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Proceeding Paper

Associations between Vitamin D Receptor (VDR) Polymorphisms and Gut Microbiota in a Spanish Population Sample †

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Abstract: This study examined the association between genetic variations in the Vitamin D Receptor (VDR) gene and the composition of gut microbiota in a Spanish population. The VDR gene plays a role in mediating the effects of vitamin D on gut microbiota composition. We analyzed 87 healthy participants from the Spanish Caucasian population and found that the genetic variations were in Hardy–Weinberg equilibrium. Through metataxonomic sequencing, we identified that the GG genotype from the VDR polymorphism rs731236 seems to be associated with the opportunistic genus *Solobacterium*, indicating that genetic variations may influence the composition of gut microbiota.

Keywords: Vitamin D Receptor (VDR) polymorphisms; gut microbiota; Spanish population; rs2731236; Lefse

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1. Introduction

1,25-Dihydroxyvitamin D (1,25(OH)2D3), the active form of vitamin D, activates the widely expressed vitamin D receptor (VDR). The VDR gene encodes the vitamin D receptor, which plays a crucial role in mediating the biological effects of vitamin D. The vitamin D3 receptor belongs to a group of ligand-inducible transcription factors known as the nuclear hormone receptor superfamily [1]. The receptor not only functions as a receptor for vitamin D3 but also acts as a receptor for a secondary bile acid called lithocholic acid [2]. The main targets regulated by the vitamin D3 receptor are primarily involved in mineral metabolism, but it also plays a role in various other metabolic pathways such as immune response and cancer [3]. The vitamin D receptor (VDR) seems a key genetic factor for shaping the host microbiome as conditional VDR deletion severely changes metabolites specifically produced from carbohydrate, protein, lipid, and bile acid metabolism. In one study conducted in mice, gender differences were also observed regarding the effect of VDR deletion [4].

Nevertheless, low levels of vitamin D or inactivating polymorphisms in VDR have been associated with inflammatory and metabolic disorders. Mutations in this gene have been associated with a condition called type II vitamin D-resistant rickets [5]. Different transcript variants of the gene have been identified. A recent study has also provided evidence of translational readthrough in this gene, resulting in the expression of an extended

isoform with a different C-terminus, utilizing an alternative translation termination codon within the gene's coding sequence [6].

The rs731236 polymorphism, also known as the TaqI restriction site polymorphism (T/C), is one of the common genetic variations in the VDR gene. There are different studies suggesting a role of this polymorphism in health status. A nonsignificant increase in mortality of 55% was observed among patients homozygous for the rare allele C [7]. On the contrary, an analysis conducted among 111 Swedish breast cancer survivors below 37 years of age found a higher survival among estrogen receptor-positive tamoxifen-treated patients homozygous for the C allele [8]. VDR has also been suggested to be a potential key player in the pathogenetic mechanism of obesity. Vasilopoulos et al. found we found a strong association between the VDR TaqI (rs731236) 'T' allele and obesity when analyzing a Greek population [9].

A metanalysis from Wang et al. suggested a role of VDR in the composition of the gut microbiome and showed that Parabacteroides are the most significant taxa correlated with the VDR gene [10]. The lack of VDR leads to dysbiosis, indicating a critical role of VDR in the conformation of gut microbiota communities [11].

In this work, we have explored the connections between the gut microbiome and the VDR gene variations in a sample of the Caucasian Spanish population. By analyzing genetic variations in the VDR gene and their potential impact on the gut microbial composition, we aim to shed light on the interplay between these factors.

2. Materials and Methods

2.1. Study Population

This study included a total of 87 healthy participants, consisting of 57 men and 30 women, with ages ranging from 18 to 48 years. Strict exclusion criteria were applied, excluding individuals with any form of pathology within six months prior to the study, those who had undergone previous gastrointestinal surgery, those who had taken antibiotics within three months prior to the study, smokers, individuals using prebiotics, probiotics, or nutritional supplements, vegetarians, or vegans, and pregnant or lactating individuals. All participants in this study were of Caucasian ethnicity.

2.2. Sample Collection

Participants were provided with the Fe-Col® Fecal Sample Collection Kit (Alpha Laboratories, Hampshire, UK), along with insulated bags and ice blocks to maintain sample integrity during transportation. Stool samples were preserved at −80 °C until further processing.

2.3. DNA Extraction

Human and bacterial DNA extraction was conducted on 100 mg of stool sample utilizing the commercial E.Z.N.A.® Stool DNA Kit (Omega Biotek, Norcross, GA, USA) and a bead-beating homogenizer (Bullet Blender Storm, Next Advance, New York, NY, USA). DNA concentration and purity were assessed using the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA, USA) and an FP-8300 spectrofluorimeter (Jasco, Tokyo, Japan). Bacterial DNA was employed for microbiota analysis, while human DNA was used for VDR genotyping.

2.4. VDR Genotyping

Applied Biosystems TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA; assay ID: C_2404008_10) were employed for allelic discrimination analysis of the TaqI VDR (rs731236) gene polymorphism. The StepOnePlus Real-Time PCR system (ThermoFisher Scientific) was utilized, with a protocol involving denaturation at 95 °C for 10 min, followed by 50 cycles of denaturation at 92 °C for 15 s, annealing/extension at 60 °C for 1 min, and a final extension step of 30 s at 60 °C. Fluorescence analysis was conducted with Allelic Discrimination 7500 software v.2.0.2. Following genotyping, participants were

classified as follows based on the VDR genotype: TT as VDR-1, TC as VDR-2, and CC as VDR-3 for subsequent analysis.

2.5. Sequencing and Bioinformatics

The hypervariable V3 and V4 regions were amplified using the primer pair 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′ and 5′ GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAG-3′. The amplicon of 459 bp was visualized in a 0.8% agarose gel stained with ethidium bromide, and bands were cut and cleaned using the MinElute Gel Extraction Kit (Qiagen, Hilden, Germany). DNA amplicons were sequenced on a MiSeq Illumina platform (Illumina, San Diego, CA, USA). Sequence outputs were analyzed using the Quantitative Insights into Microbial Ecology (QIIME2) program, v2019.10. The 16s paired reads were imported into QIIME2 and processed with the DADA2 plugin], adjusting the maximum expected error threshold to 2.0 (both forward and reverse). The taxonomy assignments were performed with the classify-sklearn method and an in-house customized classifier based on the SILVA reference database [12–14]. To build the customized reference database, sequences, according to our primers (forward primer sequence: CCTACGGGNG-GCWGCAG, reverse primer sequence: GACTACHVGGGTA TCTAATCC), were extracted from the SILVA 132 database clustered at 99% identity. The classifier was trained using our tailored reference reads and SILVA 7-levels for reference taxonomy, including the species probability (weights) likely to be observed for human stool (downloaded from https://github.com/BenKaehler/readytowear, accessed on 1 June 2023) [12,13].

2.6. Statistical Analysis

The allele and genotypes frequencies were calculated using the SNPStat program [15]. Statistical analysis was carried out using QIIME2 v2019.10, SPSS software v26.0 (SPSS, Chicago, IL, USA) and the R statistical package v4.1.1. Linear discriminant analysis coupled with effect size (LEfSe v1.0) was performed to identify bacterial associated pathways differentially represented between groups with default settings. Significance was set at $p < 0.05$.

3. Results

3.1. VDR Genotyping

Descriptive statistics were used to show the baseline characteristics of the participants in this study. Deviation from the Hardy–Weinberg equilibrium (HWE) was tested using SNPStat Software [15]. Genotyping results for the rs731236 (TaqI) polymorphism were obtained and are presented in Table 1. In comparison to the 1000 Genomes Project reference, the calculated allele frequencies (T = 0.61 and C = 0.39) were as expected for a European population sample. When examining genotypes, TT homozygotes were found at a frequency of 0.43, while TC heterozygotes and CT heterozygotes had frequencies of 0.38 and 0.20, respectively, in accordance with Hardy–Weinberg equilibrium ($p = 0.071$) (Table 1).

Table 1. Genotyping results showing the allele and genotype frequencies for the VDR rs731236 (TaqI) polymorphism (test for Hardy–Weinberg equilibrium; $p = 0.071$).

rs731236 (TaqI) (C/T)	SNP Allele Frequencies (n = 87)	Genotypes	Frequencies (H-W)
T	0.39	TT	0.43
C	0.61	TC	0.38
		CC	0.20

3.2. Bacterial Composition According to Genotypes

No differences were found in either body composition parameters or diet.

LEfSe (Linear Discriminant Analysis Effect Size) statistical analysis shows that the GG genotype is enriched in the genus *Solobacterium*, whilst the AA genotype is enriched

in the genera *Victivallaceae*, *Moryella*, *Enterococcus* (Fam. Enterococcaceae) and the families Desulfovibrionaceae, Selenomonadaceae, and Ruminococcaceae (phylum Desulfobacterota; class Desulfovibrionia, order Desulfovibrionales) (Figure 1).

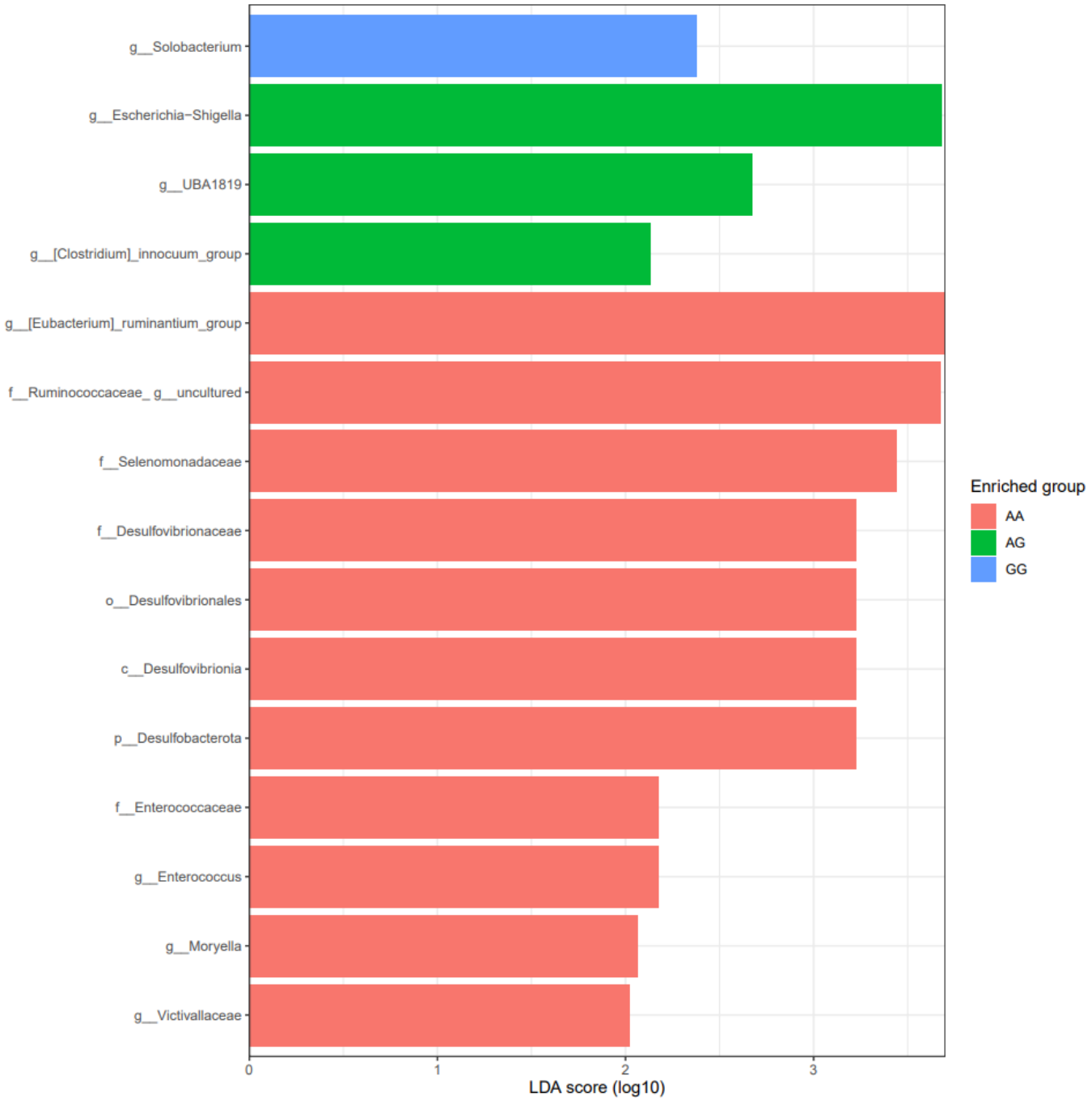

Figure 1. Lefse analysis showing bacterial composition according to genotype.

4. Discussion

The LEfSe analysis revealed a strong association between the *Solobacterium* genus and the presence of the rare GG genotype for the VDR gene. Some species of the *Solobacterium* genus are opportunistic pathogens and are part of the oral and intestinal microbiotas [16]. *Solobacterium* seems to be increased in obesity in some cases, and in a recent study, the intake of lactic acid appeared to decrease its presence [17]. However, in our study, we did not find associations between body composition and the genotypes, so further studies and analysis will be required to find consistent results showing a role of the VDR nuclear

receptor in the gut microbiota composition and whether they lead to specific changes in the microbiota, such as the presence of opportunistic pathogens as it seems.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of COMITÉ ÉTICO DE INVESTIGACIÓN CON MEDICAMENTOS REGIONAL DE LA COMUNIDAD DE MADRID (protocol number 47/560280.9/18 approved 10 September 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Data will be provided upon a collaborative research request.

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Conflicts of Interest: The authors declare no conflict of interest.

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