Gut Microbes

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/kgmi20

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To cite this article: Esteban L. Ortega-Vega, Sandra J. Guzmán-Castañeda, Omer Campo, Eliana P. Velásquez-Mejía, Jacobo de la Cuesta-Zuluaga, Gabriel Bedoya & Juan S. Escobar (2020) Variants in genes of innate immunity, appetite control and energy metabolism are associated with host cardiometabolic health and gut microbiota composition, Gut Microbes, 11:3, 556-568, DOI: 10.1080/19490976.2019.1619440

To link to this article: https://doi.org/10.1080/19490976.2019.1619440

GUT MICROBES
2020, VOL. 11, NO. 3, 556–568
https://doi.org/10.1080/19490976.2019.1619440

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RESEARCH PAPER

Variants in genes of innate immunity, appetite control and energy metabolism are associated with host cardiometabolic health and gut microbiota composition

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ABSTRACT

Identifying the genetic and non-genetic determinants of obesity and related cardiometabolic dysfunctions is cornerstone for their prevention, treatment, and control. While genetic variants contribute to the cardiometabolic syndrome (CMS), non-genetic factors, such as the gut microbiota, also play key roles. Gut microbiota is intimately associated with CMS and its composition is heritable. However, associations between this microbial community and host genetics are understudied. We contribute filling this gap by genotyping 60 variants in 39 genes of three modules involved in CMS risk, measuring cardiometabolic risk factors, and characterizing gut microbiota in a cohort of 441 Colombians. We hypothesized that CMS risk variants were correlated with detrimental levels of clinical parameters and with the abundance of disease-associated microbes. We found several polymorphisms in genes of innate immunity, appetite control, and energy metabolism that were associated with metabolic dysregulation and microbiota composition; the associations between host genetics and cardiometabolic health were independent of the participants' gut microbiota, and those between polymorphisms and gut microbes were independent of the CMS risk. Associations were also independent of the host genetic ancestry, diet and lifestyle. Most microbes explaining genetic-microbiota associations belonged to the families *Lachnospiraceae* and *Ruminococcaceae*. Multiple CMS risk alleles were correlated with increased abundance of beneficial microbiota, suggesting that the phenotypic outcome of the evaluated variants might depend upon the genetic background of the studied population and its environmental context. Our results provide additional evidence that the gut microbiota is under the host genetic control and present pathways of host–microbe interactions.

ARTICLE HISTORY
Received 8 January 2019
Revised 4 April 2019
Accepted 8 May 2019

KEYWORDS
Genetic polymorphism; SNP; gastrointestinal tract; microbiome; mestizo; Colombia; Latin America; obesity; metabolic syndrome

Introduction

Identifying the genetic and non-genetic determinants of human health has been the object of intense research for many decades.[1] Genome-wide association studies have revealed that the susceptibility to obesity and related cardiometabolic dysfunctions, such as abnormal body fat distribution, insulin resistance, atherogenic dyslipidemia, elevated blood pressure, and pro-inflammatory state, which together contribute to the cardiometabolic syndrome (CMS),[2] is partly genetically determined, with many small-effect variants at different loci adding to the risk of disease.[3] However, genetic factors do not explain the bulk of variation in CMS risk, which is further accounted for by non-genetic determinants including diet and lifestyle.[4] Another of such factors is the gut microbiota, the set of microbes that naturally colonize the human gastrointestinal tract.

Gut microbiota is intimately related to CMS.[5–8] These microbes are acquired from the environment and colonization is partially under the host genetic control.[9] Furthermore, recent studies have pinpointed specific human genetic variants that were associated with gut microbiota.[10–13] Despite these latest efforts, the relationship between gut microbiota and host genetics remains incompletely elucidated. One reason for this is that both the composition of intestinal microbes and host genetic architecture are dependent on the geographic origin

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of the studied population,[14,15] limiting the replicability of previous results. In addition, studies analyzing host genetics and gut microbiota have been restricted to a small number of human populations–mainly Americans and Europeans.[9]

We here contribute filling this gap by genotyping variants in genes of innate immunity, appetite control and energy metabolism related to CMS and gut physiology, and associating this variation with gut microbiota and CMS risk. We hypothesized that genetic polymorphisms were associated with host cardiometabolic health and gut microbiota in a consistent fashion, that is, variants increasing the risk of CMS were expected to directly correlate with detrimental levels of clinical parameters and the abundance of disease-associated microbes.

Results and discussion

We performed a cross-sectional study in which we enrolled 441 adults from Colombia (South America) living in five large cities spanning the Andes and both the Caribbean and Pacific coasts. Participants were enrolled in similar proportions according to the city of origin (Bogota, Medellin, Cali, Barranquilla and Bucaramanga), body mass index (BMI: lean, overweight, obese), sex (male, female), and age range (18–40 years, 41–62 years). In these participants, we measured numerous clinical CMS risk factors (blood chemistry, blood pressure, and adiposity), and obtained information about diet (intake of calories, macronutrients, and dietary fiber) and lifestyle (levels of physical activity, smoking status, and medicament consumption).

In 440 participants, we genotyped 60 variants in 39 genes of three modules: innate immunity (Figure S1), appetite control (Figure S2), and energy metabolism (Figure S3). One individual of our cohort could not be genotyped because we were not able to acquire DNA from blood. We obtained 26,223 genotypes informing about the host genetic variation (Table S1). We also characterized the gut microbiota through 16S rRNA gene sequencing, and obtained 14,742,223 reads that grouped into 4,719 OTUs, which informed about gut microbiota composition and diversity. We focused on the 137 OTUs with median relative abundance ≥0.001%, thus limiting the inclusion of OTUs potentially severely affected by technical artifacts (*e.g.*, sequencing errors). This set of OTUs comprised the majority of sequenced reads (83 ± 12% SD) and were sufficiently abundant to be of biological relevance. The analysis by OTUs (Table S2) produced similar results as the analysis by taxonomic levels (Table S3).

As detailed below, we detected six associations between host genetics and cardiometabolic outcomes, and 70 between host genetics and gut microbiota. These associations involved genes of the three evaluated modules (Figure 1). Fifty-one different OTUs were associated with host genetics. The relative abundance of these OTUs accounted for 17.7 ± 0.8% (SD) of the microbial community, and they were classified in 15 taxonomic families; two of these comprised the majority of associations: *Lachnospiraceae* (25) and *Ruminococcaceae* (20). We did not detect significant associations between the evaluated genetic variants and gut-microbiota alpha diversity (adjusted $p > 0.20$). Importantly, the rich set of metadata collected in our participants allowed adjusting statistical models by potential confounding such that the associations between genetic polymorphisms and cardiometabolic health were independent of gut microbiota; and the associations between host genetics and gut microbiota were independent of the host CMS risk. They were also independent of the genetic ancestry of the Colombian population (i.e., the individual contributions of European, Native American and African genetic backgrounds; see accompanying paper by Guzmán-Castañeda et al.), sex, age, and variables related to diet and lifestyle.

In what follows, we present and discuss results per evaluated module, examine the strengths and limitations of our study, and draw conclusions on this work.

Innate immunity

In this module, we analyzed 20 variants in 12 genes involved in host–microbiota interactions through pattern recognition receptors, such as the nucleotide-binding oligomerization (NOD), NOD-like receptor (NLR) and Toll-like receptor (TLR) signaling pathways. We also evaluated some genes for downstream production of pro-inflammatory cytokines, such as

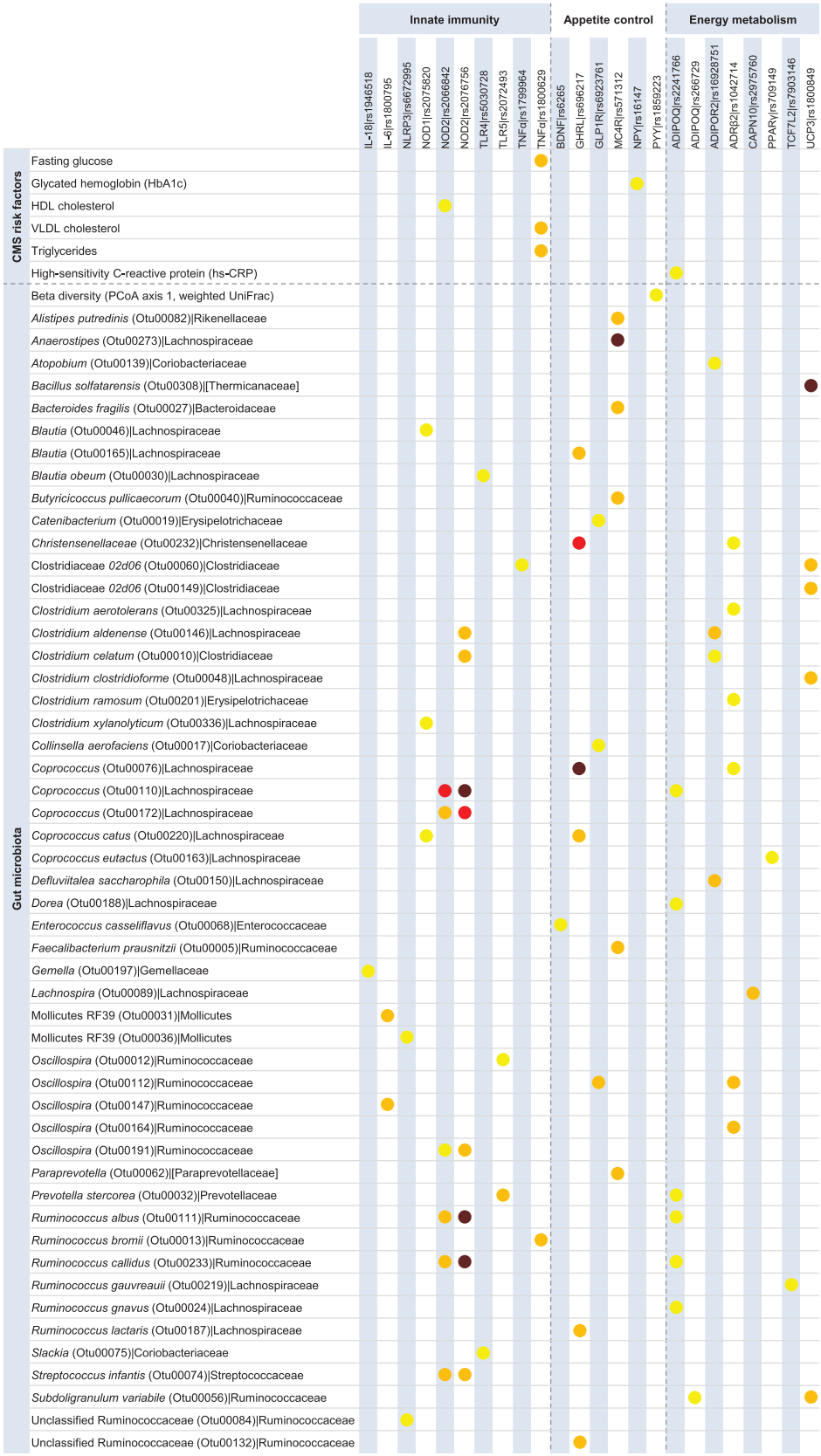

Figure 1. Associations between variants in genes of innate immunity, appetite control and energy metabolism with CMS risk factors and gut microbiota. Standardized linear regression coefficients (β) are depicted as colored dots (yellow = $\beta < 0.30$; orange = $0.30 \leq \beta < 0.45$; light red = $0.45 \leq \beta < 0.60$; dark red = $\beta \geq 0.60$). Exact values are provided as Table S2.

interleukin (IL-) 1β, IL-6, IL-12B, IL-18, and tumor necrosis factor alpha (TNFα) (Figure S1). Ten variants in eight genes of this module were significantly correlated with gut microbiota. Of these, SNPs rs1800629 in *TNFα*, rs2075820 in *NOD1*, and rs2066842 and rs2076756 in *NOD2* accounted for most of the associations (Figure 1; Table S2).

The regulatory SNP rs1800629 in *TNFα* has been well studied in the context of CMS and, in our case, was the one showing more and strongest associations with the measured clinical outcomes (Figure 1). Previous evidence in Europeans indicated that this variant was associated with low levels of total serum cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides.[16] However, it was recently shown to be associated with increased coronary artery disease risk in a Kashmiri population.[17] It was associated with higher levels of very low-density lipoprotein (VLDL) cholesterol and triglycerides in Colombians, as well as with higher levels of fasting glucose, suggesting it is involved in lipid and glycemic responses. This variant was also associated with greater abundance of an OTU classified as *Ruminococcus bromii* (*Ruminococcaceae*), a primary degrader of resistant starch[18] and a major producer of colonic butyrate.[19] Fecal levels of butyrate and other short-chain fatty acids (SCFAs) have been associated with obesity and increased energy harvesting.[20–22]

NODs are cytoplasmic receptors that recognize muramyl dipeptides, components of the bacterial cell wall. Polymorphisms in *NOD2* have been mostly associated with altered susceptibility to Crohn's disease.[23–25] We found that the missense SNP rs2066842 in *NOD2* was associated with increased levels of high-density lipoprotein (HDL) cholesterol ("good cholesterol") and higher abundance of mostly beneficial microbes (Figure 1; Table S2), including OTUs related to *Coprococcus*, a group of bacteria more abundant in healthy individuals than Crohn's disease patients;[26] *Oscillospira*, bacteria that were associated with leanness and health;[27] *Ruminococcus albus* (*Ruminococcaceae*), a species more abundant in healthy individuals than in patients with colorectal cancer;[28] and *Ruminococcus callidus* (*Ruminococcaceae*), a species that was associated with plant-based diets[29] and reduced symptoms in patients with inflammatory bowel disease.[30] However, it was also associated with increased abundance of *Streptococcus infantis*, an opportunistic pathobiont that flourished after antibiotic course.[31] *Coprococcus, Oscillospira* and *Ruminococcus* (*Ruminococcaceae*) have been shown to be heritable.[9,11,32]

The intronic SNP rs2076756 in *NOD2* was associated with the abundance of the same microbes as variant rs2066842. In addition, it was associated with increased counts of OTUs related to *Clostridium aldenense* (*Lachnospiraceae*) and *Clostridium celatum* (*Clostridicaceae*) (Figure 1; Table S2). The former species was included in a mix of 17 bacterial strains that enhanced immune regulatory T cells and induced anti-inflammatory molecules upon inoculation in germ-free mice[33] while the latter was more abundant in non-diabetic individuals than diabetic patients under metformin treatment.[34] The genus *Clostridium* (*Clostridicaceae*) and the family *Lachnospiraceae* have been shown to be heritable.[11,35]

The missense SNP rs2075820 in *NOD1* was associated with Crohn's disease[36] and to *Helicobacter pylori*-related gastric cancer.[37] In our study, it was associated with higher abundance of three OTUs related to *Blautia, Clostridium xylanolyticum* (*Lachnospiraceae*) and *Coprococcus catus* (Figure 1; Table S2). The particular OTU of *Blautia* detected here clustered in a consortium of bacteria that were associated with obesity[38] and impaired cardiometabolic health;[39] *Clostridium xylanolyticum* was correlated with lipopolysaccharide-associated metabolic endotoxemia and pathophysiological features of liver disease;[40] and *Coprococcus catus* clustered in a consortium of beneficial gut microbiota.[39] *Blautia, Coprococcus* and *Lachnospiraceae* have been shown to be heritable.[9,11]

Not only had the NOD signaling hubs associated with gut microbiota. Variants at *NLRP3, TLR4, TLR5, IL-6*, and *IL-18* were also associated with increased abundance of bacterial groups (Figure 1; Table S2), some of which are heritable, including *Mollicutes RF39*,[9] *Oscillospira*, and *Prevotella*.[11]

Appetite control

We analyzed 13 variants in nine genes that produce hormones acting in the gut-brain axis and controlling food intake (Figure S2). While the majority of associations were between gut microbiota and variants in genes encoding ghrelin

(*GHRL*), melanocortin 4 receptor (*MC4R*) and glucagon-like peptide-1 receptor (*GLP1R*), we highlight that the neuropeptide Y (*NPY*) promoter SNP rs16147 was associated with increased levels of glycated hemoglobin (HbA1c), the intronic SNP rs1859223 in the gene encoding peptide tyrosine tyrosine (*PYY*) was associated with gut-microbiota beta diversity, and the missense SNP rs6265 in the gene encoding the brain-derived neurotrophic factor (*BDNF*) was associated with greater abundance of Otu00068 (*Enterococcus casseliflavus*) (Figure 1; Table S2).

The missense SNP rs696217 in *GHRL* was associated with increased susceptibility to CMS[41] and early onset of obesity.[42] Consistently, this variant was associated with greater abundance of microbiota found in individuals with impaired health, such as *Blautia*[38] and *Ruminococcus lactaris* (*Lachnospiraceae*).[30] However, it was also associated with higher abundance of microbiota enriched in healthy individuals, such as *Christensenellaceae*,[43] *Coprococcus*,[26] *Coprococcus catus*, and unclassified *Ruminococcaceae*[39] (Figure 1; Table S2). *Blautia, Christensenellaceae, Coprococcus*, and *Lachnospiraceae* are heritable.[9]

The SNP rs571312 in *MC4R* was associated with increased levels of triglycerides, C-reactive protein and BMI.[44–46] In our study, it was associated with greater abundance of gut microbiota proper of the mucosal lining, including butyrate producers such as *Anaerostipes*,[47] *Butyricicoccus pullicaecorum*,[48] *Faecalibacterium prausnitzii*,[49] and microbes that co-occur with the beneficial mucin degrader *Akkermansia muciniphila*, such as *Alistipes putredinis, Bacteroides fragilis*, and *Paraprevotella*[39] (Figure 1; Table S2). *Butyricicoccus*[32] and *Faecalibacterium*[9] are heritable, whereas *Bacteroides* has low heritability.[11] While all these microbes are beneficial in the context of inflammatory bowel disease, the role of butyrate and other SCFAs for cardiometabolic disease is a matter of debate.[20–22]

The missense SNP rs6923761 in *GLP1R* was associated with decreased metabolic and cardiovascular biomarkers in obese females.[50] We found it was associated with higher abundance of *Catenibacterium*, a bacterium enriched in hunter-gatherers with high intake of dietary fiber;[51] *Collinsella aerofaciens*, a bacterium able to ferment a variety of dietary carbohydrates[52] that end up in the production of SCFAs; and *Oscillospira*, a heritable beneficial microbe (Figure 1; Table S2).

Energy metabolism

We analyzed 27 variants in 18 genes affecting energy expenditure (Figure S3). Genetic variants in four of the 18 genes assessed accounted for the majority of associations with CMS risk factors and gut microbial shifts, including *ADIPOQ, ADIPOR2, ADRβ2*, and *UCP3* (Figure 1; Table S2).

The synonymous SNP rs2241766 in the gene encoding adiponectin (*ADIPOQ*) has been highly studied and recent meta-analyses have associated it with CMS,[53] hypertension,[54] and coronary artery disease.[55] We found it was associated with a pro-inflammatory state (higher levels of high-sensitivity C-reactive protein: hs-CRP), as well as to increased abundance of several gut microbiota, including the same OTUs of *Coprococcus, Prevotella stercorea, Ruminococcus albus* and *Ruminococcus callidus* that were associated with variants at *NOD2* and *TLR5* (Figure 1; Table S2). As said above, many of these are purportedly beneficial microbes. In addition, it was positively associated with the abundance of *Dorea* and *Ruminococcus gnavus* (*Lachnospiraceae*), bacteria that thrive in patients with atherosclerotic cardiovascular disease,[6] adiposity, insulin resistance, and dyslipidemia.[56]

The synonymous SNP rs16928751 in the gene encoding adiponectin receptor 2 (*ADIPOR2*) was associated with type-2 diabetes[57] and cardiovascular disease.[58] We found it was associated with greater abundance of the same OTUs of purportedly beneficial *Clostridium aldenense* and *Clostridium celatum* that were associated with the SNP rs2076756 in *NOD2*. It was also associated with thriving of an OTU of *Atopobium*, abundant bacteria found in patients with atherosclerotic cardiovascular disease;[6] and, oddly, with higher counts of an OTU classified as *Defluviitalea saccharophila*, a thermophilic anaerobic bacterium isolated from slaughterhouse wastewaters (Figure 1; Table S2).

The missense SNP rs1042714 in the gene encoding beta-2 adrenergic receptor (*ADRβ2*) was associated with obesity[59] and CMS.[60] We found it was

associated with higher abundance of OTUs of *Christensenellaceae, Clostridium aerotolerans, Clostridium ramosum, Coprococcus*, and *Oscillospira* (Figure 1; Table S2). The OTUs of *Christensenellaceae* and *Coprococcus* associating with this variant were the same associating with the missense SNP rs696217 in *GHRL* (Figure 1), and are presumably beneficial. *Clostridium aerotolerans* is an anaerobic xylanolitic bacterium that clustered in a consortium that was associated with leanness and cardiometabolic regulation.[39] One of the two OTUs of *Oscillospira* found here was the same associating with the SNP rs6923761 in *GLP1R* (Figure 1); as already mentioned, *Oscillospira* is a beneficial microbe. In contrast, *Clostridium ramosum*, a member of the *Erysipelotrichi*, was associated with CMS in humans[61] and mice.[62]

Recent meta-analyses showed that the SNP rs1800849 in the 5′ untranslated region of the gene encoding the mitochondrial uncoupling protein 3 (*UCP3*) was associated with type-2 diabetes[63] and increased BMI in Asians but not in Europeans.[64] In Colombians, we found it was associated with higher abundance of OTUs related to: *Bacillus solfatarensis*, a poorly studied bacterium that branches deeply among *Bacilli* and that warrants taxonomic reclassification;[65] *Clostridium clostridioforme* (*Lachnospiraceae*), a clinically relevant bacterium involved in infection and bacteremia[66] and associated with gut microbiota with low gene diversity and CMS;[56] and *Subdoligranulum variabile*, a major butyrate-producing bacterium that was recently associated with gut microbiota of patients with atherosclerotic cardiovascular disease[6] but that, in the studied population, together with *Clostridiaceae 02d06*, clustered in a consortium of microbes that thrive in lean and cardiometabolically healthy individuals[39] (Figure 1; Table S2).

The four remaining variants were associated with higher abundance of a unique OTU: the SNP rs266729 in *ADIPOQ* was associated with an OTU related to *Subdoligranulum variabile*; the intronic SNP rs2975760 in the gene encoding calpain-10 (*CAPN10*) with an OTU related to *Lachnospira*; the intronic SNP rs709149 in the gene encoding peroxisome proliferator-activated receptor gamma (*PPARγ*) with an OTU related to *Coprococcus eutactus*; and the intronic SNP rs7903146 in the gene encoding transcription factor 7-like 2 (*TCF7L2*) with an OTU related to *Ruminococcus gauvreauii* (Figure 1; Table S2). Most of these variants and microbiota were associated with impaired cardiometabolic health[6,30,44,54,67] but the limited number of associations detected here requires further confirmation.

Strengths and limitations

Our study has important strengths. Of note, the rich set of metadata collected in the participants allowed adjusting statistical models for potential confounding such that the associations between genetic variants and cardiometabolic health were independent of the gut microbiota, and the associations between host genetics and gut microbiota were independent of the participants' CMS risk. They were also independent of the host genetic ancestry, diet intake and lifestyle features (levels of physical activity, medicament consumption and smoking status).

Limitations include our targeted gene approach, in which we focused on some genes affecting host–microbiota interactions, energy intake, and expenditure. This approach missed many genes not in our candidate list that could be associated with cardiometabolic health and gut microbiota. Furthermore, we focused on variants with past evidence of association with CMS risk and gut physiology; they were expected to have higher likelihood of association with gut microbiota and clinical outcomes. Collectively, our reduced genomic mapping precluded the attribution of associations to the tested polymorphisms. It is indeed likely that the detected associations stem from variants in strong linkage disequilibrium with the ones evaluated. Additional limitations include the cross-sectional design, which did not allow inference into causal relationships; and the possibility that unmeasured confounding by other factors could explain our results.

Conclusions

We uncovered several associations between variants in genes of innate immunity, appetite control and energy metabolism with the host cardiometabolic health and gut microbiota composition. It is noteworthy that we found many associations

between gut microbiota and genetic composition independent of the host cardiometabolic health. This and past evidence on the subject, reviewed by Goodrich et al.,[9] suggest that genetic-microbiota interactions are complex phenotypes affected by many genes with small effects. Moreover, several of the microbes exhibiting associations with host genetics have been previously shown to be heritable, providing further evidence that the composition of the gut microbiota is partly under the host genetic control. This seems to be especially true for two bacterial families: *Lachnospiraceae* and *Ruminococcaceae*, the most prevalent families of *Firmicutes*, the most abundant phylum in the studied population[39] and in others.[68] Interestingly, other taxa that were associated with health in this and other populations, such as *Akkermansia muciniphila* (*Verrucomicrobia*), and members of the families *Bacteroidaceae* (*Bacteroidetes*) and *Enterobacteriaceae* (*Proteobacteria*) showed fewer or no associations with genetic variants.

Collectively, the hypothesized consistency of associations between host genetics, cardiometabolic health and gut microbiota was unclear. In some cases, risk variants were associated with impaired cardiometabolic health and detrimental gut microbiota. However, in other cases they were associated with beneficial gut microbiota. This suggests that the phenotypic outcome of the evaluated variants might depend upon the genetic background of the studied population and its environmental context, which may be indirectly accounted for by the gut microbiota. We believe that this result represents an opportunity to reduce disease risk through personalized medicine approaches targeting specific modulation of the gut microbiota in light of individualized genetic makeups.

Materials and methods

Study population

Between July and November 2014, we enrolled 441 adult Colombians of both sexes, living in five capital cities: Bogota, Medellin, Cali, Barranquilla, and Bucaramanga (min-max distances between cities: 238–861 km). Participants were enrolled in similar proportions according to the city of residence, BMI (lean, overweight and obese), sex (male, female), and age range (18–40 years and 41–62 years). We excluded underweight participants (i.e., BMI <18.5 kg/m^2), pregnant women, individuals who had consumed antibiotics or antiparasitics in the 3 months prior to enrollment, and individuals diagnosed with neurodegenerative diseases, current or recent cancer (<1 year), and gastrointestinal diseases (Crohn's disease, ulcerative colitis, short bowel syndrome, diverticulosis or celiac disease).

This study followed the principles of the Declaration of Helsinki and had minimal risk according to the Colombian Ministry of Health (Resolution 8430 of 1993). We obtained written informed consent from all the participants. The study was approved by the Bioethics Committee of SIU–Universidad de Antioquia (act 14–24–588 dated May 28, 2014). A detailed description of the acquisition of these data can be found elsewhere.[39]

Genetic data

We isolated total genomic DNA from venous blood using the DNeasy Blood & Tissue kit (Qiagen; cat. no. 69504) following the manufacturer's instructions. This served as starting material to genotype 60 variants in 39 genes related to CMS risk and gut physiology (Table S1), clustering in three modules: innate immunity, appetite control and energy metabolism. Genes of innate immunity were targeted because this system is located at the host-microbiota interface, sensing microbes and their metabolic products (Figure S1). Impaired communication between the innate immune system and the gut microbiota has been shown to contribute to CMS.[69] Genes of appetite control and energy metabolism were selected because the former regulate food intake (Figure S2) and the latter energy expenditure (Figure S3). Imbalances between these two quantities lead to obesity and CMS.[70]

Fifty-seven out of the 60 variants mentioned above corresponded to single nucleotide polymorphisms (SNP), and three to insertion/deletion (InDel) polymorphisms (Table S1). All of them had minor allele frequencies >0.05 in the studied population (population CLM: http://www.1000gen

omes.org). SNPs were genotyped by PCR-RFLP and InDels by PCR and electrophoresis. The primers to genotype variants were obtained from previous publications or with Primer3[71] (Table S1).

In addition, we estimated the ancestral genetic composition of each participant by genotyping 40 ancestry informative markers (see accompanying paper by Guzmán-Castañeda et al.). The ancestral genetic composition of each subject served to adjust statistical models (see *Statistical analysis* below), limiting the detection of spurious associations due to hidden genetic structure produced by the recent admixture among Europeans, Native Americans and Africans of the Colombian population.[72]

Gut microbiota

Detailed laboratory and bioinformatic procedures can be found elsewhere.[39] Briefly, each participant collected a fecal sample from which the total microbial DNA was isolated using the QIAamp DNA Stool Mini Kit (Qiagen; cat. no. 51504). Afterwards, we obtained amplicons of the V4 hypervariable region of the 16S rRNA gene with the primers F515 and R806. Primers were barcoded, multiplexed, and sequenced with the Illumina MiSeq Reagent Kit v2. The raw sequenced reads were processed as previously described[39] and deposited at the SRA-NCBI (BioProject PRJNA417579).

The gut microbiota composition was summarized at different levels. First, we calculated the relative abundance of operational taxonomic units (OTUs). OTUs were obtained with the average neighbor algorithm using 97% sequence identity as threshold with Mothur v.1.36 and classified by consensus with Greengenes 13_8_99. We restricted the analyses to the set of 137 OTUs with median relative abundance ≥0.001% across participants to limit the impact of sequencing errors, chimeras and other artifacts that could have gone through our processing pipeline. Afterwards, we obtained relative abundances at the phylum, class, order, family, genus, and species levels following the Greengenes taxonomy.

The gut microbiota diversity within and between individuals (alpha and beta diversities, respectively) were also calculated. For the alpha diversity, we calculated the observed OTU richness, Shannon diversity index, inverse Simpson index, and Pielou evenness using BiodiversityR. For the beta diversity, we calculated phylogeny-based weighted UniFrac distances with GUniFrac, using a relaxed neighbor-joining tree obtained with Clearcut.

CMS risk, diet, and lifestyle

We collected clinical data informing about CMS risk: body fat distribution (BMI, waist circumference and percentage body fat), blood pressure (systolic and diastolic), blood lipids (serum levels of HDL, LDL, VLDL, total cholesterol and triglycerides), and insulin resistance (serum levels of fasting glucose, HbA1c, fasting insulin levels and the insulin-resistance index through the homeostatic model assessment: HOMA-IR). In addition, we measured the serum levels of hs-CRP informing about pro-inflammatory states, and adipokines (leptin and adiponectin). Body fat distribution and blood pressure were measured by trained personnel; blood chemistry by a professional clinical laboratory (Dinámica IPS, Medellin, Colombia).

Waist circumference, diastolic blood pressure, and the levels of triglycerides, fasting insulin, and hs-CRP were used to calculate a CMS risk scale that further served to adjust statistical models (see *Statistical analysis* below). To calculate the CMS risk scale, variables were log-transformed, centered, scaled, and added (see accompanying paper by Guzmán-Castañeda et al. for details).

In addition, each participant completed a 24-h dietary recall interview to calculate the daily caloric intake. Dietary recalls were randomly distributed in the different days of the week. Trained interviewers used validated forms, food models, geometric figures, and full-size pictures to assess portion sizes and improve accuracy. Ten percent of the participants were interviewed a second time on a different day of the week, with a minimum of 2 days between consecutive evaluations, to estimate intra-individual variability.

Levels of physical activity (number of metabolic equivalents per minute per week) were obtained with the short form of the International Physical Activity Questionnaire, and specific questionnaires

were employed for self-reporting smoking and medicament consumption.[38] For the latter, we considered all drugs taken by participants on a regular basis during the three months prior to enrollment, to the exception of over-the-counter vitamin and mineral supplements, phytotherapeutics, and contraceptives. All measurements and questionnaires were performed by trained personnel.

Statistical analysis

The associations between genetic variants and clinical parameters, and between genetic variants and gut microbiota were determined by multiple linear regressions with plink v1.07.[73] Since we analyzed quantitative, continuous response variables, we fitted additive genetic models adjusted by several covariates. We employed the – *linear* and – *standard-beta* commands to obtain standardized regression coefficients (mean 0, variance 1).

For the associations between genetic variation and clinical parameters, the statistical models were adjusted by the participants' ancestral genetic composition, city of origin, sex, and age. This because the ancestral genetic composition of Colombians affects the host cardiometabolic health and gut microbiota (see accompanying paper by Guzmán-Castañeda et al.). The city of origin is an important driver of gut microbiota composition.[39] Sex and age were considered because they affected the cardiometabolic health: males had higher CMS risk than women, and this risk was higher at older age.[38] In addition, since we wanted associations to be independent of the gut microbiota, we performed principal coordinates analysis (PCoA) on weighted UniFrac distances, and further adjusted associations by the first two components of the PCoA. We repeated the analyses with additional adjustment by caloric intake, levels of physical activity, medicament consumption, and smoking status.

For the associations between genetic variation and gut microbiota, the statistical models were adjusted by the participants' ancestral genetic composition, city of origin, sex, age, caloric intake, levels of physical activity, medicament consumption, and smoking status. In addition, since we wanted associations not to be confounded by the host cardiometabolic status, we further adjusted models by the CMS risk scale.

In all cases, p-values were adjusted for multiple comparisons using the Bonferroni correction. Associations were considered significant if they had adjusted p-values <0.05.

Acknowledgments

We thank the participants who took part in the study, and the GENMOL and Vidarium staff for their contributions during field, lab work, analysis, and discussion. We are grateful to EPS SURA and Dinámica IPS for their support throughout the study, to the Centro de Computación Científica Apolo at Universidad EAFIT for hosting supercomputing resources (http://www.eafit.edu.co/apolo), and to the University of Michigan Medical School Host Microbiome Initiative for sequencing support. Some authors of this work collaborate through the Microbiome & Health Network.

Disclosure of Potential Conflicts of Interest

We disclose that, while engaged in this project, JdlC-Z, EPV-M and JSE were employed by a food company (Grupo Empresarial Nutresa). SJG-C, ELO-V, WR, and GB had nothing to disclose.

Funding

This study was funded by Colciencias under grant 111565741349; Grupo Empresarial Nutresa, Universidad de Antioquia, Dinámica IPS, and EPS SURA. The funders of this work have not had any role in the study design; in the collection, analysis or interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

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