Speeckaert M, Huang G, Delanghe JR, Taes YEBiological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 372(1-2): 33-42

ARTICLE *in* CLINICA CHIMICA ACTA · NOVEMBER 2006

Impact Factor: 2.82 · DOI: 10.1016/j.cca.2006.03.011 · Source: PubMed

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Review

Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism

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Received 15 January 2006; received in revised form 10 March 2006; accepted 10 March 2006
Available online 12 May 2006

Abstract

The vitamin D binding protein (DBP) is the major plasma carrier protein of vitamin D and its metabolites. Unlike other hydrophobic hormone-binding systems, it circulates in a considerably higher titer compared to its ligands. Apart from its specific sterol binding capacity, DBP exerts several other important biological functions such as actin scavenging, fatty acid transport, macrophage activation and chemotaxis.

The DBP-gene is a member of a multigene cluster that includes albumin, α-fetoprotein, and α-albumin/afamin. All four genes are expressed predominantly in the liver with overlapping developmental profiles.

DBP is a highly polymorphic serum protein with three common alleles (Gc1F, Gc1S and Gc2) and more than 120 rare variants. The presence of unique alleles is a useful tool for anthropological studies to discriminate and to reveal ancestral links between populations.

Many studies have discussed the link between DBP-phenotypes and susceptibility or resistance to osteoporosis, Graves' disease, Hashimoto's thyroiditis, diabetes, COPD, AIDS, multiple sclerosis, sarcoidosis and rheumatic fever.

This article reviews the general characteristics, functions and clinical aspects of DBP.

Keywords: Vitamin D binding protein; Gc-globulin; Polymorphism; Actin scavenger system

Contents

Abbreviations: AIDS, acquired immunodeficiency syndrome; ALB, albumin; AFP, α-fetoprotein; AFM, α-albumin/afamin; BMD, bone mineral density; BMI, body mass index; CD36, thrombospondin receptor (53 kDa); CD44, heparan sulfate proteoglycan (81.5 kDa); COPD, chronic obstructive pulmonary disease; DBP, vitamin D binding protein; ERK1/2, extracellularly regulated kinases 1/2; F-actin, filamentous actin; FEV_1, forced expiratory volume in 1 s; FGF-2, fibroblast growth factor 2; FHF, fulminant hepatic failure; FVC, forced vital capacity; G-actin, globular actin; Gc, group specific component; JNK1/2, c-Jun N-terminal kinases 1/2; kDa, kiloDalton; MAF, Macrophage Activating Factor; MS, multiple sclerosis; p38, MAPK14, mitogen activated protein kinase 14; RID, radial immunodiffusion; SNP, single nucleotide polymorphism; VEGR-2, vascular endothelial growth factor receptor 2.

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doi:10.1016/j.cca.2006.03.011

1. Introduction

The vitamin D binding protein (DBP), formerly known as group-specific component of serum (Gc-globulin), is the major plasma carrier protein of vitamin D and its metabolites. Vitamin D sterols are necessary to maintain a normal serum calcium concentration and electrolyte homeostasis.

DBP is a member of the albumin, α-fetoprotein and α-albumin/afamin gene family. It is a highly polymorphic serum protein, predominantly synthesized in the liver as a single chain of glycoproteins with a molecular weight of 52–59 kDa. Apart from its specific sterol binding capacity, DBP exerts several other important biological functions, from actin scavenging to fatty acid transport and macrophage activation. DBP is involved in macrophage chemotaxis and may play a role in bone density. In this review, biological and clinical aspects of DBP will be discussed. Special interest has risen in the use of DBP as a marker for trauma, based on the actin scavenging properties of DBP.

2. General characteristics

2.1. Structure, synthesis, turnover

DBP is a serum α_2-globulin with a molecular weight of 52–59 kDa [1,2]. The human DBP-gene is localized on the long arm of chromosome 4 (4q12–q13) (Fig. 1). It extends over 35 kb DNA and contains 13 exons and 12 introns. The amino acid sequence is composed of 458 amino acids, arranged in three domains, in addition to a 16 amino acid leader sequence [1,3,4]. Two binding regions have been identified within the DBP-sequence: a vitamin D binding domain between residues 35 and 49 and an actin binding domain between residues 373 and 403 [5]. In healthy subjects, the plasma concentration of DBP is 300–600 μg/ml [2]. Its hepatic synthesis is estrogen dependent and is significantly increased during pregnancy and estrogen therapy [6–8]. The differences between DBP and other hydrophobic hormone-binding systems are its molar excess (5×10^{-6} M), compared with its major circulating ligand 25 (OH)-vitamin D_3 (5×10^{-8} M) and its rapid turnover rate. Unlike 25(OH)-vitamin D_3 (12 days), DBP has a short plasma half-life (2.5 days) (Table 1) [9,10].

The production rate of DBP is approximately 10 mg/kg per day [2]. Animal studies with homologous DBP-preparations reveal a widespread distribution into the tissues. DBP or the DBP-25(OH)-vitamin D_3 complex is removed from plasma by a variety of tissues such as kidney, liver, skeletal muscle, heart, lung, intestine, bone. Multiple proteases control the DBP-degradation, which explains the limited size of the DBP-fragments in plasma and urinary excretion of small molecular weight fractions [10]. Liver diseases, nephrotic syndrome and malnutrition are characterized by low DBP-concentrations, due to a diminished synthesis rate or excessive protein loss [9].

In contrast to other plasma proteins, DBP maintains stable plasma concentrations throughout life. No seasonal variations in DBP-plasma concentrations are observed [6].

2.2. Homology of DBP

The vitamin D binding protein gene is a member of a multigene cluster that includes albumin (ALB), α-fetoprotein (AFP), and α-albumin/afamin (AFM) (Fig. 1). All four genes are predominantly expressed in the liver with overlapping developmental profiles. Comparison of the gene structure reveals that ALB and AFP originated through gene duplication [9]. Based on several structural features of DBP, it was assumed that DBP might be a member of this gene family [1,9,11,12]. The marked homologous nucleotide and amino acid sequence with ALB and AFP and its high serum concentration support this statement [9]. Based on this and other lines of study, it has been suggested that this gene family arose by triplication of an internal 192 amino acid region of the ancestral gene leading to the generation of the DBP-gene and the ALB/AFP/AFM-primordial gene [9,13]. This triplication was estimated to have occurred 700 million years ago [14,15]. ALB, AFP and DBP possess a series of highly conserved cystein residues and a similar secondary folding structure with three internally homologous domains. The only

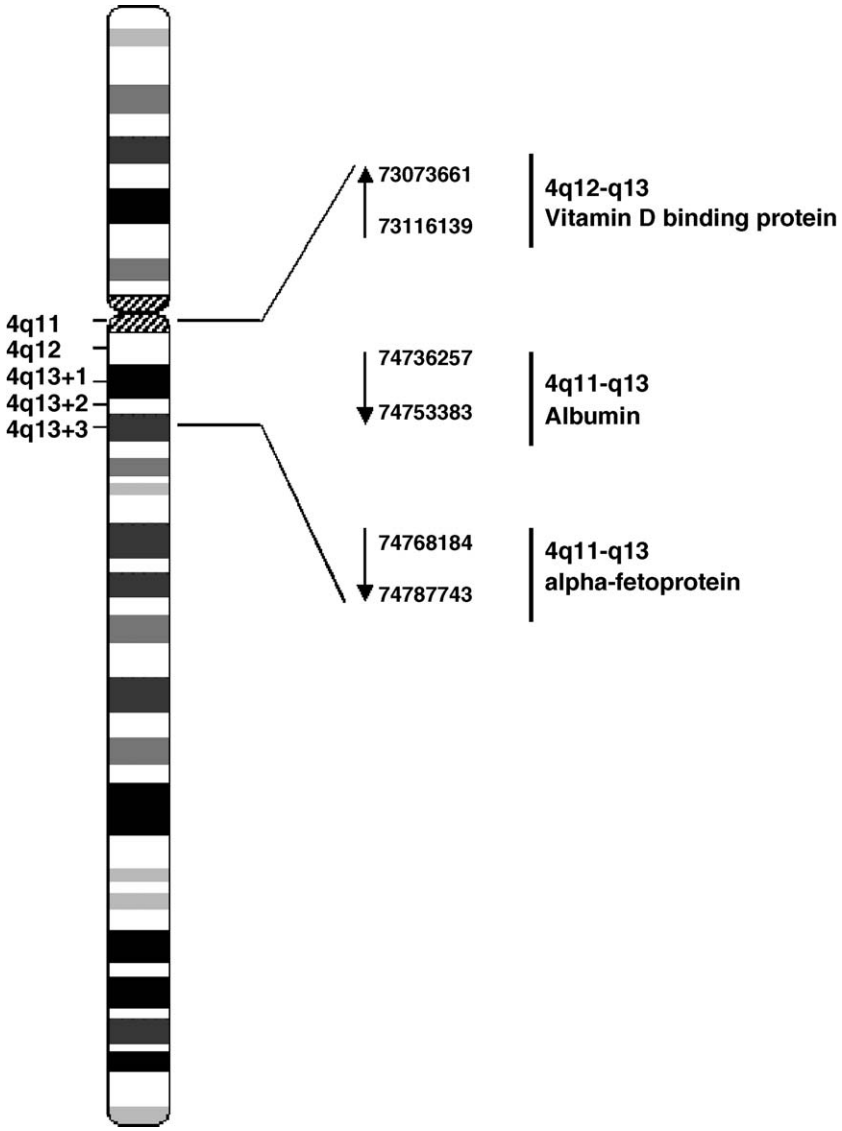

Fig. 1. Position of Gc-globulin, ALB and AFP on chromosome 4.

difference in the DBP-structure is the truncation of the third domain by 124 amino acids [11]. The ALB-, AFP-, AFM- and DBP-genes are closely associated on chromosome 4 (4q11–q13). The separation between DBP and the remaining family members is more than 1.5 Mb [13].

2.3. Polymorphism

A considerable DBP-polymorphism has been demonstrated in humans and primates using different electrophoretic methods [16]. Besides the three well-known alleles (Gc1F, Gc1S and

Table 1
Comparison DBP–TBG–CBG and SHBG

	Mass (kDa)	Gene map locus	Plasma concentration (mg/L, mean±−S.D.)	Carrier binding sites normally occupied	Fraction of total ligand concentration on carrier	% of total ligand concentration in free form
DBP	58	4q11–q13	379±280 (♂) [103] 414±331 (♀)	2%	88% [25(OH)-vitamin D_3]	0.04% [25(OH)-vitamin D_3] 0.4% [1,25$(OH)_2$-vitamin D_3]
TBG	54	Xq22.2	15.3±2.11 (♂) [104] 18.4±2.72 (♀)	50%	77% (T_4)	0.03% (T_4) 0.3% (T_3)
CBG	52	14q32.1	39.7±3.6 (♂) [105] 42.1±3.9 (♀)	50%	70% (C)	8% (C)
SHBG	95	17p13–p12	1.71±0.86 (♂) [106] 5.13±1.24 (♀)	50%	45% (T)	2% (T)

Adapted from Ref. [9].
TBG: thyroxine binding globulin; CBG: cortisol binding globulin; SHBG: sex hormone-binding globulin; C: cortisol; T: testosterone.

Gc2), more than 120 rare variants have been identified, making the DBP-locus among the most polymorphic known [17]. The Gc1-allele (Gc1F and Gc1S) encodes two bands: Gc1a [anodal, p*I* 4.84 (Gc1F), p*I* 4.85 (Gc1S)] and Gc1c [cathodal, p*I* 4.94 (Gc1F), p*I* 4.95 (Gc1S)]. The Gc1F-proteins have a faster migration rate than those encoded by Gc1S. The difference between the Gc1a- and Gc1c-isoforms has a posttranslational basis, characterized by a single *N*-acetyl-neuraminic acid residue in Gc1a which is absent in Gc1c. The Gc2-allele encodes one single band (p*I* 5.1) [7,18].

The primary structure of Gc1F and Gc1S is identical except at position 416, where aspartic acid is substituted by glutamic acid. Gc1F and Gc2 differ by a single amino acid modification (threonine vs. lysine). The basic composition of Gc1S and Gc2 is characterized by two different amino acid substitutions (positions 416 and 420). These amino acid substitutions explain the two charge differences between their iso-electric points [18,19]. Gc1-proteins are marked by an *O*-glycosylation on threonine 420 [18,20].

2.4. Analytical methods to measure DBP

The relatively high serum concentration of DBP permits measurements by simple immunochemical techniques. Radio-immunoassay, rocket immuno-electrophoresis, single radial immunodiffusion (RID), turbidimetry and nephelometry have been widely used [21].

The radioimmunoassay is more sensitive than the RID-assay and measures DBP in amounts of 1–10 ng. In contrast, the RID-assay has a detection limit of 0.2–0.8 μg. The DBP-concentration in normal plasma is sufficiently high to permit the use of the RID-assay for routine analysis. Radio-immunoassay is a good alternative if the DBP-concentration falls below the detection range of the RID-assay. Analysis of the same samples by these two immunoassays gives comparable results [22].

Using immunonephelometry offers the advantage to combine ease of use, short assay time, high sensitivity and high specificity. RID has become abundant in favour of nephelometry from clinical practice [21].

Total DBP can also be measured by inhibition-ELISA with polyclonal or monoclonal antibodies. The choice of method depends on the technical equipment and the experience of the laboratory concerned [23].

2.5. Geographical distribution

Human DBP is a highly polymorphic protein. It exhibits a geographical distribution of three common alleles and a large number of unique racial variants. Populations with a white skin have a relatively lower frequency of the Gc1F-allele and a higher frequency (50–60%) of the Gc1S-allele. The Gc1F-allele frequency is markedly higher among black Americans and black Africans. The Gc1F- and Gc1S-allele frequencies display a typical geographical cline from Southeast Asia, through Europe and the Middle East, down to Africa. A common feature of all populations is the less predominance of the Gc2-allele, in comparison with the Gc1-allele. Unlike Black populations, Caucasians have a markedly higher Gc2-allele frequency. The Tuareq Kel Kummar population of Mali from the Southern Sahara is the only community with a complete absence of the Gc2-allele. The observed variation in the Gc-allele frequencies in different geographic areas may be correlated with skin pigmentation and intensity of sun light exposure. Pigmented (black) and keratinized (yellowish) skin types are characterized by a lower rate of UV light penetration and a higher susceptibility to rickets. The higher frequency of Gc1F in dark skinned persons may be explained by its greater affinity for and more efficient transport of vitamin D metabolites. The presence of unique alleles is a useful tool for anthropological studies to discriminate and to reveal ancestral links between populations [24].

3. Functions of vitamin D binding protein

3.1. Vitamin D binding

The major function of DBP is binding, solubilization and transport of vitamin D and its metabolites [25]. Each DBP-vitamin D metabolite complex has its own affinity constant. 25 (OH)-vitamin D_3 (calcidiol) binds DBP (88% bound) with high affinity ($K_a = 5 \times 10^{-8}$ M), whereas 1,25$(OH)_2$-vitamin D_3 (calcitriol), the most active metabolite of vitamin D, is bound (85%) with a lower affinity ($K_a = 4 \times 10^{-7}$ M) [5]. Unlike other hydrophobic hormone-carrier proteins in human plasma, DBP has a high plasma concentration [0.32–0.46 g/L or 5.52–7.93×10^{-6} M] compared to its major ligand 25(OH)-vitamin D_3 (5×10^{-8} M) and a characteristic rapid turnover rate. Less than 5% of the binding sites on DBP are occupied by vitamin D sterols (Table 1) [9]. The large molar excess of DBP may play an important role in protection against vitamin D intoxication. It can serve as a buffer for the increasing concentration of free vitamin D metabolites or act as a circulating reservoir of 25 (OH)-vitamin D_3 [5,9].

Under normal physiological conditions, nearly all circulating vitamin D compounds are protein bound, which has a great influence on the vitamin D pharmacokinetic. Only 12–15% of the circulating vitamin D is associated with albumin. DBP-bound metabolites have a limited access to target cells and are less susceptible to hepatic metabolism and subsequent biliary excretion. This prolongs their half-life in circulation. Several studies reported a greater accessibility of the free form of vitamin D metabolites to target cells and therefore a higher biological response, both in vivo and in vitro [26–29].

No humans have been detected with a total absence of DBP, suggesting that one or more functions of DBP may be essential to human viability. The generation of $Dbp^{-/-}$ mice with normal fertility and size clearly demonstrated that this is not the case in mice. DBP-deficient mice received a low vitamin D diet and developed secondary hyperparathyroidism with an accelerated bone turnover. A standard diet induced no bone changes or hyperparathyroidism. Both serum 25(OH)-vitamin D_3 and 1,25 $(OH)_2$-vitamin D_3 concentrations were significantly lower in mice lacking DBP, compared to wild-type mice. After a vitamin D overload, the DBP-null mice were relatively more resistant to

hypercalcemia and the associated toxic effects than normal mice. This unexpected result can be explained by the finding that DBP and DBP-bound metabolites are filtered through the glomerulus and reabsorbed by the endocytic receptor megalin into the proximal tubular cells. Megalin mediated endocytosis of DBP-bound 25(OH)-vitamin D_3, appears to be the major pathway to preserve circulating levels of 25(OH)-vitamin D_3 and to activate 25(OH)-vitamin D_3 to 1,25$(OH)_2$-vitamin D_3. Due to the high urinary excretion of 25(OH)-vitamin D_3 and DBP, megalin null mice elicit severe vitamin D deficiencies and bone diseases. In the absence of DBP, the major pathway of renal uptake and activation of 25(OH)-vitamin D_3 to 1,25$(OH)_2$-vitamin D_3 is blunted, preventing hypercalcemie and 1,25$(OH)_2$-vitamin D_3 toxicity [26,30].

In healthy women, serum 1,25$(OH)_2$-vitamin D_3 concentrations correlate positively with serum DBP-concentrations [6]. Pregnancy and estrogen treatment increase significantly the serum DBP-concentrations with concomitant increases in total serum 1,25$(OH)_2$-vitamin D_3 concentrations. The free 1,25 $(OH)_2$-vitamin D_3 fraction, which is biologically active, remains unchanged [31].

In a recent study, Lauridsen et al. described that the DBP-phenotype determines the median plasma concentration of 25 (OH)-vitamin D_3 and 1,25$(OH)_2$-vitamin D_3. The concentration of both vitamin D metabolites decreases in order of being highest in Gc 1-1, intermediate in Gc1-2, and lowest in Gc2-2. The DBP-plasma concentration shows an identical pattern. The authors suggest that the lower concentration of DBP and 25 (OH)-vitamin D_3 in Gc2-2 phenotypes, are related to a faster metabolism of Gc2 in comparison with Gc1. The 1,25$(OH)_2$-vitamin D_3 concentration correlates with the DBP-concentration, whereas each DBP-phenotype has its own specific amount of 25(OH)-vitamin D_3 [32,33].

The DBP-concentration follows a specific pattern. The morning is characterized by a decline, followed by a rapid increase to a plateau during the day. The diurnal rhythm of DBP is correlated with the rhythm of 1,25$(OH)_2$-vitamin D_3 and with the plasma albumin concentration. Standardized blood sampling, according to the time of day is essential [34].

Arnaud et al. reported a higher affinity for binding 25(OH)-vitamin D_3 to Gc1, compared with Gc2. This results in a relatively high concentration of free 25(OH)-vitamin D_3 in the Gc2-2 phenotype [18]. Other authors could not confirm this finding [35,36].

A variety of disorders of mineral and skeletal homeostasis (including primary osteoporosis, primary and secondary hyperparathyroidism, rickets, osteomalacia and vitamin D intoxication) are accompanied by a normal serum DBP-concentration [37]. Unlike vitamin A, which facilitates the hepatic secretion of the retinol-binding protein, vitamin D sterols or other calciotropic hormones do not regulate the plasma DBP-concentration [9].

3.2. Actin scavenging action

Actin, a highly conserved cytoskeletal element, has two molecular forms: a globular, monomeric (G-actin) and a filamentous, polymeric form (F-actin). Tissue injury and cell death release actin into the circulation. In the extracellular compartment, G-actin polymerizes into F-actin filaments. This may cause vascular obstruction and organ dysfunction. DBP and gelsolin, members of the extracellular actin scavenger system, co-operate to protect from these complications. DBP binds G-actin with high affinity (Kd = 10 nM) and inhibits filament formation. The major DBP-phenotypes have an equal binding affinity [5,38–40]. Mc Leod et al. investigated the influence of changing conditions (ionic concentrations, pH and temperature) on this interaction. Increased ionic strength and Mg^{2+} favour intracellular and intravascular polymerization of G-actin. Buffers containing 50 mM KCl, 100 mM NaCl, 1 mM $MgCl_2$ or a combination of these salts had no significant effect on the high affinity DBP–G-actin binding. Unlike a pH of 8.6 with a Kd of 0.9 nM, decreasing the pH from 7.4 to 6.8 affected the binding affinity significantly [Kd = 1.1 nM (pH 7.4); Kd = 1.9 nM (pH 6.8)]. The DBP–G-actin complex was unaffected by the range of temperature from 4 to 37 °C [40]. Gelsolin forms 1:2 molar complexes with F-actin and stimulates its depolymerization [41]. Human platelet profilin, another G-actin sequestrant, has a 1000-fold less potent binding capacity ($Ki = 1.9 \times 10^{-6}$ M) to G-actin, in comparison with DBP. DNase I also binds G-actin, forming a DBP–actin–DNase I triprotein complex [7,40,42,43]. The ability of DBP to rapidly sequester free actin might be the proposed crucial role for DBP accounting for its great molar excess compared with its sterol ligands [5].

Severe cell or tissue loss lowers the DBP-serum level. The degree of reduction correlates with the development of organ dysfunction, respiratory failure, hematologic failure, sepsis which may help to identify patients at increased risk of mortality after injury [44].

3.3. Fatty acid transport

A third major function of DBP is the binding of mainly monounsaturated and saturated fatty acids. Less than 5% of the total amount of fatty acids, bound to human DBP, presents in a polyunsaturated form [45,46]. The affinity of 25(OH)-vitamin D_3 and 1,25$(OH)_2$-vitamin D_3 for DBP is decreased by mono- and polyunsaturated fatty acids, but is unaffected by saturated fatty acids [47]. Calvo et al. described strong complexes between bovine DBP and arachidonic/palmitic acid with association constants of respectively 6×10^{-5} and 7×10^{-5} M. These fatty acids induce their own conformational changes in DBP, which may explain the different competition strength with 25(OH)-vitamin D_3 for binding to DBP [arachidonic acid (a C_{20} polyunsaturated fatty acid) > palmitic acid (a C_{16} saturated fatty acid)] [48].

3.4. DBP-macrophage activating factor (MAF)

Several in vitro studies (cell lines originating from mouse/rat peritoneal nonadherent cells) identified DBP as a molecule implicated in macrophage activation, when DBP is deglycosylated by T- and B-cell glycosidases to DBP-MAF [49,50]. Studies on two nonallelic mutations in rats (osteopetrosis and incisors absent) proposed a significant role of DBP in

macrophage activation and osteoclast differentiation. DBP may control bone morphogenesis and remodeling [51–53]. In a case-control study (three infantile osteopetrosis patients), Yamamoto et al. supported this thesis by demonstrating an impaired activity of β-galactosidase (B-lymphocytes) and Neu-1 sialidase (T-lymphocytes) in peripheral blood mononuclear cells of the patient population. This resulted in a decreased activation of monocytes/macrophages [54]. DBP-MAF therapy partly corrected the skeletal defects in osteopetrosis by upregulation of the oxidative metabolism in mutant cells, by increasing the number of osteoclasts and by correcting their structure [51]. DBP-MAF and a derived synthetic peptide (14 amino acids), based on the amino acid sequence of the glycosylation site in the third domain of the native human DBP, have a similar anabolic effect on the skeletal system. This could be useful in the treatment of osteoporosis and other bone diseases [53]. Gumireddy et al. investigated the effect of DBP-MAF in a macrophage cell line. Stimulating p38 and JNK1/2 pathway, DBP-MAF induced apoptosis of those cells by increasing the activity of pro-apoptotic enzymes. This mechanism could also take place during inflammation [55]. In addition to the ability to activate tumoricidal macrophages, several in vitro and in vivo studies call attention to its (in)direct antiangiogenic effects on endothelial cells of different species (human, porcine, murine) and tissues (aorta, brain, cornea, pancreas, umbilical cord). This may be mediated through the CD36 receptor and inhibits VEGR-2 and ERK1/2 signaling cascades [56–58].

3.5. Chemotaxis

During inflammation chemotaxis attracts neutrophils to the site of inflammation. DBP is reported to augment the chemotactic effect of complement derived C5a and C5a des Arg. C5a is rapidly converted to C5a des Arg, by the removal of the carboxyterminal arginine. C5a des Arg is 100 times less active in provoking neutrophil and macrophage chemotaxis, but becomes a nearly equivalent chemoattractant in serum due to the presence of a cochemotactic factor, identified as DBP by several authors [59,60]. DBP has to bind to a cell surface receptor to fulfill its cochemotactic activity. McVoy et al. demonstrated that CD44, a chondroitin sulfate proteoglycan on the neutrophil plasma membrane, is this indispensable receptor. Annexin A2 is associated with CD44 and supports the cochemotaxis [61]. No influence of DBP on the expression level of neutrophil C5a-receptors has been reported. Gc1F, Gc1S and Gc2 have a comparable cochemotactic activity [62]. Raymond et al. reported that DBP-release at sites of endothelial injury exerts a chemotactic function on vascular smooth muscle cells and acts as a growth factor. 25(OH) vitamin D_3 and 1,25$(OH)_2$ vitamin D_3 inhibit this chemotaxis by competing for the same binding site on DBP [63].

4. Clinical aspects

Many researchers have made attempts to link the expression of the DBP-alleles with susceptibility or resistance to disease.

4.1. Bone metabolism

Eichner et al. examined the influence of DBP-phenotypes on the bone mineral density (BMD) in a group of 258 non-black older women (age 65–90) and thus the correlation between the DBP-phenotype and susceptibility to osteoporosis. There was no statistical significant relationship found between bone mineral density of the proximal/distal radius or calcaneus and the DBP-phenotype. Adjustments for age and degree of obesity had no influence on these results [64].

Lauridsen et al. showed that the DBP-phenotype is linked with premenopausal bone fracture risk in perimenopausal white women (595 subjects, age 45–58). There was a significant difference in bone fracture risk among women with different DBP-phenotypes (relative risk of 0.32 in Gc2-2, compared with Gc1-1). Bone modeling/remodeling may be guided by DBP-MAF, with an influence on the risk of bone fractures [65].

Rapado et al. demonstrated a positive correlation of DBP with both lumbar spine and femoral neck BMD in 140 elderly males (age 55–90) [66]. Experiments on male osteoporosis [26 men with symptomatic vertebral fractures (age 27–72) and 21 male control subjects (40–77)] could not confirm the effect of the DBP-phenotype on BMD. $(TAAA)_n$-Alu repeat polymorphism was associated with a different BMD and vertebral fracture risk [67]. Several single nucleotide polymorphisms (SNP) within the DBP-gene in 384 adult Japanese women (age 32–69) were associated with a low radial BMD and a higher relative risk of osteoporosis [68]. Malnutrition may be associated with a decreased DBP- and vitamin D ligand concentration. This could partially explain the link between malnutrition and the development of metabolic bone diseases. The affinity of DBP for 25(OH)-vitamin D_3 is not influenced [69]. The importance of DBP for the skeletal system has also been confirmed by disrupting the megalin gene in mice, associated with an elevated urinary excretion of DBP, bone deformation and decreased bone density [31].

4.2. Thyroid autoimmunity

Graves' disease and Hashimoto's thyroiditis are the most common autoimmune thyroid disorders. Experiments in animal models and in humans emphasize the critical role of 1,25$(OH)_2$-vitamin D_3 in the prevention of those pathologies. Pani et al. investigated the association of DBP-gene polymorphism and thyroid autoimmunity in 187 Caucasian families (561 participants). Intron 8 $(TAAA)_n$-Alu repeat polymorphism correlated with Graves' disease, but not with Hashimoto's thyroiditis. Both diseases showed no significant interaction between DBP-genotypes and HLA-haplotypes. Unlike Hashimoto's thyroiditis, Graves' disease is characterized by certain allelic variants of the DBP-gene [70].

4.3. Diabetes

There is some controversy about the link between genetic DBP-variations and the occurrence of diabetes. A study of 82 Japanese with normal glucose tolerance, demonstrated an

association of DBP-polymorphism in exon 11 [Asp(GAT)/Glu (GAG) at codon 416, Thr(ACG)/Lys(AAG) at codon 420] and insulin resistance, the hallmark of type II diabetes. Besides its strongest correlation with the Gc1S-allele, fasting serum insulin concentration, a marker of insulin resistance, was markedly higher in the presence of Gc2, in comparison with Gc1F [71,72]. In a study of 208 NIDDM patients and 209 healthy Japanese, NIDDM showed a significant lower frequency of the Gc1F-allele in contrast with the excess of Gc1S- and Gc2-alleles [73]. Other studies in white patients of American or European origin could not confirm the relation between genetic variants of the DBP-gene and the susceptibility to type II diabetes [74–76]. The influence of intron 8 $(TAAA)_n$-Alu repeat polymorphism was studied by Pani et al. No association was found between the DBP-alleles of 527 individuals and the susceptibility to type I diabetes [77].

4.4. Obesity

Recent studies focus on the relation between vitamin D and obesity. The absolute fat mass has an inverse relation with the serum 25(OH)-vitamin D_3 concentration and correlates positively with the serum PTH-level. Less sun exposure and an increased sequestration of 25(OH)-vitamin D_3 in adipose tissue might explain those associations. An increased PTH-concentration and a decreased amount of serum 25(OH)-vitamin D_3 and serum $1,25(OH)_2$-vitamin D_3 can increase intracellular calcium in adipocytes. This stimulates the lipogenesis and predisposes to further weight gain. The body mass index (BMI) has a negative correlation with peak serum vitamin D_3 [78–80].

Taes et al. described a positive relationship between DBP-concentrations, BMI and leptin concentrations in 211 elderly men (age 71–86). DBP may intervene in the relation between fat mass and vitamin D metabolism. A possible link between leptin and DBP has not yet been studied [81].

4.5. Pulmonary disease

Independent studies demonstrate that DBP-polymorphism is significantly correlated with susceptibility to and with the severity of chronic obstructive pulmonary disease (COPD) [82]. Kueppers et al. (114 COPD patients and 114 control subjects) and Schellenberg et al. (75 COPD patients and 64 nonobstructed controls) reported a decreased frequency of homozygous Gc2-phenotype in COPD [83,84]. Kauffman et al. studied 88 patients with decreased lung function and were not able to confirm these results [85].

Neutrophils play an important role in parenchymal destruction and airway inflammation. DBP is a cochemotactic factor for C5a. However, DBP-polymorphism presents no difference in neutrophil chemotaxis [84].

Another important function of DBP is its deglycosylation to DBP-MAF. The absence of a glycosylated residue at position 420 in Gc2 inhibits this conversion. This may be a partial explanation of its protective effect [86]. Unlike the Gc2-allele, the homozygous Gc1F-phenotype is a significant risk factor for the development of COPD [87,88]. Although the Gc1F-allele has no effect on the age of onset of COPD, the annual decline in FEV_1 is significantly higher in patients with this allele. High resolution CT-parameters show that Gc1F-allele carriers suffer from more severe emphysema [86]. Black et al. recently analysed serum 25(OH)-vitamin D_3 concentration, FEV_1 and FVC of 14091 subjects (age $\geq$ 20) and demonstrated a significant correlation between these parameters [89].

4.6. Liver disease

Plasma DBP sequesters actin, released into the circulation after massive hepatocyte necrosis, but is greatly depleted in the process. In fulminant hepatic failure (FHF), DBP is present in serum, both as a complex with actin and as unbound protein, the latter becoming exhausted. In 47 cases with FHF, measurement of the DBP-level predicted all patients dying of this pathology [90]. Alcoholic liver cirrhosis is characterized by an increased Gc1-allele frequency. Furthermore, an unusual sialilation of the serum DBP is associated with the Gc1-allele [91].

4.7. AIDS

In 1987 a possible correlation was proposed between the homozygous Gc1F-phenotype and susceptibility to HIV-infection/severity of HIV-related disease. Gc2-2 on the contrary, should perform a protective role [92]. Several later studies refuted this statement [93–95].

4.8. Multiple sclerosis

The prevalence of multiple sclerosis (MS) grows with increasing latitudes. Niino et al. suggested a protective effect of sufficient vitamin D and the occurrence of MS. They investigated the association between two DBP-polymorphisms (codon 416 and codon 420) and MS in a Japanese case-control study (107 patients and 109 controls). DBP-phenotypes do not correlate with the incidence of this disease [96].

4.9. Sarcoidosis

Sarcoidosis is characterized by an abnormal vitamin D- and immunoglobulin-production. Patients have no significant difference in distribution of DBP-phenotypes. Milman et al. found no link between the DBP-phenotype of 44 sarcoidosis patients and the presentation or course of the disorder [97].

4.10. Rheumatic fever

Rheumatic fever is characterized by a pronounced activity of B cells, resulting in an extensive amount of antibody to the Group A streptococcus. Bahr et al. suggested that DBP, associated with membrane immunoglobulin on B-cell membranes, could play a role in activation of those cells. They found a strong correlation between the Gc2-allele and the development of rheumatic fever in a 39 Arab children. The relative risk of Gc2 was 2.25 in comparison with the normal population [98].

4.11. Trauma

Clinical usefulness of DBP as a marker for trauma has been evaluated in several studies. The DBP-serum level is reduced by severe cell or tissue loss. Admission levels of DBP in trauma patients predict the chance of survival. Dahl et al. showed that follow-up of the DBP-concentration may help to identify patients at increased risk of mortality after injury [99–102].

5. Conclusion

DBP has recently received increasing attention. DBP is recognized as a member of a multigene family that includes albumin, α-fetoprotein and α-albumin/afamin. This highly polymorphic serum protein exhibits a geographical distribution with several exotic alleles, resulting in numerous phenotypes.

DBP is synthesized in liver as a single polypeptide and exhibits multifunctional properties. Besides the transport of vitamin D-metabolites, DBP binds G-actin with high-affinity and sequesters monomeric actin, released into the serum after injury or disease. DBP plays an important role in macrophage activation and enhances the chemotactic function of C5a and C5a des Arg. DBP is also involved in the fatty acid transport.

Several studies have linked the expression of the DBP-polymorphism and susceptibility or resistance to a spectrum of diseases.

Further research is still necessary to clarify the physiological role of DBP.

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