Original Research

Lack of Vitamin D Receptor Causes Dysbiosis and Changes the Functions of the Murine Intestinal Microbiome

Dapeng Jin, MS[1]; Shaoping Wu, PhD[1]; Yong-guo Zhang, PhD[1]; Rong Lu, PhD[1]; Yinglin Xia, PhD[2]; Hui Dong, PhD[3]; and Jun Sun, PhD[1]

[1]Department of Biochemistry, Rush University Medical Center, Chicago, Illinois; [2]Department of Biostatistics and Computational Biology, University of Rochester, Rochester, New York; and [3]Department of Gastroenterology, Xingqiao Hospital, The Third Military Medical University, Chongqing, China

ABSTRACT

Purpose: The microbiome modulates numerous aspects of human physiology and is a crucial factor in the development of various human diseases. Vitamin D deficiency and downregulation of the vitamin D receptor (VDR) are also associated with the pathogenesis of diseases such as inflammatory bowel disease, cancers, obesity, diabetes, and asthma. VDR is a nuclear receptor that regulates the expression of antimicrobial peptides and autophagy regulator ATG16L1. Vitamin D may promote a balanced intestinal microbiome and improve glucose homeostasis in diabetes. However, how VDR regulates microbiome is not well known. In the current study, we hypothesize that VDR status regulates the composition and functions of the intestinal bacterial community.

Methods: Fecal and cecal stool samples were harvested from *Vdr* knockout ($Vdr^{-/-}$) and wild-type mice for bacterial DNA and then sequenced with 454 pyrosequencing. The sequences were denoised and clustered into operational taxonomic units, then queried against the National Center for Biotechnology Information database. Metagenomics were analyzed, and the abundances of genes involved in metabolic pathways were compared by reference to the Kyoto Encyclopedia of Genes and Genomes and Clusters of Orthologous Groups databases.

Findings: In the $Vdr^{-/-}$ mice, *Lactobacillus* was depleted in the fecal stool, whereas *Clostridium* and *Bacteroides* were enriched. Bacterial taxa along the Sphingobacteria-to-Sphingobacteriaceae lineage were enriched, but no genera reached statistical significance. In the cecal stool, *Alistipes* and *Odoribacter* were depleted, and *Eggerthella* was enriched. Notably, all of the taxa upstream of *Eggerthella* remained unchanged. A comparison of $Vdr^{-/-}$ and wild-type samples revealed 40 (26 enriched, 14 depleted) and 72 (41 enriched, 31 depleted) functional modules that were significantly altered in the cecal and fecal microbiomes, respectively (both, $P < 0.05$), due to the loss of Vdr. In addition to phylogenetic differences in gut microbiome with different intestinal origins, we identify several important pathways, such as nucleotide-binding oligomerization domain–like receptor, affected by Vdr status, including amino acid, carbohydrate, and fatty acid synthesis and metabolism, detoxification, infections, signal transduction, and cancer and other diseases.

Implications: Our study fills knowledge gaps by having investigated the microbial profile affected by VDR. Insights from our findings can be exploited to develop novel strategies to treat or prevent various diseases by restoring VDR function and healthy microbe–host interactions. (*Clin Ther.* 2015;37:996–1009)

Accepted for publication April 9, 2015.
http://dx.doi.org/10.1016/j.clinthera.2015.04.004
0149-2918/$ - see front matter

Key words: *Bacteroides*, *Clostridium*, dysbiosis, immunity, inflammation, intestine, microbiome, NOD-like receptor, vitamin D, vitamin D receptor.

INTRODUCTION

Microbial habitats in the human body include the skin surface and the mucosa covering the mouth, pharynx, respiratory tract, urogenital tract, and gut, which accommodate and interact with commensal bacteria to fulfill various physiologic functions.[1] The gastrointestinal tract, in particular, harbors the most abundant microflora (100 trillion). The human genome contains over 23,000 genes; however, it is vastly underestimated if we take the microbiome into consideration, which outnumbers the human cells by an order of magnitude.[2] The symbiotic relationship that coevolves over time bestows humans with functions that do not need to be encoded within their own genomes, or at least not completely,[3] and contributes to interindividual differences.[4,5] The gut microbiome not only has been correlated with disorders such as inflammatory bowel diseases (IBD), obesity, and diabetes[6–8] but has also been shown to have extended effects in other, distant organs, including autism spectrum disorder and Alzheimer disease,[9,10] conditions previously thought irrelevant to gastrointestinal bacteria, thus heralding a new era of microbiome studies.

Vitamin D/vitamin D receptor (VDR) deficiency has been associated with various effects in humans, including higher risks for IBD, including ulcerative colitis and Crohn disease.[11,12] Vitamin D supplementation has been reported to have clinical benefit in reducing IBD occurrence and relapse and in improving outcomes.[12] VDR regulates the expression of cathelicidin antimicrobial peptides (CAMPs), β-defensins, and autophagy regulator ATG16L1[13–15] and therefore possesses some antibiotic properties. For example, [1,25$(OH)_2D_3$] leads to up-regulation of CAMPs and the killing of intracellular *Mycobacterium tuberculosis* in human monocytes.[16] These findings give rise to the inference that vitamin D/VDR signaling may dramatically change the bacterial landscape in the gut. Recent studies have reported that the absence of VDR is associated with shifts in the bacterial load and profile.[15,17] However, an accurate characterization of VDR regulation of the microbiota remains unavailable.

In the present study, we hypothesized that VDR status regulates the composition and functions of the bacterial community in the intestine. We investigated fecal and cecal stool samples from whole-body *Vdr* knockout ($Vdr^{-/-}$) and wild-type (WT) mice, aiming to profile the intestinal microbiomes of animals of different Vdr status. Our study may greatly enrich our understanding of the mechanisms underlying defects caused by VDR deficiency and help us to better navigate therapeutic interventions targeting host–bacteria interactions.

METHODS AND MATERIALS

Statement of Ethics

All animal work was approved by the Committee on Animal Resources, Rush University Medical Center (Chicago, Illinois).

Mice

WT and $Vdr^{-/-}$ C57BL/6 mice (purchased from Jackson Laboratory, Bar Harbor, Maine) were bred as previously described.[18] Tail snips were collected 4 weeks after the mice were born. Littermates 6 to 8 weeks old were chosen from each group and cohoused until the experiments were performed.

Microbial Sampling and Sequencing

The tubes for microbial sampling were autoclaved and then irradiated with ultraviolet light to destroy the contaminating environmental bacterial DNA. The mice were then anesthetized and dissected. Fresh cecal and fecal stools were isolated from the gut and placed into the specially prepared tubes. The samples were kept at low temperature with dry ice and were mailed to the Research and Testing Laboratory for 454 pyrosequencing. The sequences were denoised and subjected to quality checks. Taxonomic identifications were assigned by queries against the National Center for Biotechnology Information database. Initially, 249,435 reads were generated. After denoising, the number was reduced to 173,119, which was then diminished to 160,248 after quality checking. On alignment, 151,543 operational taxonomic units (OTUs) were obtained. For each sample, the number ranged from 1853 to 9264, with a mean of 4736.

454 Pyrosequencing

The V4–V6 region of the samples was amplified for pyrosequencing in the Research and Testing