ISSN 0120-4157

Biomédica

Revista del Instituto Nacional de Salud

PUBLICACIÓN ANTICIPADA EN LINEA

El Comité Editorial de *Biomédica* ya aprobó para publicación este manuscrito, teniendo en cuenta los conceptos de los pares académicos que lo evaluaron. Se publica anticipadamente en versión pdf en forma provisional con base en la última versión electrónica del manuscrito pero sin que aún haya sido diagramado ni se le haya hecho la corrección de estilo.

Siéntase libre de descargar, usar, distribuir y citar esta versión preliminar tal y como lo indicamos pero, por favor, recuerde que la versión impresa final y en formato pdf pueden ser diferentes.

Citación provisional:

Silverio CS, Bonilla C. Vitamin D-associated genetic variants in the Brazilian population: investigating potential instruments for Mendelian randomization. Biomédica. 2024;44 (1).

Recibido: 03-04-23

Aceptado: 29-01-24

Publicación en línea: 30-01-24

Vitamin D-associated genetic variants in the Brazilian population: investigating potential instruments for Mendelian randomization

Variantes genéticas asociadas con la vitamina D en la población brasileña: investigación de potenciales instrumentos para aleatorización mendeliana

Vitamin D-associated genetic variants in the Brazilian population

Caroline de Souza Silverio [1,2], Carolina Bonilla [1]

[1] Departamento de Medicina Preventiva, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

[2] Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, São Paulo, Brazil

Corresponding author:

Carolina Bonilla, Departamento de Medicina Preventiva, Faculdade de Medicina, Universidade de São Paulo, Av. Dr. Arnaldo 455, São Paulo, CEP 01246-903, Brazil

Phone: +55 11 3061 8610

cxbonilla@usp.br

Author contributions:

Caroline de Souza Silverio: SNP search, article search, table creation, writing the manuscript.

Carolina Bonilla: design, conceptualization, writing the manuscript.

Introduction. Vitamin D is required for bone and mineral metabolism, and participates in the regulation of the immune response. It has also been linked to a number of chronic diseases and conditions, usually in populations of European descent. A high prevalence of vitamin D deficiency and insufficiency has been reported in Brazil, despite the widespread availability of sunlight in the country. Thus, it is important to investigate the role of vitamin D as a risk factor for disease and to establish causal relationships between vitamin D levels and health-related outcomes in the Brazilian population.

Objective. To examine genetic variants identified as strong determinants of serum vitamin D in genome-wide association studies (GWAS) of Europeans and check whether the same associations are present in Brazil. If so, these SNPs could be developed locally as proxies to use in genetically-informed causal inference methods, such as Mendelian randomization.

Materials and methods. SNPs associated with vitamin D levels were extracted from the GWAS catalog. A literature search was then run to select papers ascertaining these variants and vitamin D concentrations in Brazil.

Results. *GC* was the gene with the strongest association with vitamin D levels, in agreement with existing findings in Europeans. However, *VDR* was the most investigated gene, in spite of it not being associated with vitamin D in GWAS.

Conclusions. More research is needed to validate sound proxies for vitamin D levels in Brazil, for example, prioritizing *GC* rather than *VDR*.

Keywords: vitamin D; genome-wide association study; polymorphisms, single nucleotide; vitamin D-binding protein; vitamin D3 24-hydroxylase; 25-hydroxyvitamin D3 1-alpha-hydroxylase; Brazil.

Introducción. La vitamina D es necesaria para el metabolismo óseo y mineral, y participa en la regulación de la respuesta inmunitaria. También se la ha relacionado con enfermedades crónicas, normalmente en poblaciones europeas. En Brasil existe una elevada prevalencia de deficiencia e insuficiencia de vitamina D, a pesar de la amplia disponibilidad de luz solar. Por lo tanto, es importante investigar el papel de la vitamina D como factor de riesgo de enfermedades y establecer relaciones causales entre los niveles de vitamina D y los problemas de salud en la población brasileña.

Objetivo. Examinar variantes genéticas fuertemente asociadas a la vitamina D sérica en estudios de asociación genómica (GWAS) de europeos y comprobar si las mismas asociaciones estaban presentes en Brasil. De ser así, estos SNP podrían utilizarse como proxies en métodos de inferencia causal, tales como la aleatorización mendeliana.

Materiales y métodos. SNPs asociados con los niveles de vitamina D fueron extraídos del catálogo de GWAS. Luego se realizó una búsqueda bibliográfica para identificar artículos que evaluaran estos SNPs y la concentración de vitamina D en Brasil.

Resultados. *GC* fue el gen más fuertemente asociado con los niveles de vitamina D, en concordancia con los resultados existentes en europeos. Sin embargo, el gen *VDR* fue el más investigado, pese a no estar asociado con la vitamina D en GWAS.

Conclusiones. Se necesita más investigación para validar proxies genéticos de los niveles de vitamina D en Brasil, y se recomienda priorizar *GC* en lugar de *VDR*.

Palabras clave: vitamina D; estudio de asociación del genoma completo; polimorfismos de nucleótido simple; proteína de unión a la vitamina D; vitamina D3 24-hidroxilasa; 25-hidroxivitamina D3 1-alfa-hidroxilasa; Brasil.

Vitamin D is a steroid hormone and a fat-soluble vitamin that is required by the human body for physiological bone and mineral metabolism (1), and plays a role in the regulation of the immune response (2), among other functions. When vitamin D levels are low, its insufficiency or deficiency may contribute to various adverse health outcomes, from skeletal disorders such as rickets and osteomalacia, to extraskeletal conditions like cancer, infections, and cardiovascular, autoimmune and neuropsychiatric diseases (3), although evidence of a causal effect is still scarce for many of these health problems.

The main source of vitamin D is sunlight. Pre-vitamin D_3 is converted from 7-dehydrocholesterol by ultraviolet radiation (UVR) B in the skin, and then transported to the liver and other tissues where it is metabolized to 25-hydroxy-vitamin D (25OHD), the major circulating form, by the enzyme CYP2R1. 25OHD is then further metabolized to 1,25 dihydroxy-vitamin D ($1,25(OH)_2D$), primarily in the kidney, by the enzyme CYP27B1. $1,25(OH)_2D$ is the active metabolite of vitamin D, responsible for most of its biological actions, which are achieved via binding to a specific nuclear vitamin D receptor (VDR) and eliciting the transcriptional regulation of target genes. The inactivation and catabolism of 25OHD and $1,25(OH)_2D$ are carried out by the enzyme CYP24A1, whereas circulation in the blood stream of pre-vitamin D_3 and vitamin D metabolites occurs by means of the vitamin D binding protein (VDBP) and albumin (4).

The US Endocrine Society has defined concentrations of 25OHD above 30 ng/ml as sufficient, between 20 and 30 ng/ml as insufficient, and below 20 ng/ml as deficient vitamin D levels, or their equivalent in nmol/L (1 ng/ml=2,5 nmol/L). Cut-off values may differ between studies depending on whether they follow the recommendations of the US Endocrine Society, the US Institute of Medicine,

which uses 12 ng/ml and 20 ng/ml as the thresholds for deficiency and sufficiency, respectively, or the UK Scientific Advisory Committee, which set a level below 10 ng/ml to establish vitamin D deficiency (1,5). The proposed minimum thresholds are defined by criteria that include the suppression of parathyroid hormone (PTH) secretion, increased calcium absorption, good musculoskeletal health and reduction in fractures and falls (1).

Identifying causal associations of vitamin D with disease using observational methods can be difficult because of confounding and other biases that often afflict these studies. Mendelian randomization (MR) has been devised as a tool to improve causal inference in epidemiology by employing genetic variants strongly associated with an exposure, known in this context as instrumental variables, which are unlikely to suffer the same observational biases (6). MR has become quite popular in the past decade, clarifying cause and effect relationships between many risk factors and disease outcomes (7). However, this success has been largely limited to populations of European descent, where most research is carried out. For MR to be effectively applied in Brazil (and other non-European populations) it is crucial to select genetic variants that are instrumental variables for exposures in the local populations.

For that reason, we investigated single nucleotide polymorphisms (SNPs) strongly associated with vitamin D in circulation, initially detected in Europeans, to assess whether they can be used as proxies for vitamin D in the Brazilian population to determine causal relationships between vitamin D levels and chronic diseases using MR.

Materials and methods

SNPs associated with 25OHD (from now on, vitamin D) levels in blood were identified using the publicly available GWAS catalog (8). A list of the top ~30 SNPs, and the genes where they are located, most strongly associated with vitamin D (with p value < $5x10^{-8}$) was generated. From this list, scientific papers reporting on the SNPs and/or genes in relation to vitamin D concentration in the Brazilian population were sought from the databases PubMed (9), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS (10)), Scopus (11), Scientific Electronic Library Online (SciELO (12)), and Biblioteca Digital Brasileira de Teses e Dissertações (BDTD (13)). The search was carried out using the SNP rs code or the name of the gene where the SNP lies in, together with the terms "Brazil" and "vitamin D". In addition, SNPs located in the vitamin D receptor (*VDR*) gene, which has been extensively studied in populations across the world, were included in the search. We selected studies where the association of genotypes with circulating vitamin D was ascertained, and the reporting had been made in English, Portuguese or Spanish.

Information on the SNP effect on vitamin D levels, the effect allele, allele frequencies, sample size, prevalence of vitamin D deficiency and insufficiency, % female, mean age, % White ethnicity, type of study, Hardy-Weinberg equilibrium test, adjustment for population stratification, and target population was extracted from the chosen papers.

Results

Twenty-eight SNPs strongly associated with vitamin D in blood, mainly in European populations, were obtained from the GWAS catalog (table 1). Eighteen extra SNPs in the *VDR* gene were additionally considered (table 2).

GC vitamin D binding protein gene (GC)

GC is located on chromosome 4q13.3 and encodes for the VDBP. Nine SNPs in this gene were amongst the 28 variants most robustly associated with serum vitamin D in previous GWAS (i.e. rs11723621, rs1352846, rs145432346, rs222020, rs2282679, rs3755967, rs3775150, rs4588, rs7041), of which only rs2282679, rs4588 and rs7041 were analyzed in the Brazilian population (Supplementary table 1). We found a total of 6 published studies in Brazil, three in Porto Alegre, the capital of the state of Rio Grande do Sul (RS), and one each in the states of Rio de Janeiro (RJ), Paraná (PR) and São Paulo (SP). The target populations were diverse and involved women of reproductive age, university civil servants and individuals affected by chronic diseases such as hepatitis C and cirrhosis, but their minor allele frequencies were quite similar (table 3).

Overall, we uncovered evidence of association of the *GC* gene with vitamin D concentrations in Brazil, with the rs4588 A allele, the rs7041 T allele and rs2282679 C allele underlying lower vitamin D levels.

Vitamin D receptor gene (VDR)

Despite not being one of the genes identified in earlier GWAS as associated with vitamin D levels, *VDR* has been examined in numerous human groups, often in studies conducted before the GWAS era. Our search of the literature discovered 12 publications that assessed circulating vitamin D in relation to *VDR* genotypes in Brazil (Supplementary table 2). The SNPs rs1544410 (G/A), rs2228570 (C/T), rs731236 (T/C), rs7975232 (T/G), which were formerly detected using restriction enzymes Bsm I, Fok I, Taq I and Apa I, respectively, were ascertained in most analyses, encompassing a variety of populations

across the country (table 4). However, unlike what was observed with *GC*, results were inconsistent, with respect to finding an effect (or not) and on the direction of that effect. For instance, while the A allele of SNP rs1544410 was associated with lower levels of vitamin D in young children from Acre (14), it was found to increase vitamin D in girls from south Brazil (15). The C allele at SNP rs731236 was reported in association with higher serum vitamin D in girls from south Brazil and pregnant women from Bahia, but appeared to have the opposite effect in type 1 diabetes patients from Pará state (16).

Other genes

Twenty-five SNPs in 8 genes other than *GC* and *VDR* were detected amongst the top predictors of vitamin D levels in the GWAS catalog, however, just 4 of these genes have been explored in Brazil (i.e. *CYP2R1*, *CYP24A1*, *CYP27B1, NADSYN1*) (Supplementary table 3). Several polymorphisms in *CYP2R1* and *CYP24A1* were associated with serum vitamin D and vitamin D insufficiency in a study of ~800 young people from deprived areas in Salvador, Bahia (17). In contrast, smaller studies investigating the same genes but different SNPs and populations did not find evidence of an effect (18-20).

Discussion

Despite the widespread availability of sunlight across Brazil, and UVR levels that ensure vitamin D synthesis in the skin (21), a high prevalence of vitamin D deficiency and insufficiency has been reported in numerous Brazilian studies (22). Since 2017 the Brazilian Society of Endocrinology and Metabology (SBEM) and the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML) recommend a 25OHD level equal or above 20 ng/ml for individuals up to 60 years old, and a range of 30 to 60 ng/ml for at-risk groups (23).

Considering other sources of vitamin D such as diet and supplementation, vitamin D intake in Brazil is limited, fortification of food products is not common, and there is infrequent use of vitamin D supplements (≤ 10%)(23). To that extent, the SBEM only recommends supplementation for specific groups at risk of deficiency, for example, pregnant and lactating women, individuals with osteoporosis, elderly people and patients with conditions that affect vitamin D metabolism (24).

In general, our findings showed limited local research on the genetic determinants of vitamin D levels, with a predilection towards investigating the *VDR* gene, but with sounder evidence accumulating on the effects of *GC*. This agrees with GWAS data indicating that *GC*, the gene that encodes for the binding protein, is amongst the strongest genetic predictors of vitamin D concentrations in European, Asian and African-ancestry populations (25-30). Conversely, a look-up of *VDR* in the GWAS catalog returned associations with a number of different traits, but not with vitamin D levels (Supplementary table 4). More research should be carried out in Brazil to confirm the role of *GC* (and clarify that of *VDR*), and to reveal other genetic variants robustly associated with serum vitamin D, since the identification of reliable proxies will allow us to reveal causal associations with disease and promote the use of appropriate polygenic risk scores for predictive purposes.

Additionally, we would like to suggest a few improvements to future studies and their reporting, especially if we are to employ them as the basis for meta-analyses. For example, it is important to describe all findings (significant and non-significant), and provide them as supplementary material if necessary, assess Hardy-Weinberg equilibrium and report its test results, and, given

Brazil's admixed genetic background, adjust for markers of population stratification or related variables (i.e, race/ethnicity, socioeconomic status) when these are not available.

Among the limitations of our own study there is still the chance that we have missed relevant publications that were not covered by our search parameters, or that SNPs associated with vitamin D in the GWAS catalog, outside the top 30, had in fact been analysed in Brazil, although this is rather unlikely. In addition, given the limited number of studies found and the heterogeneity of the samples included in them, it was not possible to run a meta-analysis to obtain an indication of the strength and direction of the effect of *GC* variants on the levels of vitamin D, making implementation of any action in clinical practice linked to our results unfeasible.

In conclusion, there is a lot of interest in vitamin D as a potential risk factor for several chronic diseases of public health impact, and therefore, it is essential to be able to identify causal relationships between vitamin D levels and disease outcomes. One way of improving causal inference would be to apply MR and use genetic variants to proxy or instrument the exposure (i.e. serum vitamin D), so that unbiased estimates of these relationships are obtained. However, the instruments should be appropriate for the study population, either having been discovered or validated locally. We noticed that there has not been enough research carried out in Brazil (or in South America) on vitamin D proxies, whilst the focus has been placed more on the study of *VDR* as a genetic risk factor for disease, which may or may not produce changes in circulating vitamin D.

Conflicts of interest

CB was an expert advisor on ancestry and diversity for the Global Health Equity Advisory Board of Roche/Genentech from March 2021 until March 2022. CdSS declares no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Financial support

CdSS was a recipient of a 2021 Programa Unificado de Bolsas fellowship from the University of São Paulo. CB receives support from the Brazilian National Council for Scientific and Technological Development (CNPq).

References

1. **Holick MF.** Optimal vitamin D status for the prevention and treatment of osteoporosis. Drugs Aging. 2007;24:1017–29. https://doi.org/10.2165/00002512-200724120-00005

2. **Charoenngam N, Holick MF.** Immunologic effects of vitamin D on human health and disease. Nutrients. 2020;12:2097. https://doi.org/10.3390/nu12072097

3. **Pilz S, Grübler M, Gaksch M, Schwetz V, Trummer C.** Vitamin D and mortality. Anticancer Res. 2016;36:1379–88.

4. **Bandera Merchan B, Morcillo S, Martin-Nuñez G, Tinahones FJ, Macías-González M.** The role of vitamin D and VDR in carcinogenesis: Through epidemiology and basic sciences. J Steroid Biochem Mol Biol. 2017;167:203–18. https://doi.org/10.1016/j.jsbmb.2016.11.020

5. **Vieth R, Holick MF.** The IOM—Endocrine Society controversy on recommended vitamin D targets. In: Vitamin D: Volume One: Biochemistry,

Physiology and Diagnostics. 4th Edition. Academic Press; 2018. p. 1091–107. https://doi.org/10.1016/B978-0-12-809965-0.00059-8

6. **Davey Smith G, Ebrahim S.** 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?*. Int J Epidemiol. 2003;32:1–22. https://doi.org/10.1093/ije/dyg070

7. **Bennett DA, Du H.** An overview of methods and exemplars of the use of Mendelian randomisation in nutritional research. Nutrients. 2022;14:3408. https://doi.org/10.3390/nu14163408

8. **Global Core Biodata Resource.** GWAS Catalog. Consulted: October 1, 2021. Available: https://www.ebi.ac.uk/gwas/

9. **National Library of Medicine.** PubMed. Consulted: January 10, 2022. Available: https://pubmed.ncbi.nlm.nih.gov/

10. **Panamerican Health Organization**. LILACS, scientific health information from Latin America and the Caribbean countries. Consulted: January 10, 2022. Available: https://lilacs.bvsalud.org/

11. **Elsevier B. V.** Scopus. Consulted: January 10, 2022. Available: https://www.scopus.com/

12. **Red SciELO.** Scielo, Scientific Electronic Library Online. Consulted: January 10, 2022. Available: https://scielo.org/

13. **Instituto Brasileiro de Informação em Ciência e Tecnologia.** Biblioteca Digital Brasileira de Teses e Dissertacoes. Consulted: January 10, 2022. Available: http://bdtd.ibict.br/vufind/

14. **Cobayashi F, Lourenço BH, Cardoso MA.** 25-hydroxyvitamin D3 levels, BsmI polymorphism and insulin resistance in Brazilian Amazonian

children. Int J Mol Sci. 2015;16:12531-46. https://doi.org/10.3390/ijms160612531

15. **Santos BR, Mascarenhas LPG, Satler F, Boguszewski MCS, Spritzer PM.** Vitamin D deficiency in girls from South Brazil: a cross-sectional study on prevalence and association with vitamin D receptor gene variants. BMC Pediatr. 2012;12:606. https://doi.org/10.1186/1471-2431-12-62

16. **Ferraz RS, Silva CS, Cavalcante GC, de Queiroz NNM, Felício KM, Felício JS, et al.** Variants in the VDR gene may influence 25(OH)D levels in type 1 diabetes mellitus in a Brazilian population. Nutrients. 2022;14:1010. https://doi.org/10.3390/nu14051010

17. **Galvão AA, de Araújo Sena F, de Andrade Belitardo EMM, de Santana MBR, Costa GN de O, Cruz ÁA, et al.** Genetic polymorphisms in vitamin D pathway influence 25(OH)D levels and are associated with atopy and asthma. Allergy Asthma Clin Immunol. 2020;16:62. https://doi.org/10.1186/s13223-020-00460-y

18. **Bezerra FF, Normando P, Fonseca ACP, Zembrzuski V, Campos-Junior M, Cabello-Acero PH, et al.** Genetic, sociodemographic and lifestyle factors associated with serum 25-hydroxyvitamin D. Cad Saúde Publica. 2022;38:e00287820. https://doi.org/10.1590/0102-311X00287820

19. **Brait BJ, da Silva Lima SP, Aguiar FL, Fernandes-Ferreira R, Oliveira-Brancati CIF, Ferraz JAML, et al.** Genetic polymorphisms related to the vitamin D pathway in patients with cirrhosis with or without hepatocellular carcinoma (HCC). Ecancermedicalscience. 2022;16:1383. https://doi.org/10.3332/ecancer.2022.1383

20. **Vidigal VM, Silva TD, de Oliveira J, Pimenta CAM, Felipe AV, Forones NM.** Genetic polymorphisms of vitamin D receptor (VDR), CYP27B1 and CYP24A1 genes and the risk of colorectal cancer. Int J Biol Markers. 2017;32:e224–30. https://doi.org/10.5301/jbm.5000248

21. **de Paula Corrêa M, Pires LCM. Doses of erythemal ultraviolet radiation observed in Brazil.** Int J Dermatol. 2013;52:966–73. https://doi.org/10.1111/j.1365-4632.2012.05834.x

22. **Pereira-Santos M, dos Santos JYG, Carvalho GQ, Santos DB, Oliveira AM.** Epidemiology of vitamin D insufficiency and deficiency in a population in a sunny country: Geospatial meta-analysis in Brazil. Crit Rev Food Sci Nutr. 2019;59:2102–9. https://doi.org/10.1080/10408398.2018.1437711

23. **Mendes MM, Hart KH, Botelho PB, Lanham-New SA.** Vitamin D status in the tropics: Is sunlight exposure the main determinant? Nutr Bull. 2018;43:428–34. https://doi.org/10.1111/nbu.12349

24. **Maeda SS, Borba VZC, Camargo MBR, Silva DMW, Borges JLC, Bandeira F, et al.** Recomendações da Sociedade Brasileira de Endocrinologia e Metabologia (SBEM) para o diagnóstico e tratamento da hipovitaminose D. Arq Bras Endocrinol Metabol. 2014;58:411–33. https://doi.org/10.1590/0004-2730000003388

25. **Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, et al.** Genome-wide association study of circulating vitamin D levels. Hum Mol Genet. 2010;19:2739–45. https://doi.org/10.1093/hmg/ddq155

26. **Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al.** Common genetic determinants of vitamin D insufficiency: a

genome-wide association study. Lancet. 2010;376:180–8. https://doi.org/10.1016/S0140-6736(10)60588-0

27. **Hong J, Hatchell KE, Bradfield JP, Bjonnes A, Chesi A, Lai CQ, et al.** Transethnic evaluation identifies low-frequency loci associated with 25-hydroxyvitamin D concentrations. J Clin Endocrinol Metab. 2018;103:1380–92. https://doi.org/10.1210/jc.2017-01802

28. **Kim YA, Yoon JW, Lee Y, Choi HJ, Yun JW, Bae E, et al.** Unveiling genetic variants underlying vitamin D deficiency in multiple Korean cohorts by a genome-wide association study. Endocrinol Metab. 2021;36:1189–200. https://doi.org/10.3803/EnM.2021.1241

29. **Parlato LA, Welch R, Ong IM, Long J, Cai Q, Steinwandel MD, et al.** Genome-wide association study (GWAS) of circulating vitamin D outcomes among individuals of African ancestry. Am J Clin Nutr. 2023;117:308-16. https://doi.org/10.1016/j.ajcnut.2022.12.001

30. **Manousaki D, Mitchell R, Dudding T, Haworth S, Harroud A, Forgetta V, et al.** Genome-wide association study for vitamin D levels reveals 69 independent loci. Am J Hum Genet. 2020;106:327–37. https://doi.org/10.1016/j.ajhg.2020.01.017

Table 1. SNPs associated with vitamin D levels found in the GWAS catalog.

variant	risk allele	p-value	risk allele frequency (RAF)	beta	95%CI	mapped gene	chr	location (bp) GRCh38	study accession
rs145432346	C	7x10-286	0.826	0.108617 unit increase	[0.1-0.11]	GC	4	71709300	GCST90019543
rs2282679	C	2x10-49	0.260	0.38 unit decrease	[0.32-0.44]	GC	4	71742666	GCST000664
rs2282679		2x10-14	0.290			GC	4	71742666	GCST001560
rs2282679	T	1x10-187				GC	4	71742666	GCST005366
rs2282679	T	4x10-63	0.770	16.628 (z-score) increase		GC	4	71742666	GCST005782
rs2282679	T	5x10-62				GC	4	71742666	GCST005782
rs3755967	T	5x10-343		0.089 unit decrease	[0.084-0.094]	GC	4	71743681	GCST005367
rs3755967		1x10-300		0.206767 unit decrease	[0.2-0.21]	GC	4	71743681	GCST90000618
rs3755967	T	5x10-343		0.089 unit decrease	[0.084-0.094]	GC	4	71743681	GCST90019526
rs3755967		1x10-300		0.206767 unit decrease	[0.2-0.21]	GC	4	71743681	GCST90019540
rs11723621	G	3x10-1689	0.291	0.18693 unit decrease	[0.18-0.19]	GC	4	71749645	GCST90019526
rs1352846	A	1x10-300	0.709	0.194 unit decrease	[0.19-0.2]	GC	4	71752058	GCST90019527
rs1352846	G	1x10-300	0.290	0.2335 unit increase	[0.23-0.24]	GC	4	71752058	GCST90019528
rs1352846	G	1x10-300	0.290	0.1887 unit increase	[0.18-0.2]	GC	4	71752058	GCST90019532
rs1352846	A	1x10-300	0.709	0.193471 unit increase	[0.19-0.2]	GC	4	71752058	GCST90019534
rs1352846	G	1x10-297	0.290	0.1216 unit decrease	[0.12-0.13]	GC	4	71752058	GCST90019541
rs4588	T	2x10-263	0.283	0.25nmol/l decrease	[0.23-0.27]	GC	4	71752606	GCST90019546
rs7041	C	1x10-7	0.170	5.3 (z-score) increase		GC	4	71752617	GCST005782
rs3775150	C	4x10-295	0.262	0.090838 unit decrease	[0.086-0.096]	GC	4	71775033	GCST90019542
rs10832254	G	1x10-320	0.370	0.132 unit increase	[0.13-0.14]	RRAS2, COPB1	11	14413152	GCST90019526
rs10832254	G	1x10-300	0.370			RRAS2, COPB1	11	14413152	GCST90019533
rs577185477	C	2x10-342	0.015	0.379366 unit decrease	[0.36-0.4]	PSMA1	11	14591017	GCST90019526
rs10832289	T	2x10-266	0.410	0.068522 unit decrease	[0.065-0.072]	PDE3B	11	14647950	GCST90019545
rs188480917	G	5x10-275	0.011	0.343291 unit decrease	[0.32-0.36]	PDE3B	11	14764324	GCST90019544

rs116970203		1x10-300		0.365538 unit decrease	[0.35-0.38]	PDE3B	11	14855172	GCST90019529
rs116970203	G	1x10-300	0.973	0.376873 unit increase	[0.36-0.39]	PDE3B	11	14855172	GCST90019535
rs116970203	G	1x10-300	0.973	0.377 unit decrease	[0.37-0.39]	PDE3B	11	14855172	GCST90019537
rs1894100		1x10-300		0.102148 unit decrease	[0.097-0.107]	ACTE1P	11	14855172	GCST90019530
rs117913124	A	2x10-775	0.028	0.354126 unit decrease	[0.34-0.37]	CYP2R1	11	14879385	GCST90019526
rs12794714	G	1x10-300	0.578	0.0878964 unit increase	[0.084-0.092]	CYP2R1	11	14892029	GCST90019536
rs12794714	G	1x10-300	0.578	0.089 unit decrease	[0.085-0.093]	CYP2R1	11	14892029	GCST90019538
rs10741657	A	2x10-38				CALCB, CYP2R1	11	14893332	GCST005366
rs10741657	A	2x10-46		0.031 unit increase	[0.027-0.035]	CALCB, CYP2R1	11	14893332	GCST005367
rs10741657	A	2x10-6				CALCB, CYP2R1	11	14893332	GCST005782
rs10741657	A	3x10-11	0.421	2.1mmol/l increase		CALCB, CYP2R1	11	14893332	GCST012014
rs11023379		5x10-226		0.0652928 unit decrease	[0.061-0.069]	CALCB	11	14908414	GCST90019549
rs11233933		1x10-300		0.115785 unit decrease	[0.11-0.12]	NADSYN1	11	71419297	GCST90019531
rs12803256	G	9x10-407	0.771	0.100325 unit increase	[0.096-0.105]	ACTE1P	11	71421822	GCST90019526
rs12803256	A	1x10-300	0.223	0.105 unit decrease	[0.1-0.11]	ACTE1P	11	71421822	GCST90019539
rs12785878	T	4x10-62		0.036 unit increase	[0.032-0.04]	NADSYN1	11	71456403	GCST005367
rs12800438	A	1x10-16				NADSYN1	11	71459957	GCST005782
rs4944957	A	1x10-16				NADSYN1	11	71459957	GCST005782
rs12278461	C	5x10-228	0.210	0.1294 unit decrease	[0.12-0.14]	NADSYN1	11	71471139	GCST90019548
rs3829251	A	3x10-9	0.190	0.18 unit decrease	[0.12-0.24]	NADSYN1	11	71483513	GCST000664
rs200454003	T	4x10-256	0.265	0.0867 unit decrease	[0.082-0.092]	NADSYN1	11	71517944	GCST90019547
rs10745742	T	1x10-7				AMDHD1	12	95964751	GCST005366
rs10745742	T	2x10-20		0.019 unit increase	[0.015-0.023]	AMDHD1	12	95964751	GCST005367
rs17216707	T	1x10-14				CYP24A1, BCAS1	20	54115823	GCST005366
rs17216707	T	8x10-23		0.026 unit increase	[0.021-0.031]	CYP24A1, BCAS1	20	54115823	GCST005367
rs17216707	T	6x10-48	0.817	0.038 unit decrease	[0.032-0.044]	CYP24A1, BCAS1	20	54115823	GCST90000616

Same SNPs identified in different studies are shown in colour.

bp = base pairs

Table 2. *VDR* SNPs examined in relation to vitamin D levels in the Brazilian population.

variant	allele 1	allele 2	chromosome	location (bp) GRCh38	gene position
rs9729	C	A	12	47842840	3'UTR
rs739837	G	T	12	47844438	3'UTR
rs731236	G	A	12	47844974	Ile352Ile
rs7975232	C	A	12	47845054	intron
rs1544410	T	C	12	47846052	intron
rs7963776	G	A	12	47849594	intron
rs7967152	A	C	12	47850401	intron
rs2189480	G	T	12	47870045	intron
rs2228570	A	G	12	47879112	Met1Thr
rs2853564	C	T	12	47884704	intron
rs7965274	T	C	12	47886384	intron
rs2853561	C	T	12	47887474	intron
rs10875694	T	A	12	47887877	intron
rs59128934	G	T	12	47891025	intron
rs11168287	G	A	12	47891631	intron
rs4328262	G	T	12	47891865	intron
rs4237855	G	A	12	47893420	intron
rs11568820	A	G	12	47908762	-

Table 3. Allele frequencies of *GC* SNPs tested in association with vitamin D levels in the Brazilian population.

rs4588	**city, state**	**allele 1**	**allele 2**	**allele 1 frequency**
adult patients with chronic hepatitis C genotype 1	Porto Alegre, RS	T	G	0.213
women with no evidence of clinical disease	Porto Alegre, RS	A	C	0.293
women of reproductive age	Porto Alegre, RS	A	C	0.230
healthy female students	Curitiba, PR	A	C	0.267
patients with cirrhosis	São Jose do Rio Preto, SP	A	C	0.300
controls (cirrhosis)	São Jose do Rio Preto, SP	A	C	0.280
rs7041				
adult patients with chronic hepatitis C genotype 1	Porto Alegre, RS	C	A	0.461
women with no evidence of clinical disease	Porto Alegre, RS	G	T	0.484
women of reproductive age	Porto Alegre, RS	G	T	0.535
healthy female students	Curitiba, PR	G	T	0.485
patients with cirrhosis	São Jose do Rio Preto, SP	G	T	0.460
controls (cirrhosis)	São Jose do Rio Preto, SP	G	T	0.510
rs2282679				
university civil servants	Rio de Janeiro, RJ	C	A	0.222
women with no evidence of clinical disease	Porto Alegre, RS	C	A	0.283
women of reproductive age	Porto Alegre, RS	C	A	0.225

PR = Paraná
RJ = Rio de Janeiro
RS = Rio Grande do Sul
SP = São Paulo

Table 4. Allele frequencies of *VDR* SNPs most frequently tested in association with vitamin D levels in the Brazilian population.

rs1544410 (BsmI)	**city, state**	**allele 1**	**allele 2**	**allele 1 frequency**
adolescents without chronic disease	João Pessoa, PB	A	G	0.395
adult male patients with Chagas disease	Botucatu, SP	A	G	0.400
patients with polycystic ovary syndrome (PCOS)	Porto Alegre, RS	A	G	0.400
non-hirsute women with regular ovulatory cycles	Porto Alegre, RS	A	G	0.350
children aged ≤ 10 years	Acrelândia, AC	T	C	0.406
healthy girls	Curitiba, PR/Porto Alegre, RS	A	G	0.323
type 1 diabetes (T1D) patients	Belém, PA	A	G	n/a
controls (T1D)	Belém, PA	A	G	n/a
type 2 diabetes (T2D) patients	Belo Horizonte, MG	A	G	0.401
controls (T2D)	Belo Horizonte, MG	A	G	0.411
colorectal cancer (CRC) cases	São Paulo, SP			n/a
controls (CRC)	São Paulo, SP			n/a
rs2228570 (FokI)				
children with persistent primary teeth (PPT)	Riberão Preto, SP	A	G	0.250
controls (PPT)	Riberão Preto, SP	A	G	0.269
children with delayed tooth eruption (DTE)	Riberão Preto, SP	A	G	0.296
controls (DTE)	Riberão Preto, SP	A	G	0.379
adolescents without chronic disease	João Pessoa, PB	T	C	0.332
adult male patients with Chagas disease	Botucatu, SP	T	C	0.440
children aged ≤ 10 years	Acrelândia, AC	A	G	0.299
T1D patients	Belém, PA	T	C	0.308
controls (T1D)	Belém, PA	T	C	0.331

T2D patients	Belo Horizonte, MG	T	C	0.245
controls (T2D)	Belo Horizonte, MG	T	C	0.306
rs731236 (TaqI)				
pregnant women	Santo Antônio de Jesus, BA	G	A	0.300
adult male patients with Chagas disease	Botucatu, SP	G	A	0.260
patients with PCOS	Porto Alegre, RS	G	A	0.396
non-hirsute women with regular ovulatory cycles	Porto Alegre, RS	G	A	0.354
children aged ≤ 10 years	Acrelândia, AC	G	A	0.396
healthy girls	Curitiba, PR/Porto Alegre, RS	C	T	0.314
T1D patients	Belém, PA	C	T	0.315
controls (T1D)	Belém, PA	C	T	0.283
T2D patients	Belo Horizonte, MG	C	T	0.332
controls (T2D)	Belo Horizonte, MG	C	T	0.403
rs7975232 (ApaI)				
pregnant women	Santo Antônio de Jesus, BA	C	A	0.400
patients with PCOS	Porto Alegre, RS	C	A	0.447
non-hirsute women with regular ovulatory cycles	Porto Alegre, RS	C	A	0.400
children aged ≤ 10 years	Acrelândia, AC	C	A	0.431
healthy girls	Curitiba, PR/Porto Alegre, RS	G	T	0.429
T1D patients	Belém, PA	G	T	0.362
controls (T1D)	Belém, PA	G	T	0.446
T2D patients	Belo Horizonte, MG	C	A	0.245
controls (T2D)	Belo Horizonte, MG	C	A	0.210
colorectal cancer (CRC) cases	São Paulo, SP			n/a
controls (CRC)	São Paulo, SP			n/a

rs739837				
children with persistent primary teeth (PPT)	Riberão Preto, SP	G	T	0.400
controls (PPT)	Riberão Preto, SP	G	T	0.500
children with delayed tooth eruption (DTE)	Riberão Preto, SP	G	T	0.417
controls (DTE)	Riberão Preto, SP	G	T	0.366
SCAALA cohort	Salvador, BA	G	T	0.484
rs11568820 (Cdx2)				
children aged ≤ 10 years	Acrelândia, AC	A	G	0.399
asthmatic children	Curitiba, PR	A	G	0.284
non-asthmatic children	Curitiba, PR	A	G	0.295

AC = Acre
BA = Bahia
MG = Minas Gerais
PA = Pará
PB = Paraíba
PR = Paraná
RS = Rio Grande do Sul
SP = São Paulo

Supplementary Table 1. Scientific articles on *GC* polymorphisms and vitamin D levels in the Brazilian population.

Supplementary Table 2. Scientific articles on *VDR* polymorphisms and vitamin D levels in the Brazilian population.

Supplementary Table 3. Scientific articles on polymorphisms in genes other than *GC* and *VDR* and vitamin D levels in the Brazilian population.

Supplementary Table 4. SNPs in the *VDR* gene associated with complex traits according to the GWAS catalog.