

REVIEW ARTICLE

## Common variants of the vitamin D binding protein gene and adverse health outcomes

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### Abstract

The vitamin D binding protein (DBP) is the major plasma carrier for vitamin D and its metabolites, but it is also an actin scavenger, and is the precursor to the immunomodulatory protein, Gc-MAF. Two missense variants of the DBP gene – rs7041 encoding Asp432Glu and rs4588 encoding Thr436Lys – change the amino acid sequence and alter the protein function. They are common enough to generate population-wide constitutive differences in vitamin D status, based on assay of the serum metabolite, 25-hydroxyvitamin D (25OHD). Whether these variants also influence the role of vitamin D in an immunologic *milieu* is not known. However, the issue is relevant, given the immunomodulatory effects of DBP and the role of protracted innate immune-related inflammation in response to tissue injury or repeated infection. Indeed, DBP and vitamin D may jointly or independently contribute to a variety of adverse health outcomes unrelated to classical notions of their function in bone and mineral metabolism. This review summarizes the reports to date of associations between DBP variants, and various chronic and infectious diseases. The available information leads us to conclude that DBP variants are a significant and common genetic factor in some common disorders, and therefore, are worthy of closer attention. In view of the heightened interest in vitamin D as a public health target, well-designed studies that look simultaneously at vitamin D and its carrier in relation to genotypes and adverse health outcome should be encouraged.

**Abbreviations:** **AFM:**  $\alpha$ -albumin/afamin; **AFP:**  $\alpha$ -fetoprotein; **AIDS:** acquired immunodeficiency syndrome; **ALB:** albumin; **ALS:** amyotrophic lateral sclerosis; **BCC:** basal cell carcinoma; **BMD:** bone mineral density; **COPD:** chronic obstructive pulmonary disease; **DBP:** vitamin D binding protein/group-specific component/Gc-globulin; **FALS:** familial ALS; **GC:** vitamin D binding protein gene; **Gc-MAF:** vitamin D binding protein-macrophage activating factor; **HIV:** human immunodeficiency virus; **IBD:** inflammatory bowel disease; **MS:** multiple sclerosis; **SNPdb:** single nucleotide polymorphism (SNP) database; **TDI:** transmission disequilibrium test; **UTR:** untranslated region; **VDR:** vitamin D receptor; **25(OH)D:** 25-hydroxycalciferol; **1,25(OH)<sub>2</sub>D<sub>3</sub>:** 1,25-dihydroxycholecalciferol; **1,25(OH)<sub>2</sub>D<sub>2</sub>:** 1,25-dihydroxyergocalciferol; **24,25(OH)<sub>2</sub>D:** 24,25-dihydroxycalciferol

### Introduction

#### DBP structure and function

The vitamin D binding protein (DBP), originally known as the Group-specific component (Gc-globulin), is a 51–58 kDa multifunctional serum glycoprotein synthesized in large

quantities by hepatic parenchymal cells and secreted into the circulation as a monomeric mature peptide of 458 residues and three structural domains<sup>1–3</sup>. Two binding regions are well characterized – a vitamin D/fatty acid binding domain located between residues 35–49, and an actin binding domain positioned between residues 350–403. Recently, DBP cell surface binding sites have been localized to N-terminal and C-terminal domains (domain I and III), which may be necessary to mediate DBP cellular functions<sup>4</sup>.

The functions of DBP are still being defined, but they include the transport of vitamin D and its metabolites in the

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circulation<sup>5,6</sup>, as well as actin scavenging and fatty acid binding<sup>7–12</sup>. Proteomic analysis shows that DBP is a hepatic acute phase reactant and is upregulated in patients with diabetes mellitus<sup>13</sup>, early-stage breast cancer<sup>14</sup>, oral squamous cell carcinoma<sup>15</sup>, aggressive periodontitis<sup>16</sup>, idiopathic temporal lobe epilepsy<sup>17</sup>, Alzheimer and Parkinson disease<sup>18</sup> and myopia<sup>19</sup>. It is downregulated in patients with sepsis<sup>20</sup>, neuromyelitis optica<sup>21</sup>, cutaneous malignant melanoma<sup>22</sup>, hepatocellular carcinoma<sup>23,24</sup>, primary non-metastatic breast cancer<sup>25</sup>, Klinefelter syndrome<sup>26</sup> and type I diabetes<sup>27</sup>. These observations provide an attractive prospect for clinically relevant biomarker status, but further characterization is needed to establish its usefulness in patient care.

### The GC gene encodes DBP

DBP is encoded by the single copy *GC* gene (NCBI GENE ID2638) located on chromosome 4q12-q13. The genomic sequence is 35 kb in length with a single start site, 13 exons with flanking untranslated regions at both ends and a key-enhancing motif (DNase Hypersensitivity Site IV) in intron 1 (Figure 1). The DBP gene is a member of a multigene family that includes albumin (*ALB*),  $\alpha$ -fetoprotein (*AFP*), and  $\alpha$ -albumin/afamin (*AFM*), tandemly linked in the following order: centromere–*DBP*–*ALB*–*AFP*–*AFM*–telomere<sup>28</sup>. Despite tight linkage, *DBP*, in an opposite direction of gene transcription, is separated from *ALB* by a non-transcribed sequence 1.5 Mb in length and is under autonomous regulatory control<sup>29</sup>.

A number of genetic variants are known (Figure 1). The two most common polymorphisms [c.1296T>G encoding D432E (SNPdb rs7041) and c.1307C>A encoding T436K (SNPdb rs4588)] are found in exon 11 in complete linkage disequilibrium, so only six haplotypes are observed in any significant frequency.

### Genotype-phenotype associations and vitamin D metabolism

Mature serum DBP concentration is a quantitative trait with a significant heritable component (66%), as established by twin studies published at least a decade ago<sup>30,31</sup>. Functional studies of the protein polymorphisms were initiated prior to molecular identification since the three protein variants, Gc1S, Gc1F and Gc2, can be distinguished by electrophoretic migration differences<sup>32,33</sup>. This is related to the differences in glycosylation pattern, resulting from replacement of the *O*-glycosylation moiety by a positively charged lysine residue at the T436K position as well as small differences in amino acid charge in the D432E variant (Figure 2). The full functional consequences of these variants (which probably include protein half-life and cell transit times) have not yet been delineated, although their impact on serum DBP and 25(OH)D concentrations has been demonstrated. Mean serum DBP concentrations stratified by DBP electrophoretic phenotypes were examined in 586 women<sup>34</sup>. Serum DBP levels did not differ between Gc1F and Gc1S alleles (D432E); however, Gc2 (T436K) showed a significantly lower level

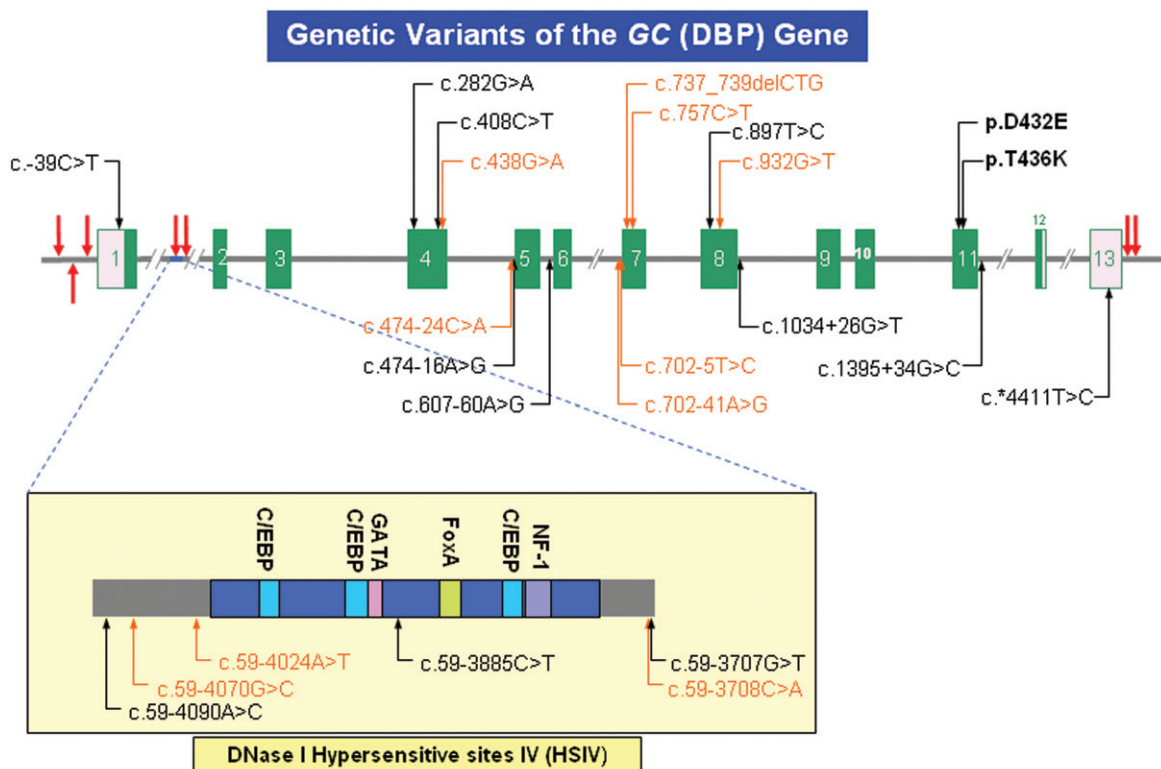


Figure 1. Genetic variants of the vitamin D-binding protein gene (*GC*). Shown in this schematic are the 13 exons (coding regions as green bars and untranslated sequences as pink boxes), separated by variable length introns (horizontal grey line, interrupted). Also shown are the DNase I hypersensitive sites (vertical red arrows). Extensively involved in control of gene expression, Site IV (HSIV), located in Intron I, is depicted in greater detail. Binding elements specific for Ccaat-enhancer-binding proteins (C/EBP, blue), GATA transcription factors (GATA, pink), hepatocyte nuclear factor 3- $\alpha$  (FoxA, lime) and nuclear factor-1 (NF-1, purple) are indicated. Besides the common missense SNPs – c.1296T>G specifying p.D432E, and c.1307C>A specifying p.T436K – there are a number of other well documented (black) and novel (orange) single-nucleotide variants scattered throughout the gene of relevance to future genetic association studies.

compared with Gc1 (Gc1F and Gc1S combined) ( $p=0.001$ ). The highest DBP serum concentration was in Gc1-1 subjects ( $272 \pm 2$  mg/L), the lowest in Gc2-2 ( $226 \pm 2$  mg/L) and intermediate levels were found in heterozygous subjects ( $249 \pm 3$  mg/L)<sup>34</sup>. Other investigators have confirmed these findings<sup>30,35</sup>. Similarly, our study also confirmed that the lowest serum DBP concentration was in the subjects with 436KK genotype (encoding the Gc2-2 protein variant)<sup>36</sup>.

Conclusions based on this type of candidate gene approach have been confirmed and strengthened by the results of genome-wide association studies (GWAS). In populations of European ancestry, two independent studies found that an intronic SNP rs2282679 which is in tight linkage disequilibrium with D432E had the strongest genome-wide significant associations with 25(OH)D concentrations<sup>37,38</sup>.

As noted before, the physiological relationship between the genetically determined differences in circulating DBP molecules and overall DBP concentrations is uncertain, but it seems likely that they specify not only the D432E and T436K substitutions in the peptide, but also affect glycosylation status<sup>39,40</sup>, and consequently, macrophage stimulating activity. While these common polymorphisms are associated with DBP status, others have demonstrated

association between genotype and serum vitamin D metabolite levels.

A recent study examining a cohort of 595 Danish Caucasian menopausal women showed that after adjusting for confounders, DBP phenotype is an independent predictor of 25(OH)D ( $p=0.016$ )<sup>41</sup>. Similar analysis of a large Han Chinese cohort ( $n=3210$ ) found four SNPs at the *GC* locus (D432E, T436K, rs2282679 and rs1155563) significantly associated with lower plasma 25(OH)D<sup>42</sup>. Of note, is the finding that T436K and rs2282679 remained significant after adjusting for the other two SNPs. A cross-sectional study examining 741 premenopausal white women of French-Canadian descent demonstrated that each additional copy of the 432D or 436 K alleles reduced 25(OH)D concentrations significantly ( $p<0.0003$  and  $p<0.0001$ , respectively)<sup>43</sup>. Similarly, in a study population of 98 healthy adult women, we found that the 436KK homozygotes exhibited reduced baseline vitamin D serum levels<sup>44</sup>.

Of more immediate clinical interest, perhaps, was our observation that this allele was also correlated with the response of serum 25(OH)D levels to long-term vitamin D supplementation. The subjects with 436KK genotype showed the largest proportional increase in response to a vitamin D supplement. The population-wide implications of these findings for vitamin D in public health are substantial, given that

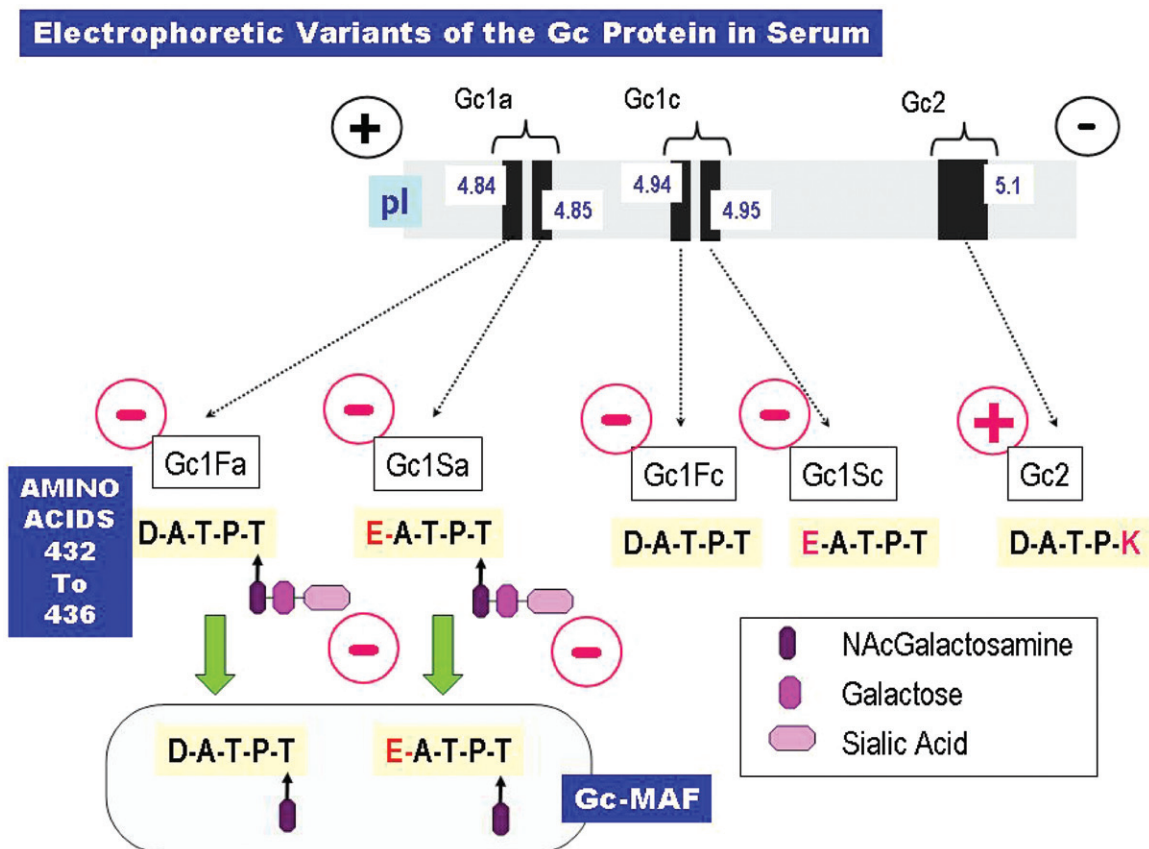


Figure 2. Common electrophoretic variants of the Gc protein (DBP). Shown at the top is the relative electrophoretic separation of the various Gc species, based on their isoelectric points (pI). The corresponding molecular structures are given below that. Only five residues of primary amino acid sequence (residues 432–436 = Asp–Ala–Thr–Pro–Thr = D–A–T–P–T) are depicted, along with their *O*-linked saccharides. Note that the D432E mutation results in a very small separation ( $\Delta pI = 0.01$ ), since the carboxyl side chains of the wild-type aspartate residue (in the Gc1F species) and the mutant glutamate residue (in Gc1S) have such similar dissociation constants. There is larger separation of the two Gc1 species if the negatively charged sialic acid residue (generating the anodal form, Gc1a) is removed, generating Gc1c, the cathodal form. Gc-MAF, shown at the bottom, arises as a result of sequential deglycosylation removing first the sialic acid and then the galactose residues. At the T436 position, the genetic variant Gc2 shows a more marked cathodal shift in electrophoretic migration due to replacement of the *O*-glycosylation site by a positively charged lysine residue (436 K).



the 436 K allele is less prevalent in Africans (15%) than in Caucasians (23%), Hispanics (20%) or Asians (29%)<sup>45</sup>. Indeed, we have recently shown that this is mirrored in immigrant sub-populations found in large North American cities<sup>46</sup>.

### Vitamin D, DBP and innate immunity – an emerging theme

The role of DBP in the biology of vitamin D as it relates to the endocrine status of bone and mineral metabolism is well established and the reader is referred to reviews<sup>47,48</sup>, monographs<sup>49</sup> and authoritative web sites<sup>50</sup>. DBP is thought to regulate the bioavailability of 25-hydroxycholecalciferol [25(OH)D<sub>3</sub>], acting as the main transporter from liver to kidney for the synthesis of the principal active metabolite, 1,25-dihydroxycalciferol [1,25(OH)<sub>2</sub>D]. Alternatively, 25(OH)D delivered to the kidney may undergo catabolic hydroxylation there to the considerably less active 24,25-dihydroxycalciferol [24,25(OH)<sub>2</sub>D] metabolite, which is then subject to further degradation and renal excretion.

In contrast, the role of vitamin D and DBP in innate immunity has been appreciated only more recently, either through its involvement in the vitamin D transport pathway or by independent mechanisms<sup>51</sup>. The vitamin D<sub>3</sub> form, 1,25-dihydroxycholecalciferol [1,25(OH)<sub>2</sub>D<sub>3</sub>], and the vitamin D<sub>2</sub> form, 1,25-dihydroxyergocalciferol [1,25(OH)<sub>2</sub>D<sub>2</sub>], exhibit similar properties not only for skeletal effects, but also for their anti-inflammatory actions<sup>52</sup>. Moreover, epidemiologic evidence for mitigating chronic diseases such as cancer<sup>53</sup>, and for upregulation of antimicrobial peptide synthesis<sup>54,55</sup> applies to both vitamins.

Interestingly, DBP has also been shown to play a more direct role in the inflammatory cascade by enhancing the leukocyte chemo-attractant effect of complement activation peptide C5a, one of the most potent of the early-acting chemo-attractants<sup>56–60</sup>. The role of DBP as a C5a adjuvant is apparently not strictly vitamin D independent as 1,25(OH)<sub>2</sub>D<sub>3</sub> can mitigate the synergistic effect of DBP and C5a<sup>60</sup>. The stepwise glycohydrolysis promoted by T and B cells leads to loss of the *O*-glycosylated oligosaccharide moiety in the DBP peptide, transforming it into a potent macrophage activating factor (Gc-MAF)<sup>61,62</sup>.

Finally, while DBP plays a proximate role in innate immune regulation, it may also act more distantly through actin scavenging to protect the host from the effects of cell damage due to tissue ischemia, inflammation or mechanical injury. Whereas excessive globular actin released from damaged cells can cause intravascular coagulation resulting in multi-organ dysfunction and cardiac arrest, DBP functions to bind and sequester actin in circulation released from damaged cells, thus potentially mitigating adverse outcomes of tissue injury<sup>7,11,63,64</sup>.

The various roles that DBP plays in different inflammatory processes suggests that functional polymorphisms in the DBP gene, *GC*, may therefore predict adverse outcomes in a wide range of chronic and infectious diseases associated with these processes. Below we summarize the evidence supporting this contention, and examine the strength of the findings to date (Table 1).

### DBP genotypes and chronic disease

#### Diabetes

Type I diabetes mellitus results from the autoimmune destruction of insulin producing pancreatic  $\beta$ -cells while type II diabetes is associated with insulin resistance and elevated levels of inflammatory mediators<sup>65,66</sup>. Vitamin D may play a role in the prevention and treatment of diabetes through its anti-inflammatory actions or by modifying calcium homeostasis to improve insulin secretion or enhance insulin sensitivity<sup>67</sup>. As such, polymorphisms in the DBP gene might provide insight into the role of vitamin D and diabetes.

Several studies have examined whether pre-diabetic phenotypes are associated with DBP variants. In a Japanese study group, composed of 47 males and 35 females with normal glucose tolerance, carriers of Gc1S-2 and Gc1S-1S had significantly higher fasting plasma insulin levels than homozygotes for Gc1F ( $p < 0.01$  and  $p < 0.03$ , respectively)<sup>68</sup>. These findings were supported in an ethnically distinct population made up of 144 Dogrib Indians of the Northwest Territories in Canada<sup>69</sup>.

On the other hand, Baier et al. found no association with insulin or fasting plasma glucose concentrations in a Pima cohort, although they did report that exon 11 polymorphisms were associated with blood glucose responses to an oral glucose challenge in non-diabetics<sup>70</sup>. Specifically, individuals homozygous for Gc1F had the highest increment in glucose concentration at 30 and 60 min and the lowest glucose concentration at 180 min. Moreover, carriers homozygous for Gc2 had significantly lower mean incremental glucose concentrations at 60 and 120 min<sup>70</sup>.

Similarly, in a population-based case-control study composed of Hispanic and Anglo participants from the San Luis Valley Diabetes Study, DBP variants were not associated with insulin levels<sup>71</sup>. Also, and in contrast with Baier et al., DBP variants were not associated with postprandial glucose, but were associated with fasting glucose levels ( $F = 2.46$ ;  $p = 0.033$ ), the Gc1F genotype being more commonly observed in individuals with the highest levels of plasma glucose<sup>71</sup>.

Other studies have examined the association between genetic variants of DBP and clinical diabetes. Results from a Japanese study, composed of 208 non-insulin-dependent diabetes mellitus patients and 209 control subjects with normal glucose tolerance, showed that Gc1F-1F homozygosity was less frequently and Gc1S/2 heterozygosity more frequently observed in patients compared to controls ( $p < 0.001$  and  $p < 0.02$ , respectively)<sup>72</sup>. Sequence analysis identified D432E and T436K genotypes in a cohort of 912 Pima Indians (578 diabetic; 334 nondiabetic) from Gila River Indian Community in Arizona, USA<sup>70</sup>, but no association was reported between either variant and the prevalence of type II diabetes.

In a family-based study, the tetranucleotide repeat in intron 8<sup>73</sup> and D432E and T436K polymorphisms were studied in 152 Caucasian families of German origin with at least one child developing type I diabetes<sup>66</sup>. Transmission disequilibrium testing (TDT) showed neither allelic distortion of intron 8 or exon 11 alleles between cases and controls, nor of their

Table 1. Association between common genetic variants of the vitamin D binding protein gene (GC) and various diseases.

Disease	Reference	Sample size (N)		Country	Ethnicity	GC polymorphisms studied	Findings
		Cases	Controls				
<b>Cancer</b>							
Breast Cancer	Abbas et al. <sup>160</sup>	1402	2608	Germany	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	Genotype Gc2-2 was associated with decreased risk of postmenopausal breast cancer, [OR = 0.72 (CI = 0.54–0.96, <i>p</i> = 0.04)], when compared to the most frequently observed genotype, Gc1S-1S. Gc2 carriers had an OR of 0.88 (CI = 0.77–1.01, <i>p</i> = 0.02) compared to non-carriers.
	Anderson et al. <sup>161</sup>	1560	1633	Canada	Caucasian	rs7041, rs4588	TT genotype of rs7041 was associated with breast cancer, OR = 1.23 (CI = 1.01–1.51, <i>p</i> < 0.05). Resulting genotype Gc2-2 had OR = 1.22 (CI = 0.93–1.59), when compared to genotype Gc1-1.
Gastrointestinal Cancer (includes Colorectal Cancer)	McCullough et al. <sup>159</sup> Hibler et al. <sup>169</sup>	500 1439	500 N/A	United States United States	99% Caucasian <sup>b</sup> Caucasian	rs7041, rs4588 rs7041 and six other SNPs in <i>GC</i> gene	NS NS
	Poynter et al. <sup>168</sup>	1806	2879 (siblings)	United States, Canada, Australia	87% non-Hispanic White; remainder Black, Asian and Other	rs7041, rs4588 and other SNPs in the <i>Gc</i> gene	NS
	Zhou et al. <sup>170</sup>	964	1187	China	Han Chinese	D432E, T436K	Homozygous KK genotype of T436K had elevated risk for colorectal cancer in comparison to 436 TT genotype, OR = 3.41 (CI = 1.85–6.57, <i>p</i> < 0.001). When all gastrointestinal cancer types were combined, KK genotype had 1.15 fold increased risk over TT (CI = 1.02–1.30, <i>p</i> = 0.020) after adjustment for age, sex and smoking status. Carriers of D432-436 K haplotype (associated with Gc2 allele) also had an increased risk for developing gastrointestinal cancer, OR = 1.22 (CI = 1.04–1.39, <i>p</i> = 0.015)
Prostate Cancer	Ahn et al. <sup>166</sup>	749	781	United States	Non-Hispanic White	rs7041, rs4588 and other SNPs in the <i>Gc</i> gene	NS
	Corder et al. <sup>167</sup>	181	181	United States	182 White, 180 Black	Gc1F, Gc1S, Gc2	NS
	Dimopoulos et al. <sup>165</sup>	115	155	Greece	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	Increased disease risk for carriers of Gc2 allele; RR = 1.81 ( <i>p</i> < 0.01)

(continued)

Table 1. Continued.

Disease	Reference	Sample size (N)		Country	Ethnicity	GC polymorphisms studied	Findings
		Cases	Controls				
Skin Cancer, Basal Cell Carcinoma (BCC)	Flohil et al. <sup>171</sup>	7983	N/A	Netherlands	Caucasian	rs7041, rs4588	Heterozygous Gc1F carriers were significantly more likely to develop a first BCC compared to non-carriers, HR = 1.40 (CI = 1.11–1.78). Homozygote Gc1S carriers aged less than 65 years old has significantly lower risk of BCC compared to non-carriers, HR = 0.53 (CI = 0.31–0.91).
	Wjst et al. <sup>117</sup>	947 (201 families)	191	Germany and Sweden	Caucasian <sup>a</sup>	rs7041, rs4588 and other SNPs in the GC gene	NS. Weak association with D432E (rs7041) and total serum IgE ( $p = 0.0249$ )
Chronic Diseases Asthma	Li et al. <sup>116</sup>	467	288	China	Chinese Han	rs7041, rs4588	Gc2 allele was associated with asthma susceptibility, OR = 1.35 (CI = 1.01–1.78, $p = 0.006$ ); Gc2-2 genotype was strongly associated with risk of asthma compared to Gc1-1, OR = 13.13 (CI = 2.42–7.13, $p = 0.001$ )
	Horne et al. <sup>90</sup>	104	413	Canada	Caucasian	Gc1F, Gc1S, Gc2	Gc2 allele may confer disease protection; Highest disease risk in Gc1F-1F genotype (RR = 4.8), and lowest risk in Gc2-1F genotype (RR = 0.5)
Chronic Obstructive Pulmonary Disease (COPD)	Ito et al. <sup>93</sup>	103	88	Japan	Japanese	T436K, D432E	Increased risk of COPD for Gc1F-1F genotype; significantly larger proportion of Gc1F homozygotes in patients (32%) compared to healthy smokers (17%) $p = 0.014$ ; OR for Gc2-2 is 2.3 (CI = 1.2–4.6).
	Janssens et al. <sup>97</sup>	262	152	Belgium	Caucasian <sup>a</sup>	rs7041, rs4588	Individuals with homozygous TT at rs7041 have increased risk for COPD; OR = 2.11 (CI = 1.2–3.7, $p = 0.009$ ) for COPD. No association with heterozygous T carriers.
	Kasuga et al. <sup>98</sup>	532 (low lung function)	114	Canada	Caucasian	Gc1F, Gc1S, Gc2	NS
	Kueppers et al. <sup>91</sup>	114	114	United States	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	Gc2-2 may offer a protective advantage; Gc2-2 genotype was under-represented in the COPD group (1%) versus the control group (5%), $p = 0.049$ .
	Schellenberg et al. <sup>89</sup>	75	64	Canada	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	Homozygous Gc2 appears protective against disease. OR for Gc2-1 = 1.01 (CI = 0.49–2.10) OR for Gc2-2 = 0.17 (CI = 0.03–0.83)

Shen et al. <sup>95</sup>	100	100	China	Chinese Han	Gc1F, Gc1S, Gc2	Decreased COPD disease risk for Gc2. OR for Gc1F-IF = 3.08 (CI = 1.498–6.347, $p = 0.003$ ) OR for Gc2-2 = 0.215 (CI = 0.06–0.772, $p = 0.0017$ )
Wood et al. <sup>92</sup>	611 (471 with AATD and 140 with COPD)	480	United Kingdom	Caucasian	rs7041, rs4588 and other SNPs in Gc gene	Gc2 decreased risk of COPD, OR = 0.79 (CI = 0.65–0.99, $p = 0.048$ ). Gc2 had increased risk of bronchiectasis, OR = 1.51 (CI = 1.02–2.22, $p = 0.034$ ). rs7041 was associated with bronchiectasis in $\alpha 1$ -antitrypsin deficiency (AATD), OR = 0.52 (0.29–0.93, $p = 0.027$ ). No association with rs4588.
Cooper et al. <sup>224</sup>	8517	104 381 and 1933 families	United Kingdom <sup>c</sup>	Caucasian	rs7041, rs4588	Association between rs4588 and type 1 diabetes was observed in the case/control dataset, OR = 0.95 (CI = 0.91–1.00, $p = 0.05$ ). No associations were observed in the family collection data ( $p = 0.071$ ). No association with D432E.
Hodge et al. <sup>225</sup>	103	N/A	United States	Caucasian	Gc1F, Gc1S, Gc2	Excess of Gc2-1 and Gc1-1 phenotypes ( $0.05 > p > 0.02$ ); however, association not significant when corrected for multiple testing.
Pani et al. <sup>66</sup>	527 (152 families with at least one affected offspring)	N/A	Germany	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2; number of repeats for intron 8 [(TAAA) <sub>n</sub> ]	NS
Baier et al. <sup>70</sup>	578	595	United States	Pima Indians	D432E, T436K	Gc genotypes differed in plasma glucose concentrations in response to oral glucose tolerance test, highest concentrations observed in Gc1F and lowest in Gc2 ( $p < 0.028$ , repeated measures ANOVA).
Hirai et al. <sup>72</sup>	208	209	Japan	Japanese	Gc1F, Gc1S, Gc2	Reduced risk for individuals with Gc1F allele. Gc1F-IF genotype was less frequent in cases versus controls (11% versus 19%, $p < 0.001$ ). Gc1S-2 genotype was more common in cases than controls (48% versus 28%, $p < 0.02$ ).
Hirai et al. <sup>68</sup>	N/A	82	Japan	Japanese	Gc1F, Gc1S, Gc2	Fasting insulin concentrations differed in Gc alleles ( $p < 0.05$ ); individuals with Gc1S allele had highest concentrations, followed by Gc2 allele carriers, the lowest fasting insulin was observed in Gc1F carriers. HOMA was highest in Gc1S carriers ( $p < 0.03$ ).

(continued)

Table 1. Continued.

Disease	Reference	Sample size (N)		Country	Ethnicity	GC polymorphisms studied	Findings
		Cases	Controls				
Endometriosis	Iyengar et al. <sup>71</sup>	N/A	468	United States	Anglos (N = 289); Hispanic Americans (N = 179)	Gc1F, Gc1S, Gc2	Gc1F allele associated with highest levels of plasma glucose (p = 0.033).
	Klupa et al. <sup>74</sup>	396 (Type I, N = 181; Type II, N = 215)	163	United States	Caucasian	Gc1F, Gc1S, Gc2	NS
Thyroid Autoimmune Disease	Malecki et al. <sup>76</sup>	231	162	Poland	Caucasian	Gc1F, Gc1S, Gc2	NS
	Szathmary <sup>69</sup>	144	N/A	Canada	Dogrib Indians	Gc1F, Gc1S, Gc2	Gc1F-1F had the lowest fasting glucose level. After adjustment for BMI, Gc genotype was the only variable that had a significant effect on insulin concentrations (p = 0.040).
Ischemic Stroke	Ye et al. <sup>75</sup>	237	143	France	Caucasian	Gc1F, Gc1S, Gc2	NS
	Faserl et al. <sup>124</sup>	57	24	United States	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	Gc alleles differed between cases and controls (p = 0.006). 18% of cases expressed Gc2 allele alone, but none of the control cases did.
Inflammatory Bowel Diseases (IBD)	Kurylowicz et al. <sup>196</sup>	332	185	Poland	Caucasians	D432Eand T436E; Variable tandem (TAAA) <sub>n</sub> -Alu repeat in Intron8	Increased risk for carriers of K allele at T436K, OR = 1.5 (CI = 1.13-1.99, p = 0.005). OR = 1.63 for homozygous 436KK genotype (CI = 0.80-3.32, p = 0.01).
	Pani et al. <sup>195</sup>	187 nuclear families with an offspring affected by Graves (N = 95) or Hashimoto thyroiditis (N = 92)	N/A	Germany and Italy	Caucasian	Gc1F, Gc1S, Gc2; Variable tandem (TAAA) <sub>n</sub> -Alu repeat in Intron8	Association with intron 8(*8) polymorphisms in Graves Disease patients (p < 0.03). No association with Hashimoto thyroiditis.
Inflammatory Bowel Diseases (IBD)	Wang et al. <sup>119</sup>	3550	6560	United States, Europe, China	Varied by study; primarily Caucasians and Chinese	rs7041, rs4588	NS
	Eloranta et al. <sup>125</sup>	636 (232 with ulcerative colitis; 404 with crohn disease)	248	Switzerland	Caucasian <sup>a</sup>	D432E, T436K	Homozygous 436KK genotype was more common in control group than in IBD patients (p = 0.006). Association was significant in separate comparisons of healthy controls with ulcerative colitis patients (p = 0.022) and Crohn disease patients (p = 0.016). DBP_2 TA haplotype (D432-436K, Gc2 allele) was found at higher frequencies in healthy controls than in IBD cases [OR = 2.53, (CI = 1.48-4.34, p = 0.0005)], particularly



Liver Disease	Constans et al. <sup>35</sup>	17 (15 cirrhosis; 2 hepatitis)	100	France	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	when comparing healthy controls to the ulcerative colitis cases [OR = 4.39, (CI = 1.87–10.31, $p = 0.0003$ )] Gc1 was overrepresented in the cases versus healthy controls. An unusual electrophoretic form of Gc1 was found in many of the cases (10 out of 17 cases), perhaps differentiated by the presence of two sialic acid residues.
Osteoporosis	Al-oanzi et al. <sup>87</sup>	56	114	United Kingdom	Caucasian	Variable tandem (TAAA) <sub>n</sub> -Alu repeat in Intron8	Allele *10 was associated with lower risk of osteoporosis, OR = 0.39 (CI = 0.25–0.64, $p < 0.0005$ ). Allele 11 was also associated with reduced risk, OR = 0.09 (CI = 0.01–0.67, $p < 0.0007$ ). Allele *8 and *9 both showed increased susceptibility.
	Eichner et al. <sup>226</sup>	258	N/A	United States	Caucasian <sup>a</sup> (non-Black)	Gc1F, Gc1S, Gc2	No association between Gc alleles and BMD. Homozygous Gc1F phenotype was associated with higher bone mass density, but only 7 individuals with this phenotype.
	Ezura et al. <sup>85</sup>	384	N/A	Japan	Japanese	rs7041, rs4588 and 12 other SNPs in the Gc gene	Several SNPs were associated with bone mass density (BMD); D432E (rs7041) in conjunction with IVS1 + 827C > T showed the strongest relationship with BMD ( $r^2 = 0.029$ , $p = 0.005$ ).
	Fang et al. <sup>88</sup>	6181	N/A	Netherlands	Caucasians	rs7041, rs4588	Gc variants were not associated with fracture risk in entire study. In a subgroup of individuals with calcium intake < 1.09 g/day, the hazard ratio for fracture risk for the Gc1S homozygote was 1.47 (CI = 1.06–2.05), compared to non-carriers.
	Lauridsen et al. <sup>77</sup>	595	N/A	Denmark	Caucasian	Gc1F, Gc1S, Gc2	Lowest fracture risk in Gc2-2 group (14%) compared to Gc1-2 (27%) and Gc1-1 (34%) ( $p = 0.017$ ). Fracture risk OR for Gc2-2 = 0.32 (CI = 0.13–0.80) Compared to the other genotypes, none of the Gc2-2 individuals had any low energy fractures ( $p = 0.021$ ).
	Papiha et al. <sup>86</sup>	26	21	United Kingdom	Caucasian	Gc1F, Gc1S, Gc2; Variable tandem (TAAA) <sub>n</sub> -Alu repeat in Intron8	Intron 8*Alu repeat had significant association with lumbar spine and femoral neck BMD. BMD was observed to be lower in men with 10/8 genotype than 10/10 genotype ( $p < 0.05$ ).

(continued)

Table 1. Continued.

Disease	Reference	Sample size (N)		Country	Ethnicity	GC polymorphisms studied	Findings
		Cases	Controls				
<b>Sarcoidosis</b> <b>Infectious Diseases</b> Human Immunodeficiency Virus (HIV)	Milman et al. <sup>192</sup>	44	44	Denmark	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Alonso et al. <sup>216</sup>	318 (at risk or infected)	187	Spain	Caucasian	Gc1F, Gc1S, Gc2	NS
	Cleve et al. <sup>217</sup>	97	1523	United Kingdom	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Eales et al. <sup>219</sup>	203 (at risk males)	177 (50 homo-sexual and 122 hetero-sexual males)	United Kingdom	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	30% of AIDS patients were homozygous for Gc1F compared to 0.8% of controls ( $p < 0.0001$ ). Gc2-2 individuals were overrepresented in sero-negative contacts of AIDS patients ( $p < 0.05$ ).
	Pronk et al. <sup>212</sup>	447 (96 AIDS patients and 351 homosexual men)	86	Netherlands	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
Rheumatoid Fever	Putkonen et al. <sup>218</sup>	125 (85 AIDS patients and 40 couples with 1 infected partner)	3394	Sweden	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Rosberger et al. <sup>227</sup>	121	1011	United States	Caucasians and African Americans	Gc1F, Gc1S, Gc2	NS
	Bahr et al. <sup>208</sup>	39	90	Kuwait	Arabic	Gc1F, Gc1S, Gc2	An association was observed between Gc2 allele with rheumatic fever ( $p = 0.0024$ ); 56% of all rheumatic fever patients were carriers of Gc2 allele.
	Bahr et al. <sup>208</sup>	41	90	Kuwait	Arabic	Gc1F, Gc1S, Gc2	No association with GC alleles and TB, although an association was observed between Gc2 allele with rheumatic fever ( $p = 0.0024$ ); 56% of all rheumatic fever patients were carriers of Gc2 allele.
<b>Neurodegeneration</b> Amyotrophic Lateral Sclerosis (ALS)	Martineau et al. <sup>206</sup>	123 (United Kingdom); 130 (Brazil); 281 (South Africa)	140 (United Kingdom); 78 (Brazil); 182 (South Africa)	United Kingdom	Varied based on study location <sup>d</sup>	Gc1F, Gc1S, Gc2	Associations between GC polymorphism and TB only observed in Gujarati Asians from United Kingdom. Gc2-2 associated with susceptibility to active TB compared to wild type (Gc1-1): OR = 2.81 for Gc2-2 versus Gc1-1 (CI = 1.19-6.66, $p = 0.009$ ). No associations were observed in the populations from Brazil and or South Africa.
	Palma et al. <sup>182</sup>	11 (7 ALS, 4 other muscular disorders)	4	Portugal	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	Gc2 may represent a disease risk, Gc2 was present in all familial ALS patients and 2 patients with other muscular diseases but in none of the healthy controls.

Multiple Sclerosis (MS)	Hollisberg et al. <sup>176</sup>	95	227	Denmark	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Lindblom et al. <sup>177</sup>	88	3394	Sweden	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Niino et al. <sup>173</sup>	107	109	Japan	Japanese	D432E, T436K	NS
	Orton et al. <sup>179</sup>	1364	1661 (first degree relatives)	Canada	Caucasian <sup>a</sup>	rs7041, rs4588 and 2 other SNPs in the Gc gene	NS
Parkinson's Disease	Simon et al. <sup>178</sup>	214	428	United States	Various (Nurses Health Study)	rs7041, rs4588	NS
	Steckley et al. <sup>174</sup>	236 sib pairs (1 affected, 1 not)	N/A	Canada	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Suzuki et al. <sup>191</sup>	137	N/A	Japan	Japanese <sup>a</sup>	Gc1F, Gc1S, Gc2	No association with GC and severity of Parkinson disease as evaluated by Hoehn & Yahr (HY) stages and Unified Parkinson's Disease Rating Stage (UPDRS).
Schizophrenia	Beckman et al. <sup>187</sup>	47	2361	Sweden	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Fananas et al. <sup>189</sup>	162	365	Spain	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Lange, <sup>184</sup>	222	176	Germany	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	Excess of Gc1-1 and a deficit of Gc2-1 and Gc2-2 ( $p < 0.01$ ) in cases versus controls
	Papiha et al. <sup>185</sup>	215	402 (203 family & 199 unrelated)	United Kingdom	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS with Gc2 allele. However, lower frequency of Gc1S in female cases than controls (50% versus 63%, $p < 0.025$ )
	Rudduck et al. <sup>188</sup>	152	3384	Sweden	Caucasian <sup>a</sup>	Gc1F, Gc1S, Gc2	NS
	Saha and Tsoi, <sup>186</sup>	423 males	595	Singapore	Asian <sup>a</sup>	Gc1F, Gc1S, Gc2	Gc1S appears to offer protection against schizophrenia. Excess of Gc2 allele over Gc1 ( $p < 0.005$ ) and lack of Gc1S in cases ( $p < 0.001$ ) was observed. RR for Gc1F, Gc1S, and Gc2 estimated as 1.12, 0.76, and 1.15, respectively.

Acronyms used in table: CI = Confidence Interval (95%); OR = Odds Ratio; RR = Relative Risk. Abbreviations used in table: NS = no significant association between polymorphisms studied and disease risk; AATD =  $\alpha$ 1-antitrypsin deficiency; HOMA (Homeostatis model assessment) is a measure used to quantify insulin resistance and beta cell function. In the cited study, HOMA(R) was calculated as [the fasting plasma glucose concentration (mg/dL)]  $\times$  [fasting plasma insulin concentration ( $\mu$ U/mL)]/405<sup>68</sup>.

<sup>a</sup>In these studies, ethnicity was not clearly provided so they are assumed to be representative of the ethnic majority of the country where the study originates.

<sup>b</sup>Sample was 99% Caucasian; the remainder of the sample was comprised of African-American, Asian, Hispanic and other ethnicities.

<sup>c</sup>Family collections included samples from Ireland, Romania, Norway and families from the Human Biological Data Interchange.

<sup>d</sup>Ethnicity included Gujarati Asians (United Kingdom), White, Black, Mixed Ethnicity (Brazil), Xhosa and Cape Colored (South Africa).

combined haplotypes<sup>66</sup>. Similarly, allele, genotype and haplotype frequencies of SNPs in exon 11 did not differ between non-diabetic controls ( $n = 163$ ) and type I ( $n = 181$ ) or type II ( $n = 215$ ) diabetics in a Caucasian American population<sup>74</sup>. These findings were substantiated in French and Polish Caucasian case-control studies that additionally reported that phenotypes, defined by haplotype combinations, were equally distributed between Type II diabetics and non-diabetics<sup>75,76</sup>.

In aggregate, these studies fail to support a strong, consistent role for DBP in the pathobiology of diabetes. It is likely that ethnicity and lifestyle factors contribute to the lack of concordance among these studies.

### Osteoporosis

Osteoporosis is a common disorder of aging that disproportionately affects post-menopausal women. It is characterized by low bone mass and micro-architectural changes in bone which contribute to increased bone fragility and risk of fracture<sup>77</sup>. The primary clinical marker of osteoporosis, lower bone mineral density (BMD), is associated with vitamin D insufficiency<sup>78–80</sup>. However, the links between DBP and bone biology are not well understood. Whereas Gc-MAF is shown to increase phagocytosis and free radical (superoxide) production in macrophages<sup>61,81</sup>, further work has implicated it as a potent activator of osteoclasts<sup>82</sup>. Indeed, osteopetrotic patients (characterized by excessively dense bone and high BMD) appear to lack the ability to generate Gc-MAF<sup>83</sup>. Osteopetrotic rats treated with Gc-MAF showed a reduction in BMD and associated skeletal defects<sup>84</sup>.

In the ongoing 20-year Danish Osteoporosis Prevention Study comprised of 595 white postmenopausal women, Scandinavian investigators found a three-fold lower fracture risk associated with Gc2-2 compared with Gc1-1 genotype ( $p = 0.014$ , OR = 0.32, 95% CI = 0.13–0.80)<sup>77</sup>. A more comprehensive set of SNPs distributed across promoter, intronic and exonic regions of DBP was analyzed in 384 Japanese in a search for association with BMD<sup>85</sup>. Six SNPs (–39C>T, IVS1+827C>T, IVS1+1916C>T, IVS1-1154A>G, D432E and IVS11+1097G>C) were either significantly associated or suggestively associated with BMD, the IVS11+1097G>C SNP in intron 11 showing the strongest correlation ( $p = 0.006$ ). Furthermore, a common haplotype (T-C-C-G-T-C) derived from the six SNPs showed significant association with adjusted radial BMD ( $r = 0.15$ ,  $p = 0.008$ ). The functional consequence(s) of these SNPs/haplotypes are not known.

Another polymorphism in the form of an (TAAA)<sub>n</sub>-Alu repeat or repetitive element downstream of intron 8 was examined in a cohort of Caucasian men from northeast England for association with DBP serum levels, BMD and osteoporosis<sup>86</sup>. The (TAAA)<sub>8</sub> (GC-I8\*8) allele is the most prevalent one in this study group. Upon analyzing 26 men with vertebral fractures and 21 controls, the GC-I8\*10/\*8 genotype was found to be associated with decreased BMD lumbar spine and femoral neck BMD and increased vertebral fractures compared with GC-I8\*10/\*10 ( $p < 0.05$ , OR = 56; 95% CI = 7–445)<sup>86</sup>. Interestingly, GC-I8\*10/\*8 was also associated with elevated serum DBP ( $p = 0.049$ ) which, in turn, may provide greater potential for Gc-MAF activation of

osteoclasts in Gc1 carriers. Results of this study remained consistent in another male cohort made up of 56 individuals with idiopathic osteoporosis and 114 controls, but the odds ratio was much more modest<sup>87</sup>. Carriers of the GC-I8\*10/\*8 genotype and the GC-I8\*9 allele were at increased risk of osteoporosis (OR = 2.88, CI = 1.31–6.32,  $p = 0.013$  and OR = 1.86, CI = 1.07–3.24,  $p = 0.038$ , respectively). Moreover, combining all genotypes that include GC-I8\*8 is associated with a two-fold risk of osteoporosis (OR = 2.38, CI = 1.24–4.58,  $p = 0.014$ ). However, GC-I8\*10 allele carriers and GC-I8\*10/\*10 homozygotes showed increasingly substantial protection from osteoporosis (OR = 0.40, CI = 0.25–0.64,  $p = 0.0005$  and OR = 0.13, CI = 0.05–0.36,  $p = 0.0005$ , respectively). Whether this effect is related in part to linkage disequilibrium with the nearby functional polymorphisms (D432E and T436K) has not been examined.

In a common aging disorder like osteoporosis, many genes are involved and interaction with non-genetic factors (e.g. calcium and vitamin D intake) is common. A recent prospective study examining more than 6100 elderly Caucasians found no association between DBP phenotype and osteoporosis; however, a 33% increased risk of fracture was detected in Gc1S carriers homozygous for polymorphisms in the 3' untranslated region (UTR) of the vitamin D receptor (VDR) in haploblock 5 compared with non-carriers ( $p = 0.005$ )<sup>88</sup>. Moreover, a hazard ratio of 1.47 (95% CI = 1.06–2.05) was observed for homozygous Gc1S carriers (compared with non-carriers) with low dietary calcium intake (<1.09 g/day).

Collectively, these studies emphasize the importance of gene-gene and gene-environment interactions in complex trait analysis and underscore the need to characterize the role of non-coding genetic variants associated with osteoporosis and related phenotypes.

### Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by decreased pulmonary elastic recoil, expiratory obstruction and hyperinflation secondary to inflammation of peripheral airways<sup>89</sup>. The role of DBP as a modulator of inflammation suggests that it plays a role in the chronic pulmonary inflammation characteristic of COPD. Consistent with this hypothesis, an association study in a Caucasian population of 104 COPD patients and 413 controls indicated that Gc1F-IF and Gc1S carriers (at least one copy) were seven-fold and two-fold more likely, respectively, to have COPD than individuals with at least one Gc-2 allele<sup>90</sup>.

In another study of 75 Caucasian COPD patients, Gc-2 homozygosity was found to be underrepresented in COPD patients compared to 64 controls (OR = 0.17, CI = 0.03–0.83)<sup>89</sup>. This is in agreement with an earlier report examining 114 COPD cases in a match pair design study showing an excess of Gc2 homozygotes in the control group ( $p = 0.049$ )<sup>91</sup>.

Similarly, a recent study of 471 unrelated Caucasian subjects confirmed that the Gc2 variant was associated with a decreased risk of COPD (OR = 0.79, CI = 0.65–0.99;  $p = 0.048$ ), though it was associated with an increased risk of bronchiectasis in patients with  $\alpha 1$ -antitrypsin deficiency (OR = 1.51, CI = 1.02–2.22). The authors suggested this may

be due to reduced capacity to produce Gc-MAF, thereby stimulating macrophage-induced pathogen clearing from the airway<sup>92</sup>.

A Japanese case-control study (103 cases, 88 controls) found that *Gc1F* homozygotes were more susceptible to chronic bronchitis and emphysema due to COPD than were healthy smokers ( $p = 0.01$ , OR = 2.3, 95% CI = 1.2–4.6)<sup>93</sup>. In a second Japanese cohort, *Gc1F* homozygotes were overrepresented among COPD patients (36.5%) compared to controls (20.7%; OR = 2.2; 95% CI = 1.1–4.6). However, no association was found with diffuse panbronchitis, also characterized by chronic airflow limitation<sup>94</sup>. In a case-control study of a Han Chinese cohort (100 cases, 100 controls), the COPD patient group was found to be enriched for *Gc1F* homozygous individuals while the frequency of *Gc2* was significantly lower<sup>95</sup>.

Together, these findings are consistent with evidence suggesting that the presence of the 436 K minor variant (*Gc2*) precludes the conversion of DBP to Gc-MAF, potentially reducing macrophage-related inflammation<sup>96</sup>. Another recent report indicates that, 25(OH)D deficiency is more common in COPD patients from Belgium and correlates with disease severity<sup>97</sup>. In the same study, homozygous T436 subjects were found to have a 25% reduction in vitamin D serum levels ( $p < 0.0001$ ) and were enriched amongst the COPD patient population (OR = 2.11; CI = 1.20–3.71). It is conceivable, therefore, that anti-inflammatory actions exerted downstream of 25(OH)D<sup>52,97</sup> are reduced in patients. The nature by which DBP influences COPD pathogenesis deserves further investigation, however unlikely it is to affect the rate or onset of deteriorating lung function<sup>98,99</sup>.

### Asthma

Asthma is a chronic inflammatory condition characterized by airway obstruction and elevated serum IgE levels<sup>100</sup>, most often found to be a consequence of enhanced acquired reactions to external allergens. It is associated with various reactive pro-inflammatory states including allergic rhinitis and eczema. Ecological studies indicate that vitamin D deficiency is associated with asthma and asthma related phenotypes<sup>101–105</sup>, but some studies contradict these findings reporting a positive association<sup>106,107</sup>, whereas others show no association<sup>108</sup>. Further evidence suggesting an inverse correlation between vitamin D and asthma is provided by genetic<sup>109,110</sup> and epidemiologic (geospatial and solar radiation) studies<sup>111</sup>. However, in another study, Hughes et al. reported no association between ultraviolet radiation (UVR) or vitamin D serum levels; yet, cod liver oil supplementation earlier in life was associated with an increased risk of asthma<sup>112</sup>.

Overall evidence suggests that vitamin D protects against asthma, but the precise nature of this relationship is ambiguous and inconsistent findings are likely a function of epidemiological context. A molecular mechanism by which vitamin D may affect asthma is unknown and its relationship to atopy is unclear. However, emerging evidence suggests that 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibits Th2-related asthmatic inflammation either directly or via immunosuppressive T-regulatory cell induction<sup>113–115</sup>. However, these findings were not substantiated in a recent case-control study examining 467 asthmatic patients and 288 unrelated healthy controls in a Chinese Han

population, which showed an enrichment of *Gc2-2* in asthma cases (OR = 1.35; CI = 1.01–1.78;  $p = 0.006$ )<sup>116</sup>. A TDT of more than 200 families from the German Asthma Family Study found no significant association with asthma, but rs222040 and rs7041 (D432E) were both weakly associated with total serum IgE ( $p < 0.02$  and  $p < 0.03$ , respectively)<sup>117</sup>.

### Ischemic stroke

Risk of ischemic stroke is associated with elevated levels of vascular inflammation<sup>118,119</sup> and lower 25(OH)D<sup>120</sup>, both of which are thereby linked to DBP. In six study populations from the United States, Europe and China (3550 cases, 6560 controls), a recent study evaluated 105 variants in 64 inflammation- and cardiovascular-related genes for association with ischemic stroke. Among the SNPs evaluated were DBP D432E and T436K, but neither was significantly associated<sup>119</sup>.

### Endometriosis

Endometriosis is a uterine disorder in which large overgrowths of endometrium accumulate inside or adjacent to the uterus<sup>121</sup>. The precise etiology remains unclear, but one theory holds that the peritoneal epithelia can differentiate into endometrial tissue as a result of chronic inflammation<sup>122</sup>. Furthermore, macrophage activation has been associated with disease progression<sup>123</sup>, thereby highlighting a potential role for DBP. In a recent cross-sectional study of premenopausal women undergoing laparoscopy, analysis of specific *Gc* allele products using nano-scale liquid chromatography-electrospray ionization-mass spectrometry indicated that the *Gc-2* allele product was enriched in serum samples in women with endometriosis ( $p = 0.006$ )<sup>124</sup>. The authors speculate that the lack of activated macrophages' phagocytic function in those *Gc2* carriers may increase the risk for implantation of endometriosis tissue in the peritoneal cavity. Further investigation of this hypothesis is warranted.

### Inflammatory bowel disease

Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis, both of which are characterized by chronic inflammation of the gastrointestinal tract<sup>125</sup>. Data collected as part of the Swiss Inflammatory Bowel Disease Cohort Study was used to test for associations between common DBP polymorphisms and risk of IBD<sup>125</sup>. Homozygotes for the K allele of T436K SNP were more common in non-IBD controls than in IBD patients ( $p = 0.006$ ). Significant associations were observed between SNP variants and disease risk in separate comparisons of healthy controls with ulcerative colitis ( $p = 0.022$ ) and Crohn's disease patients ( $p = 0.016$ )<sup>125</sup>. The *DBP-2* haplotype (consisting of D432-436 K) was found at higher frequencies in healthy controls than in IBD cases (OR = 2.53, CI = 1.48–4.34,  $p = 0.0005$ ), especially when comparing healthy controls to the ulcerative colitis cases (OR = 4.39, CI = 1.87–10.31,  $p = 0.0003$ ).

### Cancer

Several observational studies have demonstrated an inverse relationship between UVB irradiance (required for the



cutaneous synthesis of vitamin D) or vitamin D status [serum 25(OH)D concentrations] and the incidence of mortality from endometrial<sup>126</sup>, lung<sup>127,128</sup>, breast<sup>127,129–133</sup>, prostate<sup>134–137</sup>, ovarian<sup>138,139</sup> and colorectal cancer<sup>140–149</sup>. The underlying mechanism by which vitamin D status affects cancer risk is unknown, however, 1,25(OH)<sub>2</sub>D<sub>3</sub> has been shown to modulate cell proliferation and differentiation of both normal and malignant cells. Alternatively, inflammatory modulation effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> or Gc-MAF may play important roles in cancer pathogenesis.

### Breast cancer

Ecological case-control studies indicate an inverse correlation between vitamin D status and breast cancer risk, however, the relationship is less clear in longitudinal studies<sup>150,151</sup>. Much work has been done to elucidate a vitamin D-related mechanistic role in breast cancer. Gc-MAF exhibits anti-breast tumor activities in mice<sup>152</sup> and shows immunotherapeutic properties in metastatic breast cancer patients<sup>153</sup>. Exposure to Gc-MAF significantly reduces vimentin expression in human breast cancer cells, suggesting a reversal of breast cancer progression<sup>154</sup>. Alternatively, the active metabolite of vitamin D, calcitriol, has been shown to inhibit cell proliferation and differentiation and promote apoptosis of breast tumor tissue<sup>155–158</sup>.

Genetic studies examining the relationship between functional DBP variants and breast cancer (*in situ* and invasive) have yielded inconsistent findings. In a nested case-control study derived from the Cancer Prevention Study II Nutrition Cohort (500 cases, 500 controls), D432E and T436K were not associated with postmenopausal breast cancer risk<sup>159</sup>. Interestingly, a German population-based case-control study comprised of 1,402 post-menopausal women with *in situ* or invasive breast cancer and 2608 population controls reported a protective effect of *Gc2-2* carriers (OR = 0.72, 95% CI = 0.54–0.96)<sup>160</sup>. Both these studies argue against a role for Gc-MAF or vitamin D in breast cancer pathogenesis. In contrast, subjects with the rs7041 TT genotype were at increased risk of breast cancer (OR = 1.23; 95% CI = 1.01–1.51) based on a study of 1560 invasive breast cancer patients and 1633 controls from the Ontario Women's Diet and Health Study<sup>161</sup>. Altogether, these findings underscore the complex pathogenesis of breast cancer and highlight the need to consider gene-environment interactions.

### Prostate cancer

Studies have shown that vitamin D can inhibit proliferation and differentiation of human prostatic cells *in vitro*<sup>162</sup> and 1,25(OH)<sub>2</sub>D<sub>3</sub> has marked anti-tumor effects in animal models<sup>163</sup>. Furthermore, recent work has demonstrated that Gc-MAF directly inhibits proliferation and migration of prostate cancer cells as well as expression of a tumor metastasis-associated gene, urokinase plasminogen activator receptor<sup>164</sup> consistent with an early study demonstrating the elevated risk of *Gc2* genotype and carcinoma of the prostate<sup>165</sup>. However, in a comprehensive study of 749 cases and 781 controls from the PLCO Cancer Screening Trial, Ahn et al. found no association between risk of prostate cancer and 15 SNPs in *GC*, which included D432E and

T436K<sup>166</sup>. An earlier study examining D432E and T436K in 181 prostate cancer patients (90 black and 91 white) and sex-, age- and race-matched controls also found no differences in allelic/genotypic frequencies between patients and controls; however, frequencies were strikingly different between blacks and whites<sup>167</sup>.

### Gastrointestinal and colorectal cancer

In a well established colorectal cancer cohort (Colon Cancer Family Registry), Poynter et al. explored previous epidemiological evidence suggesting a reduced risk associated with vitamin D. No evidence for association between DBP genotype and risk of colorectal cancer was found, although associations between DBP SNPs and microsatellite-unstable colorectal cancer were reported<sup>168</sup>. A more recent study examining associations between polymorphisms in the *GC* and *CASR* genes similarly found no associations in any of the tested *GC* SNPs<sup>169</sup>.

Zhou et al.<sup>170</sup> examined associations between variants of D432E (rs7041) and T436K (rs4588) and risk of four types of gastrointestinal cancers (hepatocellular, esophageal, gastric and colorectal) in a Han Chinese population. An elevated risk for colorectal cancer was observed in individuals homozygous for the 436 K allele, in comparison to individuals homozygous for the wildtype (OR = 3.41; CI = 1.85–6.57, *p* < 0.001). When all four types of cancer were combined in the analysis, the 436 K homozygotes had 1.15-fold increased risk over wildtype (CI = 1.02–1.30, *p* = 0.020) after adjustment for age, sex and smoking status. *Gc2* allele carriers also had an increased risk for developing gastrointestinal cancer (OR = 1.22; CI = 1.04–1.39, *p* = 0.015)

### Skin cancer

Associations between *GC* SNPs and basal cell carcinoma (BCC) were examined as part of the prospective cohort Rotterdam Study (*n* = 7983). The study found no significant association between the *GC* genotypes and haplotypes and risk of developing at least one BCC. However, *Gc1F* carriers were more likely to develop a first BCC compared to non-carriers (HR = 1.40; 95% CI = 1.11–1.78). When stratified by age, individuals younger than 65 years of age and homozygous for *Gc1S* had about half the risk of developing a first BCC, compared to non-carriers (HR = 0.53; CI = 0.31–0.91)<sup>171</sup>.

### Neurodegenerative disorders

#### Multiple sclerosis

Multiple sclerosis (MS) is a form of disseminated encephalomyelitis that appears to be partly autoimmune in origin though its etiology is not known. The rates of MS exhibit a pattern of increasing prevalence with increasing latitude, indicating a protective role of ultraviolet radiation in the development of MS<sup>172</sup>. Moreover, several studies have shown an inverse correlation between serum levels of vitamin D and MS<sup>173,174</sup>, suggesting that polymorphisms influencing vitamin D status might contribute to the initiation or progression of disease.

Serum DBP was found to be upregulated along with other acute phase reactive proteins in a small sample (*n* = 9) of

pediatric MS patients<sup>175</sup>, however, the clinical relevance is not known. Early reports examining the distribution of DBP phenotypes among European adults did not find significant correlations with presence of disease, disease natural history or age of onset<sup>176,177</sup>. Similarly, in 107 Japanese MS patients and 109 controls, neither DBP phenotypes nor D432E/T436K genotypes (examined independently) were associated<sup>173</sup>, a finding that was recently confirmed<sup>178,179</sup>. To control for population admixture/stratification, both SNPs were evaluated in a family-based design study of 187 Canadian families; however, the lack of association between genotype and disease was unchanged<sup>174</sup>. Ongoing interventional trials with vitamin D and association studies with larger cohorts may offer further opportunity to explore an interactive role of DBP SNPs with vitamin D in MS.

#### *Amyotrophic lateral sclerosis*

Amyotrophic lateral sclerosis (ALS) is another neuropathy of unknown etiology that is characterized by the progressive loss of motor neurons in the brain and spinal cord, leading to debilitating, usually fatal, muscle atrophy<sup>180</sup>. While the vast majority of ALS patients are sporadic, 10–15% of cases show familial clustering. Mutations in the cytosolic superoxide dismutase (*SOD1*) gene have been associated with ALS, but these account for less than 20% of all familial ALS (FALS)<sup>181</sup>. In Portuguese patients with FALS, proteomic analysis showed that the *Gc-2* phenotype (436K) was overrepresented in comparison to healthy controls<sup>182</sup>. The role of DBP in ALS is not known, but it is tempting to speculate that motor neuron damage resulting in the systemic release of actin and ensuing intravascular coagulation and local hypoxia-induced oxidative damage may be mitigated by the actin-scavenging properties of DBP. Whether the apparently reduced capacity of *Gc-2* for actin-scavenging, compared with *Gc1*<sup>30,34,35</sup>, might increase the likelihood of disease progression is not known.

#### *Schizophrenia*

A number of hypotheses have been put forth to explain the etiology of schizophrenia, but several lines of observational evidence suggest that vitamin D may be a risk factor<sup>183</sup>. The underlying molecular mechanism governing this association is not known, but prenatal vitamin D deficiency is associated with structural and functional deficits common to schizophrenic patients. Several early reports examined DBP phenotypes in relation to schizophrenia. An excess of *Gc1-1* was observed in cases over controls in a study carried out in a German study ( $p < 0.01$ )<sup>184</sup>. This association was even more compelling for the hebephrenic subgroup ( $p < 0.01$ ). On the other hand, analysis of a north east England cohort comparing 215 schizophrenic patients with healthy first-degree relatives or non-familial controls reported a significant reduction in *Gc1S* alleles ( $p = 0.025$ ) largely within the female subjects<sup>185</sup>. Findings from a Chinese male cohort of 423 schizophrenic patients also suggested a relative paucity of *Gc1S* ( $p < 0.001$ ) and an excess of *Gc2*<sup>186</sup>. However, there are several other studies reporting lack of association between DBP and schizophrenia<sup>187–189</sup>, and firm conclusions would be premature.

#### *Parkinson disease*

Parkinson disease is a progressive movement disorder, the severity of which has been found to be inversely associated with serum levels of 25(OH)D<sup>190</sup>. However, a study examining *GC* polymorphisms and severity of Parkinson disease did not identify any significant associations<sup>191</sup>.

#### **Other chronic diseases**

##### *Liver disease*

In liver disease, secretion of proteins belonging to the albumin family is usually reduced, and it is therefore not surprising that there are numerous studies reporting lower serum DBP concentrations in this group of disorders. This may also be associated with inability to sufficiently sequester cellular actin released by the dying hepatic cells themselves, thereby contributing to an increased risk of intravascular coagulation. Variants associated with DBP status might therefore be useful as biomarkers of worse outcomes in liver disease. Looking at 17 alcoholic liver cirrhosis patients and 100 healthy controls, French investigators observed an unusual electrophoretic form of non-ligand bound DBP that was characterized by the presence of an additional sialic acid moiety. This form of DBP was found only in *Gc-1* carriers and was associated with worse clinical outcome<sup>35</sup>. To date, this novel phenotype has not been reported in other studies and, therefore, the larger relevance in non-hepatic disorders is unknown.

##### *Sarcoidosis*

Sarcoidosis, a granulomatous inflammatory disease, typically affects young adults and is associated with altered immunoglobulin and increased 1,25(OH)<sub>2</sub>D<sub>3</sub> production. DBP phenotypes were examined in a small case-control study of 88 patients, but found no relationship between disease, course of disease, or presentation<sup>192</sup>. Whether 1,25(OH)<sub>2</sub>D<sub>3</sub> levels might be associated with the D432E or T436K genotypes, as others have observed, was not studied.

##### *Thyroid autoimmune disease*

Graves' disease and Hashimoto thyroiditis are the two common forms of autoimmune thyroid disease. In such disorders, 1,25(OH)<sub>2</sub>D<sub>3</sub> downregulates expression of thyrocyte-derived human leukocyte antigen class II molecules<sup>193</sup> and inhibits secretion of proinflammatory cytokines and lymphocyte proliferation<sup>194</sup>. In 95 and 92 Western European pedigrees positive for Graves' disease and Hashimoto's thyroiditis, respectively, Pani et al. looked at TDT of the *DBP* gene<sup>195</sup>. Only the *Alu* repeat in intron 8 (*I8\*8*) was found to be associated with Graves' disease ( $p < 0.03$ ), and none were associated with Hashimoto's thyroiditis. Neither D432E nor T436K was associated with disease outcome. In contrast, association of *GC* SNPs with Graves' disease was found in a case-control study evaluating 332 cases and 185 healthy controls of Polish origin. In that study, cases were significantly enriched for the T436K heterozygotes (OR = 1.50; CI = 1.13–1.99;  $p = 0.005$ )<sup>196</sup>.

## Infectious diseases

### Tuberculosis

*Mycobacterium tuberculosis*, the causative agent of tuberculosis, infects a third of the world's population and results in 3 million deaths per year<sup>197</sup>. Low serum levels of 25(OH)D have been correlated with extant tuberculosis<sup>198–204</sup> and incident infection<sup>204</sup>. Oral supplementation of vitamin D given to tuberculosis contacts enhanced immunity towards the mycobacterium<sup>205</sup>. A significant advance in our understanding of innate immunity against tuberculosis came with a seminal report showing convergence of the vitamin D metabolic pathway with the Toll-like receptor (TLR2/1) signaling cascade resulting in the induction of cathelicidin, a potent antimicrobial peptide in the innate immunity pathway<sup>54,55</sup>. Subsequently, *Gc* genotypes were examined in several ethnically diverse tuberculosis study populations<sup>206</sup>. In Gujarati Asians, the homozygous variant *Gc2-2* phenotype was strongly associated with susceptibility to active tuberculosis, compared with *Gc1-1* using logistic regression to adjust for age and sex (OR = 2.81, CI = 1.19–6.66,  $p = 0.009$ ). Furthermore, this association was observed only under conditions of low 25(OH)D, suggesting an important gene–environment interaction in this condition.

### Rheumatic fever

Rheumatic fever is a systemic inflammatory illness characterized by excessive B cell activity leading to an overproduction of antibody to Group A streptococcus. DBP is found on the surface of B-cells, often in a molecular complex with actin and surface immunoglobulin, and may play a role in B cell activation by facilitating signal transduction activity<sup>207</sup>. Alternatively, inflammatory mediation of DBP may affect the course of disease. In a study of 39 cases and 90 controls of Arab ancestry, a positive association between the *Gc-2* allele and rheumatic fever was observed ( $p = 0.0024$ )<sup>208</sup>. The implications of this finding are not altogether clear, but suggest that the anti-inflammatory properties of *Gc-2* may not be important in this disorder.

### Human immunodeficiency virus

Untreated, human immunodeficiency virus (HIV) infection often progresses to acquired immunodeficiency syndrome (AIDS) with its subsequent complications of opportunistic infection and neoplasia. Macrophages are among the first cell types to be infected by HIV-1 and serve as reservoirs for the virus in affected persons<sup>209,210</sup>. Macrophage activation via Gc-MAF may therefore be an important factor in the acquisition of HIV-1 infection and progression to AIDS. Indeed, immunotherapy of HIV-infected individuals with GcMAF appeared to be significantly curative<sup>211</sup>. Several studies have examined the association between *DBP* variants and HIV/AIDS.

DBP phenotypes were examined in a cohort consisting of 86 HIV negative hospital workers, 351 homosexual men that were either HIV negative (as determined by the absence of HIV antibodies) or HIV positive (as determined by the presence of HIV antibodies with or without HIV antigen), and 96 AIDS cases<sup>212</sup>. It is thought that the presence of HIV

antigen is a strong predictive marker of disease progression<sup>213–215</sup>. During the course of this study, a subset of 62 HIV negative homosexual men seroconverted. No significant difference in DBP distribution was observed between HIV negative heterosexuals, HIV negative homosexuals and patients with AIDS. Moreover, there was no difference in DBP phenotype distribution between homosexual men who remained HIV negative and those who sero-converted during follow-up, arguing against the involvement of DBP in susceptibility to HIV infection. Finally, DBP phenotypes were equally distributed among (i) HIV-positive homosexuals without the presence of HIV-antigen, (ii) HIV positivity with the presence of HIV antigen and (iii) AIDS – effectively excluding a role for DBP in either susceptibility or progression. Other investigators examining Spanish, German and Swedish populations also found no evidence of association between DBP variants and susceptibility to HIV/AIDS<sup>216–218</sup>. However, an excess of *Gc2* was observed in persistently seronegative sexual contacts (engaging in unprotected sex) of AIDS patients, although the sample size was small<sup>219</sup>.

In summary, there is little evidence to support a role for DBP in susceptibility to HIV/AIDS or progression to AIDS, based on these studies. The interplay between HIV and DBP, however, is complex<sup>211</sup> and likely confounds any true associations between genetic variation and infection/disease outcome. Investigators may choose to focus on identifying new genetic variants in or near the *Gc* gene that may provide further insight into a molecular mechanism bridging DBP and HIV/AIDS.

## Discussion

We present an overview of studies examining the association of genetic/phenotypic variants of *Gc* and adverse health outcomes, several of which constitute global health problems of major public health importance. It is becoming increasingly evident that the DBP plays an important role in inflammation and immunity, and that genetic variants encoding this protein may, in part, modulate these pathophysiological pathways. Despite recent progress, the nature of this regulation remains unclear. In comparison to *Gc-2* (436 K) types, non-*Gc-2* polymorphisms are associated with increased macrophage activity and elevated vitamin D serum levels required for downstream upregulation of antimicrobial peptides<sup>220</sup>. While it is tempting to speculate that Gc1-related macrophage activity results in prolonged inflammation associated with chronic diseases<sup>61</sup>, binding of active 1,25(OH)<sub>2</sub>D<sub>3</sub> metabolite to its intracellular receptor (VDR) is anti-inflammatory<sup>52,221</sup>. Included amongst those actions is the negative regulation of nuclear factor kappa B (NFκB), an essential component of the inflammatory response by recruitment of histone deacetylase 3<sup>52,222</sup>.

It should be noted that there are inherent limitations in some association studies that are related not to sample size but to intrinsic heterogeneity of disease phenotype. A case in point is schizophrenia, a disorder that is notoriously difficult to classify and has proven quite resistant to confirmation of initially promising results<sup>223</sup>. Candidate gene studies looking at DBP in this area are no exception.

Further work is needed to characterize more precisely the role of DBP in chronic and infectious disease, however,



current evidence from genotype-phenotype association studies does indicate an important role in innate-immune-related inflammation.

## Conclusion

There is growing evidence that DBP plays a role in immune modulation, either directly through Gc-MAF, or indirectly by influencing serum levels of vitamin D. It is tempting to speculate that DBP operates as a molecular switch between pro- and anti-inflammation and intervention strategies designed to modify this switch may be important in the control and prevention of chronic and infectious diseases. Nevertheless a precise understanding of the role of DBP in chronic and infectious diseases is still lacking. Correlations between functional DBP variants and adverse health outcomes have been difficult to interpret, in part because there is still much about the biology of DBP that is not well understood. Furthermore, many of the association studies were insufficiently powered and failed to account for multiple comparisons. A greater effort is therefore needed to clarify the relevant gene-gene and gene-environment interactions. Given that *GC* gene is so polymorphic, it is reasonable to consider that other SNPs in this gene may be more relevant to the disease outcomes being studied, but they have yet to be formally examined. It is becoming clear that DBP is indeed a multifunctional protein with an important auxiliary role in the relationship between vitamin D and innate immunity. The various discordant findings underscore the need for more robust genetic studies and better characterization of just how this protein functions.

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