conversion of proinsulin to insulin [24,34]. Moreover, calcium is not only necessary for insulin exocytosis but also for β-cell glycolysis, which plays a role in signalling circulating glucose concentration [34].

Vitamin D also affects insulin secretion by stimulating its synthesis by means of activation of protein biosynthesis in pancreatic islets [42]. Several other factors, such as serum phosphorus or the direct action of vitamin D on the β cells of the pancreas, have been proposed to account for the stimulation of insulin secretion by vitamin D treatment [28]. Since serum phosphorus is not altered by vitamin D supplementation [28], vitamin D has been suggested to be responsible for increased insulin secretion through other mechanisms such as the direct modulation of β-cell growth [45].

Vitamin D Status and Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is characterized by insulin resistance and altered insulin secretion, although its precise aetiopathogenesis is unknown. Environmental factors are important in such a process and, aside from their role as triggers, they may also have an accelerating or protective effect. Hypovitaminosis D, owing to depletion or relative vitamin D resistance, has long been suspected to be a risk factor for glucose intolerance. For instance, prolonged treatment of osteomalacia with vitamin D increases insulin secretion and improves glucose tolerance [17,34]. Furthermore, hyperinsulinaemia has also been suggested to be associated with increased bone mineral density in subjects with diabetes [46] and without diabetes [47]. On the other hand, administration of a single high dose of vitamin D increases blood glucose in patients with diabetes [17]. Further, no benefits in glucose tolerance have been found with vitamin D supplementation in subjects without vitamin D deficiency [34].

Data from several studies have shown that hypovitaminosis D might play an important role in the pathogenesis of type 2 diabetes in human beings. Epidemiological data showed a reduction in serum vitamin D concentration in a London Bangladeshi population at risk for type 2 diabetes compared with subjects not at risk [11]. These patients showed a higher prevalence of type 2 diabetes mellitus than British Caucasian population, suggesting that vitamin D status might contribute to the pathogenesis of the disease [17]. Short-term vitamin D replenishment in Bangladeshi Asian population increased insulin secretion without altering glycaemia, while longer vitamin D treatment also improved glucose levels [11,34]. It has also been reported that vitamin D treatment in a Bulgarian population of female patients with type 2 diabetes, with a high prevalence of hypovitaminosis D, partially normalized insulin secretion and action [42]. Also, a study in New Zealand reported that newly diagnosed patients with type 2 diabetes or impaired glucose tolerance had lower $25(OH)D_3$ levels than matched control subjects [48]. In addition, in elderly Dutch men, vitamin D status was inversely associated with glucose tolerance and insulin secretion [49]. Data from the Third National Health and Nutrition Examination Survey also showed an inverse association between vitamin D status and diabetes in non-Hispanic white and Mexican American people but not in non-Hispanic black people [50]. Moreover, serum $25(OH)D_3$ levels were not related to glucose status in an English population [51]. All these data suggest that hypovitaminosis D may be a significant risk factor for glucose intolerance in some, but not all, populations. The lack of an inverse correlation between vitamin D status and diabetes in non-Hispanic black people is striking, particularly taking into account their low serum vitamin D levels. An explanation for the lack of association could be the

existence of a variable threshold effect among different ethnic groups [50]. It is also possible that non-Hispanic black people had decreased sensitivity to vitamin D or related hormones, such as parathyroid hormone (PTH) [50,52]. In this respect, a decreased sensitivity to the effects of PTH among non-Hispanic black people in comparison with Caucasians has been reported [53].

Chiu *et al.* found that healthy normoglycaemic subjects with hypovitaminosis D had a greater prevalence of the metabolic syndrome than subjects without hypovitaminosis D [54]. They also found a positive correlation between $25(OH)D_3$ concentration and insulin sensitivity and an alteration in β-cell function associated with hypovitaminosis D, suggesting that hypovitaminosis D might be an independent risk factor for insulin resistance, type 2 diabetes and the metabolic syndrome. The role of vitamin D in the metabolic syndrome was also suggested in the prospective Coronary Artery Risk Development in Young Adults Study [24]. In that study, vitamin D treatment was more potent than either troglitazone or metformin treatment in improving insulin sensitivity [24].

The prevalence of type 2 diabetes is increased in obesity, which is often associated with hypovitaminosis D. Vitamin D is efficiently deposited in body fat stores where it is no longer bioavailable, which probably explains why a significant proportion of persons with obesity are chronically vitamin D deficient [17,18]. The vitamin D deficiency in subjects with obesity is also associated with functional alterations such as elevated PTH levels [17]. This secondary hyperparathyroidism may contribute to the production of glucose intolerance and cardiovascular diseases which, in turn, are also associated with obesity. As stated above, vitamin D stimulates insulin secretion by pancreatic β cells but inhibits PTH synthesis [22]. PTH and insulin increase vitamin D production, and thus, acute insulin deficiency in diabetes mellitus may decrease vitamin D production [22]. In support of this, it is well known that patients with hyperparathyroidism have an increased prevalence of diabetes and insulin resistance [34,50]. Moreover, after parathyroidectomy, there is a correction of abnormal insulin resistance and glucose intolerance. Thus, the relationship between hypovitaminosis D, altered insulin secretion and type 2 diabetes may be the result of several related metabolic effects.

Vitamin D and the Immune Response

Inflammatory factors have often been associated with insulin resistance and β-cell failure, both of which are key features of type 2 diabetes mellitus. An increase in acute-phase proteins, cytokines and mediators associated with endothelial dysfunction has been reported in type 2 diabetes. In type 2 diabetes mellitus, abnormalities in many systemic inflammation markers have been found, such as tumour necrosis factor (TNF)-α and TNF-β, interleukin-6 (IL-6) and its receptor, C-reactive protein and plasminogen activator inhibitor-1 [55]. Some of these immune mediators, such as TNF-α and IL-6, may directly interfere with insulin signalling, causing insulin resistance through several mechanisms [55]. Since a variety of studies reported the occurrence of these inflammatory changes many years before diabetes onset, they are regarded as contributors rather than a consequence of the disease [55].

The existence of VDRs in activated T lymphocytes, macrophages and thymus tissue raised the idea that vitamin D might function as an immune modulator [24,56,57]. It has now been shown that vitamin D has a wide range of immune actions: it promotes the differentiation of monocytes into macrophages, thus increasing their cytotoxic activity; reduces the antigen-presenting activity of macrophages to lymphocytes; prevents dendritic cell maturation; inhibits T lymphocyte–mediated immunoglobulin synthesis in B cells and inhibits delayed-type hypersensitivity reactions [1,17,56,58–60]. In contrast, vitamin D exerts an antiproliferative effect on activated lymphocytes while suppressing the generation and activity of new natural killer cells [56,61].

To our knowledge, no published data are available concerning the possible interplay between vitamin D and the immune/inflammatory mediators in type 2 diabetes mellitus. Some of the immune non-classical actions of vitamin D may point to a role of this molecule in the pathogenesis of type 2 diabetes mellitus. Interestingly, vitamin D has been reported to downregulate the production of several cytokines: IL-2, IL-6 and IL-12, interferon-γ, TNF-α and TNF-β [58,62]. To date, however, it remains to be elucidated whether the low-grade inflammation observed in type 2 diabetes mellitus might be influenced by the immune properties of vitamin D.

Vitamin D and Gestational Diabetes Mellitus

Pregnant woman with diabetes and her foetus are known to be at greater risk of reduced vitamin D levels [22]. Experimental dietary-induced vitamin D depletion has been associated with reduced foetal growth in animals, and spontaneous hypovitaminosis D in human beings has an analogous effect in newborns [34]. The presence of insulin resistance in human pregnancy is also well established. In gestational diabetes mellitus, vitamin D concentrations remain at lower levels in comparison

with those of normal pregnant women [63]. Intravenous administration of vitamin D to pregnant women with gestational diabetes transiently decreased fasting glucose levels, while, surprisingly, the level of insulin also decreased [64]. This apparently contradicts most other studies suggesting that the effect of vitamin D on glucose metabolism may be via an effect on insulin secretion [11,28]. Therefore, a different mode of action of vitamin D on glucose metabolism has been suggested to be mediated by an increase in the cellular absorption of glucose, either directly or by an increase in insulin sensitivity [64]. Supporting this hypothesis are the lower insulin levels measured during an oral glucose tolerance test and the absence of changes in glucose levels after vitamin D treatment of pregnant women [64]. In conclusion, administration of vitamin D tends to reduce fasting glucose concentration in women with gestational diabetes mellitus, probably because of increased insulin sensitivity [64].

Type 2 Diabetes Mellitus and Vitamin D–related Genetic Factors

Vitamin D Receptor Polymorphisms

Vitamin D exerts its actions on target tissues through its binding to the cytosolic/nuclear VDR, which is a member of the steroid/thyroid hormone receptor family that functions as a transcriptional activator of many genes. The VDR gene, which is located on chromosome 12q13.1, consists of 14 exons and has an extensive promoter region capable of generating multiple tissue-specific transcripts [16,59,60] (figure 3). In fact, VDR is expressed in a large number of tissues, including those involved in the regulation of glucose metabolism, such as muscle and pancreatic β cells [26,65–67]. Upon binding with vitamin D and subsequent specific phosphorylation by kinase cascades, the VDR undergoes a conformational change which facilitates its binding to the retinoid X receptor [68]. The resulting heterodimer interacts with vitamin D–responsive elements in the promoter region of target genes, thereby modifying their expression [19,61]. Recently, the existence of a putative membrane VDR (mVDR) has also been postulated [69]. Similar to other steroid hormones, vitamin D has a range of effects that are thought not to involve gene expression, such as a rise in intracellular calcium and cGMP levels and activation of protein kinase C [70]. It has been suggested that mVDR might be responsible for these effects of vitamin D [71]. Strikingly, pancreatic β cells express both the specific cytosolic/nuclear VDR and the putative mVDR [72]. However, since the mVDR has not yet been thoroughly characterized, the term VDR used hereafter will refer exclusively to the cytosolic/nuclear VDR.

Deleterious mutations in the VDR gene cause the rare monogenetic disease known as vitamin D–resistant rickets [59]. To our knowledge, this extremely rare disease has not been described in association with diabetes mellitus or any form of insulin resistance. On the other hand, more subtle sequence variations in the VDR gene, namely polymorphisms, are much more frequent in the population. However, VDR polymorphisms are generally anonymous (i.e. they are located in intronic regions or result in silent changes of the codon) and have an unknown functional effect, thus hindering most VDR association studies. These polymorphisms may be linked to truly functional polymorphisms elsewhere in the VDR gene or nearby, thus explaining the observed associations. To date, more than 25 different polymorphisms have been mapped to the VDR locus [60]. Allelic differences in the VDR gene may contribute to the genetic predisposition to certain diseases. As vitamin D modulates insulin secretion, it is feasible that genetic variants of the VDR gene may contribute to the development of type 2 diabetes mellitus. Since patients with type 2 diabetes exhibit subtle alterations in glucose

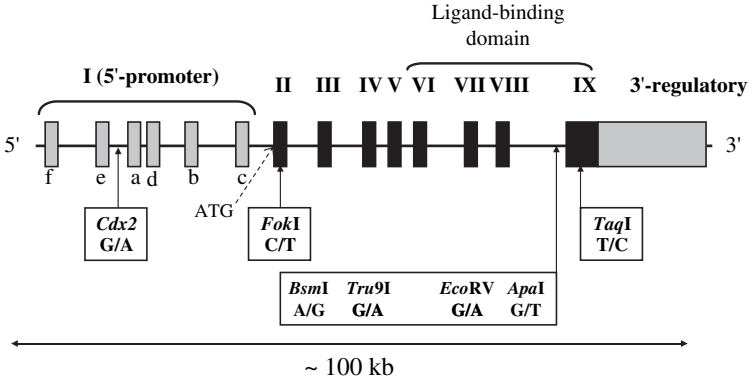

Fig. 3 Structure of the vitamin D receptors (VDR) gene on locus 12q.13.1 and position of some polymorphisms according to Uitterlinden *et al.* [59,60] and Collins *et al.* (2004) [101]. Boxes I to IX represent the VDR exons: grey boxes correspond to 5′ and 3′ untranslated regions and black boxes represent the coding regions. Only two polymorphisms are located within coding regions: *Fok*I and *Taq*I (silent).

metabolism long before onset of the disease, genetic factors contributing to its pathogenesis or development could be detected early in the disease process.

Most attempts to identify functional polymorphisms in the human VDR gene have focused on the 3′ untranslated region (3′UTR), since this region is known to be involved in gene expression regulation, especially through regulation of messenger RNA (mRNA) stability and protein translation efficiency. Four common allelic variants or polymorphisms of the VDR gene have been identified and described in detail: *Fok*I, *Bsm*I, *Apa*I and *Taq*I [19] (figure 3). The three alleles of the *Bsm*I, *Apa*I and *Taq*I polymorphisms are closely located in intron 8 and exon 9, near the 3′ end of the VDR gene and are genetically linked [59,60]. The role of these VDR polymorphisms has been thoroughly studied in patients with diabetes (table 1). Hitman *et al.* [73] showed an association between the *Apa*I polymorphism (homozygosis for the a allele) and lower insulin secretion in a healthy Bangladeshi Asian population at risk of type 2 diabetes living in London (UK) who, as stated above, have a high prevalence of vitamin D deficiency. A correlation between *Apa*I polymorphism and fasting plasma glucose and glucose intolerance was also observed in a community-based study of older adults without known diabetes [74]. Ogunkolade *et al*. corroborated these data and also showed a positive association between the *Taq*I (genotype TT) and the *Bsm*I (genotype bb) polymorphisms with reduced insulin secretory capacity in the same population [16]. All these findings have recently been replicated. Speer *et al.* reported that patients with diabetes and obesity with the BB genotype of the *Bsm*I

Table 1 Reported association studies between the pathogenesis of type 2 diabetes mellitus and VDR, DBP and *CYP1alpha* genes

Gene	Polymorphism (base change)	Genotype	Reported association*	Population	Reference
VDR	*Apa*I (T/G)	aa	+ (lower insulin secretion)	Bangladeshi Asians population at risk of DM2 living in London	Hitman *et al.* [73]
		aa	+ (fasting plasma glucose, glucose intolerance)	Older adults without diabetes (Rancho Bernardo study)	Oh and Barrett-Connor [74]
		aa	− (DM2), + (obesity)	French Caucasians	Ye *et al.* [72]
	*Bsm*I (G/A)	bb	+ (reduced insulin secretory capacity)	Bangladeshi Asian population at risk of DM2 living in London	Ogunkolade *et al.* [16]
		bb	− (DM2), + (obesity)	French Caucasians	Ye *et al.* [72]
		B−	+ (altered calcium absorption, elevated PTH, DM2 and elevated fasting glucose)	Healthy young subjects	Ortlepp *et al.* [14]
		BB	+ (higher levels of postprandial serum C-peptide)	Obese diabetic subjects	Speer *et al.* [75]
	*Fok*I (T/C)	FF	+ (increased insulin sensitivity)	Healthy glucose-tolerant Caucasians	Chiu *et al.* [54]
	*Taq*I (A/C)	TT	+ (reduced insulin secretory capacity)	Bangladeshi Asian population at risk of DM2 living in London	Ogunkolade *et al.* [16]
		T−	+ (reduced insulin secretion)	Subjects with 25(OH)D_3 insufficiency	Ogunkolade *et al.* [16]
		TT	− (DM2), + (obesity)	French Caucasians	Ye *et al.* [72]
	*Tru9*I (A/G)	u−	− (DM2)	French Caucasians	Ye *et al.* [72]
DBP		Gc−	+ (glucose tolerance), − (DM2 prevalence)	Non-diabetic Pima Indians	Baier *et al.* [87], Pratley *et al.* [13]
		Gc1−	+ (DM2)	Polynesian and Japanese subjects	Kirk *et al.* [90], Hirai *et al.* [91]
		Gc1s−	+ (fasting insulin, plasma glucose)	Subarctic Amerindians	Szathmary [92]
		Gc1f-1f	+ (lower levels of fasting insulin)	Non-diabetic Dogrib Indians from Canada	Szathmary [93]
		Gc1f-1f	− (fasting insulin levels)	Hispanic population of San Luis Valley (USA)	Iyengar *et al.* [12]
		Gc−	− (DM2)	American, French and Polish Caucasians	Klupa *et al.* [94], Ye *et al.* [69], Malecki *et al.* [99]
CYP1alpha		TC	− (DM2)	Polish Caucasians	Malecki *et al.* [99]
			+ (DM2)	Obese Polish Caucasians	Malecki *et al.* [99]

DBP, vitamin D–binding protein; DM2, type 2 diabetes mellitus; PTH, parathyroid hormone; VDR, vitamin D receptors.
*Denotes positive (+) or negative (−) association with the corresponding phenotype reported in brackets.

allele in the VDR gene presented higher levels of postprandial serum C-peptide [75], which points to a possible role in the pathogenesis of type 2 diabetes. More recently, it has been reported that the same VDR B allele, which predisposes to altered calcium absorption, elevated PTH and type 2 diabetes mellitus, is associated with elevated fasting glucose in healthy young men long before the onset of type 2 diabetes [14]. It has also been reported that in subjects with $25(OH)D_3$ insufficiency, *Taq*I polymorphism is a determinant of insulin secretion [16]. The same study suggested that *Taq*I genotype contributed independently to the determination of both VDR mRNA and VDR protein levels. However, Ye *et al.*, who studied four different polymorphisms of the VDR gene (three in intron 8, *Bsm*I, *Tru*9I and *Apa*I, and one in exon 9, *Taq*I), concluded that this was not a major predisposing gene for type 2 diabetes in French Caucasians, despite being associated with susceptibility to obesity (table 1) [69]. The same authors suggested that this effect could be related to a direct action of vitamin D on adipocyte differentiation and metabolism or to an indirect modulation of insulin secretion [69].

To be emphasized is the case of the *Fok*I polymorphism which, in contrast to other VDR polymorphisms, is located within the 5′ end of the gene near the promoter region. It consists of a T to C transition at exon 2 that eliminates the first potential ATG translation start site and allows a second one, 9 bp downstream, to be used [60]. As a consequence, two variants of the VDR protein can be translated: a long version of the VDR protein (427 amino acids, the T allele, known as the 'f' allele or M1 form, i.e. methionine at first position) and a protein shortened by three amino acids (424 amino acids, the C allele, referred to as the 'F' allele or M4 form, i.e. methionine at fourth position) [54,59,60]. Strikingly, the *Fok*I polymorphism not only affects the translation product but also appears to be functional, since it influences VDR interaction with the basal transcription factor IIB (TFIIB) [76,77]. TFIIB is a transcription factor which interacts physically and functionally with the VDR and modulates the transcriptional activity of the latter [77]. In fact, the C-terminal hormone-binding domain of the VDR contains a consensus region required for the association with TFIIB [77]. Thus, the F variant is slightly more active than the f allele [59]. In a healthy glucose-tolerant Caucasian population, subjects with the homozygous FF genotype showed increased insulin sensitivity compared with those with the f allele, although no influence on β-cell function was observed [54]. This observation shows that the *Fok*I polymorphism is an independent determinant of insulin sensitivity, which concurs with the reported association of higher vitamin D levels with increased insulin sensitivity. Moreover, the *Fok*I polymorphism can be considered an independent marker in the VDR gene since it is not in linkage disequilibrium with the 3′ polymorphisms of the gene [59].

These data provide evidence for VDR as a candidate gene contributing to the susceptibility to type 2 diabetes. In summary, genetic changes or alterations in the VDR might contribute to the pathogenesis of type 2 diabetes mellitus by at least four different pathways: alteration in calcium metabolism, modulation of adipocyte function, modulation of insulin secretion and modification of cytokine expression [78]. Vitamin D has been suggested to inhibit the expression of IL-2, which is produced by activated T lymphocytes [79]. Thus, inhibition of vitamin D binding to its receptor and subsequent signalling might alter the cytokine secretion profile.

VDR itself can directly promote insulin secretion [80], and altered transcription of the VDR gene in pancreatic β cells can modify insulin secretion [78]. Thus, two additive actions on adipocytes and pancreatic cells might lead to a higher degree of insulin resistance. However, polymorphisms located within the 3′UTR are less likely to have a pathogenic role and affect glucose metabolism. Further, these polymorphisms do not affect VDR mRNA stability [81]. It is also possible that the reported VDR polymorphisms are just markers in linkage disequilibrium with another gene which may actually be responsible for the associations observed with type 2 diabetes mellitus. It is likely that still more polymorphisms remain to be discovered. If these polymorphisms were located within the complex 5′ promoter region of the VDR gene, they could affect mRNA expression patterns and levels. This altered expression, in combination with 3′UTR sequence variations, could alter VDR protein levels and/or function depending on cell type, developmental stage and activation status.

Two separate studies in Japanese and Caucasian subjects recently identified a G to A sequence polymorphism within the VDR intestinal-specific transcription factor called Cdx2 [82,83]. The A allele of this polymorphism is more active than the G allele in binding the Cdx2 transcription factor and in having more transcriptional activity [82]. The *Cdx2* polymorphism has been suggested to play an important role in the intestinal-specific transcription of the VDR gene [59]. Since the intestine is the major area for calcium absorption, the *Cdx2* site is thought to influence the vitamin D regulation of calcium absorption [59]. To our knowledge, no studies analysing the possible association between *Cdx2* polymorphisms and type 2 diabetes mellitus have yet been published.

Vitamin D–Binding Protein and Type 2 Diabetes Mellitus

The DBP (also named Calbindin-D_{28K}), which is encoded by the *Gc* (group-specific component) gene, functions as a specific transporter of circulating vitamin D metabolites [2,84] and is essential for vitamin D endocytosis and metabolism [85]. Additionally, it has other effects which include binding of globular actin and fatty acids and immunomodulation [61]. DBP is a highly polymorphic single-chain serum glycoprotein synthesized and secreted by the liver [12]. DBP forms a complex with vitamin D, which ensures that circulating vitamin D is delivered to target tissues [12]. As a consequence, alterations in serum DBP concentration usually coincide with parallel changes in the total concentration of vitamin D [37].

The region containing the DBP on chromosome 4q11-13, unlike the region containing the VDR gene, 12q13.1, was found to be linked to fasting insulin in a genome scan in Pima Indians with type 2 diabetes [86] (table 1). Later, four genetic markers were found to be linked to fasting insulin concentrations and two to fasting glucose concentrations around this chromosomal region [87] (table 1).

Genetic variants of DBP have been associated not only with diabetes but also with prediabetic traits in several populations (table 1). Sequence analysis of the *Gc* exons identified two missense polymorphisms at codons 416 and 420 which produce three electrophoretic variants of DBP: Gc1 fast (Gc1f), Gc1 slow (Gc1s) and Gc2 [84,87,88]. These DBP variants have been suggested to affect the availability of active vitamin D forms in β cells and, subsequently, to affect insulin secretion [89].

Previous studies in non-diabetic Pima Indians showed genetic linkage between markers near the *Gc* locus and oral glucose tolerance but not with insulin response to oral glucose [13,87]. However, no association was found between DBP variants and the prevalence of type 2 diabetes in the same ethnic group [13,87]. The Gc1 allele of DBP has also been suggested to be associated with type 2 diabetes in Polynesian and Japanese subjects [90,91]. Further, studies on subarctic Amerindians indicated an association of the Gc1s allele with fasting insulin and plasma glucose [92]. In addition, Gc 1f-1f homozygous subjects showed the lowest levels of fasting insulin among non-diabetic Dogrib Indians from Canada [93]. By contrast, Iyengar *et al.* found no relationship between the Gc genotype and fasting insulin levels in a Hispanic population of the San Luis Valley [12]. Although both study groups were normoglycaemic, it has been postulated that nutritional differences between the two populations might account for the lack of relationship between the Gc genotype and glucose of homeostasis in the latter study [12]. However, other studies in American Caucasian [94], French Caucasian [72] and Polish [89] populations found no evidence of an association between polymorphisms in the DBP gene and type 2 diabetes mellitus.

These discordant findings may be related to dissimilar genetic backgrounds of the populations studied. Different ethnic groups may have differences in the frequency of susceptibility alleles. Reported associations between type 2 diabetes and DBP polymorphisms have only been observed in non-white populations [87,90,93]. Thus, the effect of DBP variants on the development of type 2 diabetes may be characteristic of non-Caucasian populations [94]. Type 2 diabetes has a complex aetiology, and its heredity is thought to be polygenic. Therefore, many different combinations of alleles may exist among patients with diabetes. As a consequence, abnormalities in insulin secretion that are associated with DBP polymorphisms might play an important role only in certain environmental or genetic backgrounds [94]. For example, it is noteworthy that Gc1f homozygosity is much more prevalent in Pima and Dogrib Indian populations than in Caucasian population [72].

It has been suggested that the different DBP variants bind the diverse vitamin D metabolites with variable affinity, thereby affecting the intracellular amount of vitamin D in the β cell [87]. Alternatively, if DBP binds to other ligands such as fatty acids, it may exert its action by means of an increase in the concentration of islet fatty acids, which may finally induce β-cell abnormalities [87]. It is also possible that the association of DBP with glucose tolerance results from variation in a closely linked gene [87].

Other Polymorphisms Associated with Type 2 Diabetes Mellitus

Vitamin D 1alpha-hydroxylase (CYP1alpha) influences the bioavailability of the active vitamin D form [95]. Severe mutations in this gene cause vitamin D–dependent rickets [96,97]. *CYP1alpha* is located on chromosome 12q13.1-13.3, where some evidence for linkage with type 2 diabetes mellitus has already been reported [98]. However, Malecki *et al.* observed that *CYP1alpha* was not a major gene for type 2 diabetes mellitus in Polish Caucasian subjects (table 1) [99]. None of the haplotypes of the T→C polymorphism in intron 6 or the rs184712 single nucleotide polymorphism, which is located around 14.5 kb upstream of exon 1, of the *CYP1alpha* gene showed an association with susceptibility to type 2 diabetes [99]. However, a slight association

between a heterozygous haplotype combination (TC) and type 2 diabetes was found when subjects were stratified in a subgroup with obesity [99]. To date, the mechanisms of this association remain unclear. Although some *in vitro* studies suggest a direct effect of this gene on vitamin D action in adipocyte metabolism [100], it cannot be ruled out that these polymorphisms may simply be in linkage disequilibrium with yet-to-be discovered genes.

Conclusions and Future Prospects

Evidence is accumulating on the possible role of vitamin D in the pathogenesis of type 2 diabetes. Alterations in vitamin D status and/or action may affect insulin sensitivity, β-cell function or both. Furthermore, several vitamin D–related genes have shown associations with different pathogenetic traits of the disease. Therefore, vitamin D and its related metabolic and immune pathways may be involved in the pathogenesis of type 2 diabetes mellitus at both environmental and genetic levels. The possible role of vitamin D in the pathogenesis of type 2 diabetes mellitus is far from being completely understood. Additionally, further knowledge on this issue may identify new candidate targets in the treatment and prevention of the disease. Therefore, further investigations on this issue are warranted.

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