Letter to the Editor

Vitamin D binding protein: A polymorphic protein with actin-binding capacity in COVID-19

To the Editor:

With interest, we read the paper of Katz et al. [1], which demonstrated a strong association between vitamin D deficiency and COVID-19, even after adjusting for sex, malabsorption, dental diseases, race, diabetes mellitus, and obesity. Besides the mentioned confounders, we would like to illustrate the potential influence of polymorphic vitamin D binding protein (DBP) with actin-binding capacity on the reported findings.

DBP or group-specific component (Gc-globulin), a member of the albuminoid family, was discovered by Hirschfeld in 1959 [2]. The human *DBP* gene is located on chromosome 4q12-q13 and is characterized by >120 variants, based on electrophoretic properties. The three most common variants are DBP1F (rs7041-T/rs4588-C), DBP1S (rs7041-G/rs4588-C), and DBP2 (rs7041-T/rs4588-A). The single nucleotide polymorphisms are in complete linkage disequilibrium, and only six haplotypes are observed with any significant frequency. The DBP polymorphism presents distinct racial distribution patterns. A common feature of all populations is the less predominance of the DBP2 allele, in comparison with the DBP1 allele. White populations carry more frequently the DBP1S and the DBP2 allele, whereas black and Asian populations are more likely to exhibit the DBP1F, with a rare presence of the DBP2 allele [3]. The major function of DBP is the transport of vitamin D metabolites in the circulation, with ~88% binding of serum 25-hydroxyvitamin D [25(OH)D] and 85% of 1,25-dihydroxyvitamin D [1,25(OH)$_2$D]. In comparison, albumin, with its substantially lower binding affinity, binds only ~12% to 15% of these metabolites, despite its 10-fold higher serum concentration than DBP. Approximately 0.03% of total 25(OH)D and 0.4% of total 1,25(OH)$_2$D are present in a free form in the serum of normal non-pregnant individuals [4]. Total and free 25(OH)D concentrations, as well as serum DBP concentrations, are affected by the DBP phenotypes, with the lowest concentrations in the DBP2−2 haplotype, which could partly explain why certain patients are more prone for COVID-19 [5,6]. Investigating the influence of the DBP phenotypes on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in 55 countries, we have demonstrated an association between the DBP1 allele frequency and a lower prevalence of and mortality due to COVID-19 [3]. The association between the DBP polymorphism and SARS-CoV-2 infection could be partly attributed to the potential protective effects of vitamin D, as DBP1 carriers have higher plasma vitamin D concentrations than patients with a

DBP2 allele. Additionally, the lower serum DBP concentrations in the DBP2 group could also be a point of attention as lower DBP levels are associated with septic shock mortality [7]. DBP is not only the major transport protein of vitamin D metabolites but exerts several other important functions, including actin scavenging, binding of fatty acids, chemotaxis, binding of endotoxins, influence on T cell response, and influence of vitamin D binding protein-macrophage activating factor (DBP-MAF). More specifically, we would like to highlight the actin scavenger function of DBP in the pathogenesis of COVID-19 [8].

Focusing on the cytoskeleton, in one round of viral life cycle, coronaviruses promote actin filament polymerization to provide forces for egress [9]. In catabolic conditions (e.g., sepsis and acute respiratory distress syndrome), tissue damage leads to the release of globular actin (G-actin) to the extracellular environment and the circulation. The accumulation of G-actin in the bloodstream results in polymerization and formation of filamentous actin (F-actin), which contribute to pulmonary microthrombi, pulmonic vessel obstruction, and endothelial damage [10]. As demonstrated by immunofluorescence microscopy, the barrier integrity of primary human pulmonary microvascular endothelial cells is disrupted after exposure to plasma from severe COVID-19 patients as compared with healthy controls. More specifically, a loss of junctional VE-cadherin and cortical actin is observed, with the formation of actin stress fibers and interendothelial gap formation [11]. Actin activates platelets, which further increases the risk for thrombi formation and obstruction of the microcirculation (particularly in the lungs), a phenomenon that is frequently observed in patients with severe COVID-19 [12]. The extracellular actin scavenger system is composed of two plasma proteins with complementary functions: gelsolin that severs F-actin filaments into G-actin monomers, which are bound subsequently by DBP in a high affinity (Kd of 10^{-9} M) 1:1 molar complex for transport and clearance primarily in the liver. In COVID-19, DBP could act as a scavenger protein to clear extracellular G-actin released from necrotic cells, which may be of relevance in severe acute lung injury [13]. However, it should be mentioned that the formation of DBP-actin complexes is a double-edged sword. In a mice model, it was demonstrated that elevated concentrations and/or prolonged exposure to DBP-actin complexes may induce endothelial cell injury and death, particularly in the lung microvasculature [14].

In conclusion, the observed association between vitamin D deficiency and the risk for COVID-19 may be influenced by the DBP polymorphism. The higher risk for and worse prognosis after SARS-CoV-2 infection in individuals with a DBP2 haplotype may be attributed to lower serum vitamin D and DBP levels. We suggest including this parameter in future studies exploring the potential role of vitamin D in the COVID-19 pandemic.

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