Gut Microbiota Differs in Composition and Functionality Between Children With Type 1 Diabetes and MODY2 and Healthy Control Subjects: A Case-Control Study

Isabel Leiva-Gea,[1]
Lidia Sánchez-Alcoholado,[2]
Beatriz Martín-Tejedor,[1]
Daniel Castellano-Castillo,[2,3]
Isabel Moreno-Indias,[2,3]
Antonio Urda-Cardona,[1]
Francisco J. Tinahones,[2,3]
José Carlos Fernández-García,[2,3] and
María Isabel Queipo-Ortuño[2,3]

Diabetes Care 2018;41:2385–2395 | https://doi.org/10.2337/dc18-0253

OBJECTIVE

Type 1 diabetes is associated with compositional differences in gut microbiota. To date, no microbiome studies have been performed in maturity-onset diabetes of the young 2 (MODY2), a monogenic cause of diabetes. Gut microbiota of type 1 diabetes, MODY2, and healthy control subjects was compared.

RESEARCH DESIGN AND METHODS

This was a case-control study in 15 children with type 1 diabetes, 15 children with MODY2, and 13 healthy children. Metabolic control and potential factors modifying gut microbiota were controlled. Microbiome composition was determined by 16S rRNA pyrosequencing.

RESULTS

Compared with healthy control subjects, type 1 diabetes was associated with a significantly lower microbiota diversity, a significantly higher relative abundance of *Bacteroides*, *Ruminococcus*, *Veillonella*, *Blautia*, and *Streptococcus* genera, and a lower relative abundance of *Bifidobacterium*, *Roseburia*, *Faecalibacterium*, and *Lachnospira*. Children with MODY2 showed a significantly higher *Prevotella* abundance and a lower *Ruminococcus* and *Bacteroides* abundance. Proinflammatory cytokines and lipopolysaccharides were increased in type 1 diabetes, and gut permeability (determined by zonulin levels) was significantly increased in type 1 diabetes and MODY2. The PICRUSt analysis found an increment of genes related to lipid and amino acid metabolism, ABC transport, lipopolysaccharide biosynthesis, arachidonic acid metabolism, antigen processing and presentation, and chemokine signaling pathways in type 1 diabetes.

CONCLUSIONS

Gut microbiota in type 1 diabetes differs at taxonomic and functional levels not only in comparison with healthy subjects but fundamentally with regard to a model of nonautoimmune diabetes. Future longitudinal studies should be aimed at evaluating if the modulation of gut microbiota in patients with a high risk of type 1 diabetes could modify the natural history of this autoimmune disease.

[1]*Pediatric Endocrinology, Hospital Materno-Infantil, Málaga, Spain*
[2]*Clinical Management Unit of Endocrinology and Nutrition, Laboratory of the Biomedical Research Institute of Málaga, Virgen de la Victoria University Hospital, Universidad de Málaga, Málaga, Spain*
[3]*Centro de Investigación Biomédica en Red (CIBER) de Fisiopatología de la Obesidad y Nutrición, Instituto Salud Carlos III, Madrid, Spain*

Corresponding author: José Carlos Fernández-García, josecarlosfdezgarcia@hotmail.com.

Received 5 February 2018 and accepted 26 August 2018.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-0253/-/DC1.

I.L.-G. and L.S.-A. contributed equally to this work.

Type 1 diabetes is a multifactorial immune-mediated disease characterized by the progressive loss of insulin-producing β-cells in the islets of Langerhans in the pancreas. The causes that lead to the appearance of type 1 diabetes have not yet been fully identified, with genetic factors playing a major role; however, environmental factors are also closely linked, such as birth delivery mode (1), diet in early life (cow milk proteins or gluten-containing cereals) (2), and widespread usage of antibiotics (3), all factors closely related to gut microbiota.

Recent studies have associated the microbiome with the development of type 1 diabetes; animal studies have demonstrated a close link between intestinal microbiota and type 1 diabetes in BioBreeding diabetes-prone rats (4) and in nonobese diabetic mice (5). Also, in children with type 1 diabetes, autoimmune positivity has been related to changes in microbiota composition (6,7).

In a previous study, we found that in comparison with healthy control subjects, children with type 1 diabetes presented large significant differences in the relative abundance of predominant phyla, families, and genera (8). Potentially, the altered microbiota profile in type 1 diabetes may be associated with alterations in the gut immune system, such as increased gut permeability (9). Recent studies have shown that commensal bacteria are crucial for the maturing and functioning of the mucosal immune system. Moreover, an impaired integrity of the intestinal barrier with an increase in permeability has been described in both animal models and human type 1 diabetes studies (10). Therefore, given that intestinal microbes may affect intestinal permeability, intestinal ecology could also play a crucial role in the development of type 1 diabetes (11). On the other hand, zonulin, a physiological modulator of intercellular tight junctions, increases gut permeability and macromolecule absorption, and previous studies claim a role for zonulin as a capital regulator of intestinal barrier function in the genesis of metabolic disorders (12).

Maturity-onset diabetes of the young (MODY) is a genetic form of diabetes that accounts for 1–2% of all diabetes cases in Europe and is associated with specific loss-of-function mutations with characteristic phenotypes (13). The most common presentation of MODY is MODY2, caused by a heterozygous inactivating mutation in the glucokinase (GCK) gene (14). To date, no studies have evaluated the gut microbiome structure in MODY2 patients. Nevertheless, MODY2 is a highly attractive model to assess the relation of gut microbiota with type 1 diabetes, as MODY2 is not normally associated with obesity, a glycemic control similar to type 1 diabetes can be achieved, and, importantly, its cause is not of autoimmune origin (15).

We hypothesize that if the fecal microbiota in type 1 diabetes differs from that of MODY2, the gut microbiota profile could constitute a novel associated risk factor for the type 1 diabetes autoimmune process. On the contrary, if the fecal microbiota were similar and different from the microbiota of healthy control subjects, this would indicate that differences in intestinal microbiota could be attributed to hyperglycemia per se.

Therefore, the aim of this case-control study is to evaluate the gut microbiota profile, functional capacity, low-grade inflammation, and gut permeability between patients with type 1 diabetes and MODY2 and healthy control subjects.

RESEARCH DESIGN AND METHODS

This study was a case-control study, including 15 children with type 1 diabetes, 15 children with MODY2, and 13 healthy control children, all under 18 years old, of Caucasian origin and with the same geographical location.

Type 1 diabetes was diagnosed according to the criteria of the American Diabetes Association (16) and the positivity of at least two persistent, confirmed anti-islet autoantibodies (anti-insulin autoantibodies, GAD autoantibodies, or tyrosine phosphatase autoantibodies). MODY2 children were diagnosed by suggestive clinical history, negative anti-islet autoantibodies, and positive genetic testing. Healthy control subjects were children with negative anti-islet autoantibodies, matched to children with type 1 diabetes and MODY2 for age, sex, race, BMI, mode of delivery, and duration of breastfeeding. In addition, patients with type 1 diabetes and MODY2 were controlled by HbA_{1c} levels. Patients with type 1 diabetes were undergoing treatment with multiple doses of insulin, whereas MODY2 patients were drug naive. Exclusion criteria to participate in this study included acute or chronic inflammatory diseases or infectious diseases or undergoing treatment with antibiotics, prebiotics, or probiotics or any other medical treatment that could potentially influence intestinal microbiota 3 months before inclusion.

The parents of all the participants completed a structured interview to obtain health status, lifestyle aspects, and dietary habits. Patients with type 1 diabetes and MODY2 were instructed to follow a standard diabetic diet, containing 40–50% of calories from carbohydrates, 20–30% from fat, and 20% from protein. Dietary intake patterns were determined from a food frequency questionnaire.

The written informed consents of the children's guardians or parents were obtained. The sampling and experimental processes were performed with the approval of the local Ethics Committee of the Regional Hospital of Málaga.

Laboratory Measurements

Serum glucose, cholesterol, triglycerides, and interleukin-1β (IL-1β) and IL-10 cytokines were measured by ELISA as previously described (17). Concentrations of IL-6, IL-13, and tumor necrosis factor (TNF)-α were quantified by ELISA assay kits (Thermo Fisher Scientific) in serum samples according to the instructions of the manufacturer. The detection limits were as follows: 7.8–500 pg/mL for IL-6, 1.6–100 pg/mL for IL-13, and 15.6–1,000 pg/mL for TNF-α.

DNA Extraction, Pyrosequencing of 16S rRNA Sequences, and Bioinformatic Analysis

Study participants collected their fecal samples in a sterile and hermetically sealed receptacle provided by the research team. Fecal samples were collected in the morning of the day of sample receipt and were immediately refrigerated in household freezers and transported to the laboratory during the following 4 h. Frozen fecal samples were transported with ice to avoid important changes of temperature that might cause bacterial DNA degradation and were subsequently stored at −80°C in the laboratory until analysis. No DNA stabilizers were added to the fecal samples.

DNA was extracted from the fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the protocol of the manufacturer. Amplification of genomic DNA was

performed using bar-coded primers that targeted the V2–V3 regions of the bacterial 16S rRNA gene. Amplification, sequencing, and basic analysis were performed using a GS Junior 454 platform according to the protocols of the manufacturer and a Titanium chemistry apparatus (Roche Applied Science, Indianapolis, IN). The 454 pyrosequencing data sets were analyzed by Quantitative Insights into Microbial Ecology (QIIME) 1.8.0 software as previously described (17). PICRUSt analysis was used to predict metagenome function by picking operational taxonomic units (OTUs) against the Greengenes database as previously described (17,18). The statistical analysis was performed in R 3.3.3. *P* values were corrected for multiple comparisons using the Benjamini-Hochberg method (17).

Intestinal Permeability

The plasma level of zonulin was determined by ELISA using commercial kits (Immunodiagnostik AG, Bensheim, Germany). The detection limit was 0.22 ng/mL.

Limulus Amebocyte Lysate Assays

Serum concentrations of lipopolysaccharides (LPS) were measured by endotoxin assay, based on a Limulus amebocyte extract with a chromogenic Limulus amebocyte lysate assay (QCL-1000; Lonza Group Ltd.) according to the instructions of the manufacturer. The sensitivity limit for the assay was 0.02 endotoxin units (EU)/mL.

Statistical Analysis

Given the exploratory nature of this study (no previous studies evaluating the differences in gut microbiota between type 1 diabetes, MODY2, and healthy control subjects), a priori sample size estimation was not performed.

The relative abundances of each OTU (taxa) were compared by a Wilcoxon signed rank test with a continuity correction using the Explicet software package specifically addressed to analyze microbiome data. All the resulting *P* values were then adjusted for multiple comparisons via the Benjamini-Hochberg false discovery rate (FDR) correction (FDR-corrected *P* value of <0.05). α- and β-diversities were achieved by QIIME, α-diversity using a nonparametric Student *t* test using a default number of Monte Carlo permutations of 999, and β-diversity with the analysis of similarities statistical method with 99 permutations. Differences in the clinical characteristics between two groups were analyzed using Mann-Whitney *U* test, and differences among the three groups were analyzed using the Kruskal-Wallis test with Bonferroni post hoc test. The Spearman correlation coefficient was calculated to estimate the linear correlations between variables. A multiple linear regression analysis was performed to identify which bacteria taxa were independent predictors for serum zonulin, LPS, inflammatory mediators, and HbA_{1c} levels in each study group. Values were considered to be statistically significant when $P \leq 0.05$.

RESULTS

Diet and Anthropometric and Biochemical Measurements

All study participants had a similar physical activity and dietary profile. No significant differences in the consumption patterns of wheat, rice, vegetables, fish, or meat were found between study groups.

The main clinical and biochemical characteristics of the study groups are shown in Table 1. As expected, glucose and HbA_{1c} levels were significantly higher in children with type 1 diabetes and MODY2 (no differences between them) when compared with healthy children. No other significant differences were observed, including breastfeeding time or mode of delivery. However, children with type 1 diabetes had significantly higher levels of IL-1β, IL-6, TNF-α, and LPS and significantly lower levels of IL-10 and IL-13 than MODY2 and healthy control subjects.

Characterization of Fecal Microbiota Pyrosequencing

The Chao index (community richness) of each group suggested a similar bacterial richness in the fecal samples from the three study groups (199.42 ± 52.26 type 1 diabetes, 195.08 ± 41.62 MODY2, 218.65 ± 51.05 healthy control subjects; $P > 0.05$). Despite a significantly lower Shannon index (microbiota diversity) in type 1 diabetes (4.76 ± 0.42) and MODY2 (4.78 ± 0.47) in comparison with healthy control subjects (5.16 ± 0.33) ($P < 0.05$), no differences in α-diversity were found between type 1 diabetes and MODY2 ($P = 0.850$).

Regarding the β-diversity of gut microbiota, weighted UniFrac principal coordinates analysis (PCoA) showed that children with type 1 diabetes had a different pattern of clustering when compared with MODY2 and healthy control subjects ($P = 0.03$ and $P = 0.02$, respectively) (Fig. 1*A* and *B*). Nevertheless, analysis of similarities with permutations revealed no significant differences between MODY2 and healthy control subjects ($P = 0.27$), as demonstrated by the two principal component scores, which accounted for 14.50% and 26.34% of total variation (Fig. 1*C*).

Taxonomy-Based Comparisons of Fecal Microbiota at the Phylum, Family, and Genus Level

The dominant phyla of all groups were Bacteroidetes and Firmicutes, followed by Proteobacteria and Actinobacteria. Individuals with type 1 diabetes showed a significant increase in the abundance of Bacteroidetes (64.69% type 1 diabetes, 39.85% MODY2, 49.85% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$) and a significant decrease in the abundance of Firmicutes (19.60% type 1 diabetes, 31.15% MODY2, 29.69% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$) and Actinobacteria (1.02% type 1 diabetes, 2.27% MODY2, 2.82% healthy control subjects; $P = 0.003$, FDR-adjusted $P = 0.007$) when compared with MODY2 and healthy control subjects. The frequency of Bacteroidetes was significantly lower in MODY2 than in healthy control subjects (FDR-adjusted $P < 0.001$). Proteobacteria was significantly lower in type 1 diabetes when compared with healthy control subjects (1.68% type 1 diabetes, 3.06% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$), but not regarding MODY2 (1.68% type 1 diabetes, 1.80% MODY2; $P = 0.699$, FDR-adjusted $P > 0.05$). The remainder of the bacterial population belonged to five other phyla that had a relative abundance $<1\%$ (Supplementary Fig. 1 and Supplementary Data). In addition, the Firmicutes-to-Bacteroidetes ratio was significantly lower in type 1 diabetes than in MODY2 and healthy control subjects (0.30% type 1 diabetes, 0.76% MODY2, 0.58% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$).

A total of 16 families were identified among the fecal samples analyzed. Within the Bacteroidetes, two different families were significantly higher in type 1 diabetes when compared with MODY2 and healthy control subjects: Bacteroidaceae (71.48% type 1 diabetes, 55.43% MODY2, 59.62% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$) and Rikenellaceae (15.26% type 1

Table 1—Baseline anthropometric and biochemical variables

	Healthy control subjects (n = 13)	Type 1 diabetes (n = 15)	MODY2 (n = 15)	P
Male/female, n	7/6	7/8	7/8	
Vaginal delivery/cesarean section, n	8/5	10/5	10/5	
Age (years)	12.25 ± 2.92	12.56 ± 3.59	13.06 ± 3.20	0.654
BMI (kg/m^2)	17.35 ± 1.82	17.89 ± 2.01	18.23 ± 1.90	0.430
Age of onset of diabetes (years)		7.35 ± 1.76	6.91 ± 1.40	0.455
Duration of diabetes (years)		5.68 ± 1.84	6.10 ± 1.97	0.551
Breastfeeding time (months)	6.58 ± 2.32	6.41 ± 2.81	6.54 ± 3.2	0.911
Birth weight (kg)	3.19 ± 0.45	3.28 ± 0.38	3.22 ± 0.55	0.249
Weight (kg)	37.35 ± 9.0	38.32 ± 8.92	36.91 ± 7.72	0.765
HbA_{1c} (%)	4.47 ± 0.21^a	6.26 ± 0.38^b	6.11 ± 0.33^b	0.001
HbA_{1c} (mmol/mol)	25.3 ± 2.3^a	44.9 ± 4.2^b	43.3 ± 3.6^b	0.001
Triglycerides (mg/dL)	52.67 ± 9.43	53.50 ± 10.15	53.88 ± 9.88	0.843
Cholesterol (mg/dL)	153.88 ± 14.64	153.62 ± 16.87	154.5 ± 17.9	0.920
IL-1β (pg/mL)	83.21 ± 28.25^b	119.41 ± 27.12^a	89.45 ± 25.31^b	0.004
IL-10 (pg/mL)	126.67 ± 9.87^b	81.03 ± 9.97^a	121.28 ± 5.46^b	0.001
IL-6 (pg/mL)	85.71 ± 6.28^b	109.89 ± 6.50^a	88.98 ± 7.49^b	0.001
TNF-α (pg/mL)	164.31 ± 16.78^b	373.46 ± 90.65^a	169.54 ± 18.3^b	0.001
IL-13 (pg/mL)	50.15 ± 8.37^b	22.83 ± 5.47^a	45.46 ± 7.31^b	0.001
LPS (EU/mL)	0.49 ± 0.10^b	1.10 ± 0.40^a	0.56 ± 0.38^b	0.001

Values are presented as means ± SD unless otherwise specified. P value was based on Kruskal-Wallis test. Mann-Whitney U test was used to compare the clinical characteristics between two groups. Different superscript letters (a,b) next to values in a row indicate that the means of the different groups are significantly different ($P < 0.05$, Bonferroni post hoc test).

diabetes, 7.01% MODY2, 11.19% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$). Prevotellaceae (2.65% type 1 diabetes, 15.41% MODY2, 1.25% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.003$) was significantly higher in type 1 diabetes than in healthy control subjects, and Prevotellaceae was significantly higher in MODY2 than in type 1 diabetes ($P < 0.001$, FDR-adjusted $P < 0.001$). In the Firmicutes, another three families were significantly higher in type 1 diabetes compared with MODY2 and healthy control subjects: Ruminococcaceae (38.19% type 1 diabetes, 25.74% MODY2, 30.84% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$), Veillonellaceae (31.94% type 1 diabetes, 20.33% MODY2, 18.03% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.003$), and Streptococcaceae (1.93% type 1 diabetes, 0.96% MODY2, 0.56% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.004$). No significant differences at the Firmicutes family level were found between MODY2 and healthy control subjects except in the abundance of Ruminococcaceae ($P < 0.001$, FDR-adjusted $P < 0.001$). In healthy children, only Lachnospiraceae (22.1% type 1 diabetes, 27.95% MODY2, 42.0% healthy control subjects; $P = 0.002$, FDR-adjusted $P = 0.015$) was significantly higher in comparison with type 1 diabetes and MODY2. In Actinobacteria, a significant enrichment of Bifidobacteriaceae (2.71% type 1 diabetes, 4.50% MODY2, 5.68% healthy control subjects; $P = 0.004$, FDR-adjusted $P = 0.017$) was found in healthy control subjects when compared with MODY2 and type 1 diabetes. Finally, for the Proteobacteria families, a significant increase of Enterobacteriaceae (25.20% type 1 diabetes, 15.03% MODY2, 13.04% healthy control subjects; $P = 0.006$, FDR-adjusted $P = 0.03$) was found in type 1 diabetes when compared with MODY2 and healthy control subjects, but no significant differences were found for Alcaligenaceae (62.18% type 1 diabetes, 49.03% MODY2, 57.07% healthy control subjects; $P = 0.019$, FDR-adjusted $P = 0.267$) (Supplementary Fig. 2 and Supplementary Data).

Twelve genera were differentially abundant between study groups. For the Bacteroidetes genera, the type 1 diabetes group was significantly enriched with sequences attributed to the genus *Bacteroides* (72.21% type 1 diabetes, 52.41% MODY2, 58.45% healthy control subjects; $P < 0.001$, FDR-adjusted $P < 0.001$). *Prevotella* was significantly increased in MODY2 and type 1 diabetes when compared with healthy control subjects (1.95% type 1 diabetes, 8.32% MODY2, 1.42% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.005$). Regarding Firmicutes, the relative abundances of four genera were significantly higher in type 1 diabetes than in MODY2 and healthy control subjects: *Ruminococcus* (17.19% type 1 diabetes, 5.74% MODY2, 8.85% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.002$), *Blautia* (15.50% type 1 diabetes, 5.73% MODY2, 3.74% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.003$), *Veillonella* (21.59% type 1 diabetes, 12.33% MODY2, 7.20% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.006$), and *Streptococcus* (4.86% type 1 diabetes, 2.64% MODY2, 1.47% healthy control subjects; $P = 0.003$, FDR-adjusted $P = 0.028$). In addition, four genera were significantly lower in type 1 diabetes and MODY2 than in healthy control subjects: *Lachnospira* (5.34% type 1 diabetes, 7.15% MODY2, 15.25% healthy control subjects; $P < 0.001$, FDR-adjusted $P = 0.012$), *Roseburia* (1.35% type 1 diabetes, 4.16% MODY2, 6.99% healthy control subjects; $P < 0.001$, FDR-adjusted

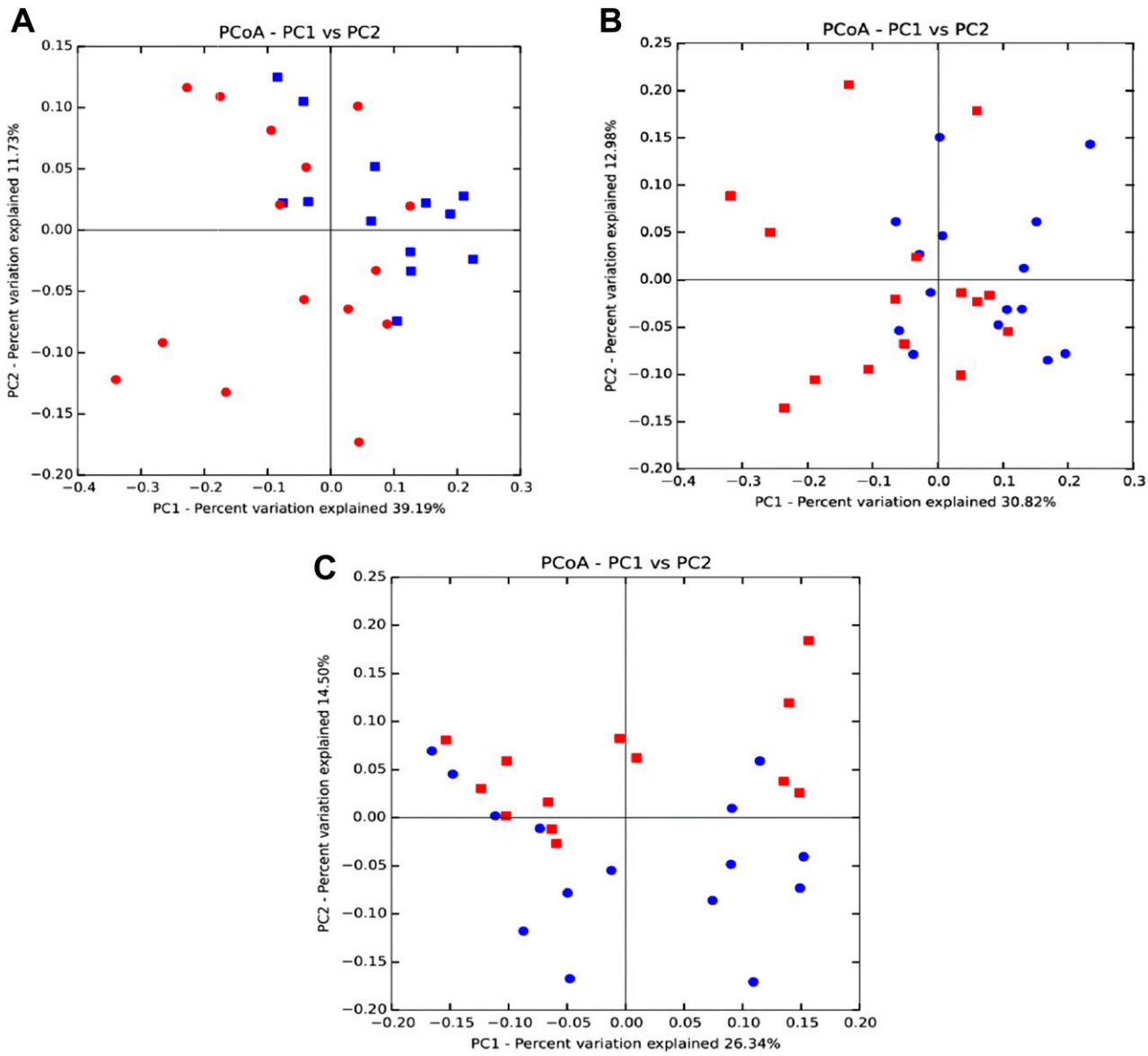

Figure 1—Clustering of fecal bacterial communities according to the different study groups by PCoA using weighted UniFrac distances. Each point corresponds to a community coded according to the child group. The percentage of variation explained by the plotted principal coordinates is indicated on the axes. *A*: Type 1 diabetes (red dots) vs. healthy control subjects (blue dots). *B*: Type 1 diabetes (blue dots) vs. MODY2 (red dots). *C*: MODY2 (blue dots) vs. healthy control subjects (red dots). PC1, principal coordinate 1; PC2, principal coordinate 2.

P = 0.015), *Anaerostipes* (2.15% type 1 diabetes, 2.97% MODY2, 5.79% healthy control subjects; $P < 0.001$, FDR-adjusted P = 0.023), and *Faecalibacterium* (4.21% type 1 diabetes, 8.08% MODY2, 13.26% healthy control subjects; $P < 0.001$, FDR-adjusted P = 0.004). In Actinobacteria, only the *Bifidobacterium* was significantly increased in healthy control subjects when compared with type 1 diabetes (1.93% type 1 diabetes, 6.75% healthy control subjects; $P < 0.001$, FDR-adjusted P = 0.017), but not regarding MODY2 (6.07% MODY2, 6.75% healthy control subjects; P = 0.503, FDR-adjusted $P > 0.05$). Finally, in the Proteobacteria genera, *Sutterella* and *Enterobacter* were significantly increased in type 1 diabetes when compared with MODY2 and healthy control subjects (61.30% type 1 diabetes, 49.32% MODY2, 57.07% healthy control subjects [P = 0.002, FDR-adjusted P = 0.027] and 16.18% type 1 diabetes, 8.25% MODY2, 6.99% healthy control subjects [$P < 0.001$, FDR-adjusted P = 0.003], respectively). No significant differences between study groups were found in the relative abundance of *Desulfovibrio*, *Haemophilus*, and *Bilophila* (FDR-adjusted $P > 0.05$) (Supplementary Fig. 3 and Supplementary Data). A cladogram showing differences in fecal microbiota between study groups is shown in Fig. 2.

Serum Zonulin Levels in Type 1 Diabetes, MODY2, and Healthy Control Subjects

Patients with MODY2 showed higher serum zonulin levels (4.80 ± 1.41 ng/mg protein) when compared with patients with type 1 diabetes (3.94 ± 1.44 ng/mg protein, P = 0.02) and healthy control subjects (3.21 ± 1.24 ng/mg protein, $P < 0.001$). Significant differences in serum

Figure 2—Cladogram showing differentially abundant taxa of the fecal microbiota in type 1 diabetes, MODY2, and healthy controls. Linear discriminant analysis effect size analysis ($P < 0.05$ for Kruskal-Wallis test) was used to validate the statistical significance and the effect size of the differential abundances of taxa in the study groups. The diameter of each circle is proportional to its abundance.

zonulin levels were also found between type 1 diabetes and control subjects ($P < 0.05$).

The Relationship Between Gut Microbiota Composition, Serum Zonulin, HbA_{1c}, Inflammatory Mediators, and LPS

Several significant correlations between the relative abundance of specific bacteria at different taxa levels and serum zonulin levels and HbA_{1c} were found in children with type 1 diabetes and MODY2, but not in healthy control subjects (Table 2). In addition, significant correlations between serum levels of IL-1β, IL-6, TNF-α, IL-10, IL-13, and LPS and gut microbiota composition were found in type 1 diabetes (Supplementary Table 1).

The linear regression analysis including all the bacterial groups showed that the increase in *Bacteroides* ($P = 0.002$, $\beta = 0.995$, $r^2 = 0.94$) and *Veillonella* ($P = 0.021$, $\beta = 0.636$, $r^2 = 0.93$) and the decrease in *Faecalibacterium* ($P = 0.041$, $\beta = -0.671$, $r^2 = 0.94$) and *Roseburia* ($P = 0.038$, $\beta = -0.694$, $r^2 = 0.93$) in type 1 diabetes and the rise in *Prevotella* in MODY2 ($P = 0.002$, $\beta = 0.682$, $r^2 = 0.90$) were associated with the increment in serum zonulin levels. On the other hand, the increase in the abundance of *Blautia* ($P = 0.043$,

Table 2—Correlation between gut microbiota composition and serum zonulin and HbA_{1c} levels in children with type 1 diabetes and MODY2

Zonulin			HbA_{1c}		
	Type 1 diabetes	MODY2		Type 1 diabetes	MODY2
Ruminococcus	−0.162 (P = 0.55)	−0.540 (P = 0.036)	*Ruminococcus*	−0.362 (P = 0.18)	−0.584 (P = 0.025)
Roseburia	−0.320 (P = 0.031)	−0.732 (P = 0.48)	Firmicutes-to-Bacteroidetes ratio	−0.561 (P = 0.029)	−0.332 (P = 0.45)
Prevotella	0.560 (P = 0.37)	0.798 (P = 0.037)	*Blautia*	0.559 (P = 0.038)	0.740 (P = 0.79)
Faecalibacterium	−0.703 (P = 0.027)	−0.547 (P = 0.49)	*Streptococcus*	0.068 (P = 0.018)	0.441 (P = 0.12)
Bacteroides	0.739 (P = 0.004)	0.350 (P = 0.090)			
Veillonella	0.570 (P = 0.033)	0.704 (P = 0.04)			

Correlations are reported as Spearman ρ (r), and P values are given in parentheses. Statistical significance was set at a P value of <0.05.

β = 0.469, r^2 = 0.93) and the decrease in the Firmicutes-to-Bacteroidetes ratio (P = 0.001, β = −0.947, r^2 = 0.91) in patients with type 1 diabetes were associated with HbA_{1c} levels, whereas in MODY2 patients, only the decrease in *Ruminococcus* (P = 0.003, β = −0.877, r^2 = 0.92) was associated with HbA_{1c} levels. In addition, the increase in the relative abundance of *Bacteroides* (P = 0.006, β = 0.991, r^2 = 0.95) and *Veillonella* (P = 0.012, β = 0.825, r^2 = 0.95) and the decrease in *Bifidobacterium* (P = 0.039, β = −0.654, r^2 = 0.94), *Roseburia* (P = 0.032, β = −0.675, r^2 = 0.95), and *Faecalibacterium* (P = 0.023, β = −0.678, r^2 = 0.94) in type 1 diabetes were associated with serum IL-1β levels. Finally, in type 1 diabetes, the increase in the abundance of *Bacteroides* (P = 0.007, β = 0.632, r^2 = 0.85) and the decrease in *Roseburia* (P = 0.029, β = −0.518, r^2 = 0.85) were associated with serum IL-6 and TNF-α levels, and the increase in *Streptococcus* (P = 0.014, β = 0.616, r^2 = 0.82) and the decrease in *Bifidobacterium* (P = 0.009, β = −0.904, r^2 = 0.82) were associated with serum IL-10 and IL-13 levels. Regarding the serum levels of LPS, only the increase in the abundance of *Veillonella* (P = 0.006, β = 0.887, r^2 = 0.83) was associated with the levels in type 1 diabetes.

Functional Differences in the Gut Microbiota of Study Groups

The PICRUSt analysis indicated that genes for energy (P = 0.015) and carbohydrate metabolism (P = 0.014) were significantly depleted in type 1 diabetes in comparison with MODY2 and healthy control subjects. Nevertheless, lipid metabolism functions (P = 0.008), together with amino acid metabolism functions (P = 0.013), were overrepresented in type 1 diabetes when compared with the other groups. In addition, pathways of lipid and carbohydrate metabolism in type 1 diabetes had a significant enrichment in the proportion of genes related to arachidonic acid and propanoate metabolism (P = 0.002 and P = 0.003, respectively), in comparison with MODY2 and healthy subjects (Fig. 3). Metagenomic comparison between study groups showed that gene families linked to amino acid metabolism, such as amino acid–related enzymes (P = 0.010), arginine and proline metabolism (P = 0.006), and valine, leucine, and isoleucine biosynthesis (P = 0.020), were significantly increased in type 1 diabetes, whereas genes for glutation metabolism (P = 0.005) were significantly depleted. Conversely, in MODY2 and healthy control subjects, in comparison with type 1 diabetes, a significant increase in genes related to glycolysis/gluconeogenesis (P = 0.018), pentose phosphate pathway (P = 0.015), and butanoate metabolism (P = 0.023), as well as in energy metabolism genes such as sulfur (P = 0.014), nitrogen metabolism (P = 0.012), and oxidative phosphorylation (P = 0.018), was detected (Fig. 3).

Finally, when compared with MODY2 and healthy control subjects, gut microbiota from patients with type 1 diabetes was significantly enriched with genes for antigen processing and presentation (P = 0.010), chemokine signaling pathways (P = 0.001), LPS biosynthesis (P = 0.008), bacterial invasion of epithelial cells (P = 0.017), and ABC transporters (P = 0.016) (Fig. 3).

CONCLUSIONS

In this study comparing the bacterial flora in type 1 diabetes, MODY2, and healthy control subjects, we show that type 1 diabetes is associated with different gut microbial composition and functional profiling, in comparison with MODY2 and healthy control subjects. Also, we report that gut permeability, determined by serum zonulin levels, is significantly increased in MODY2 and type 1 diabetes when compared with healthy control subjects. Another key finding in this study is a significantly lower diversity of the dominant bacterial community in type 1 diabetes and MODY2 when compared with healthy control subjects.

The higher loss of diversity in patients with type 1 diabetes when compared with healthy control subjects might be related to the autoimmune process. In a previous study evaluating type 1 diabetes markers, a lower microbial diversity was found in fecal samples of children with at least two positive disease-associated autoantibodies than in samples from autoantibody-negative children matched for age, sex, early feeding history, and HLA genotyping (19). Also, in longitudinal studies from birth with children at risk for type 1 diabetes, a decrease of microbial diversity occurred just before the appearance of anti-islet cell antibodies and subsequent onset of type 1 diabetes (20).

We demonstrate that there are clear differences in the gut microbiota profile of type 1 diabetes, MODY2, and healthy control subjects, given that in the OTU-based PCoA plot analysis, patients with type 1 diabetes clustered separately when compared with MODY2 patients and healthy control subjects. Accordingly, children with type 1 diabetes showed a significant increase in the relative abundance of *Bacteroides*, *Ruminococcus*, *Veillonella*, *Blautia*, *Enterobacter*, and *Streptococcus* genera and a decrease in the relative abundance of *Bifidobacterium*, *Roseburia*, *Faecalibacterium*, and *Lachnospira*, when compared with MODY2 and healthy control subjects. On the other hand, MODY2 was related to a significant increase in

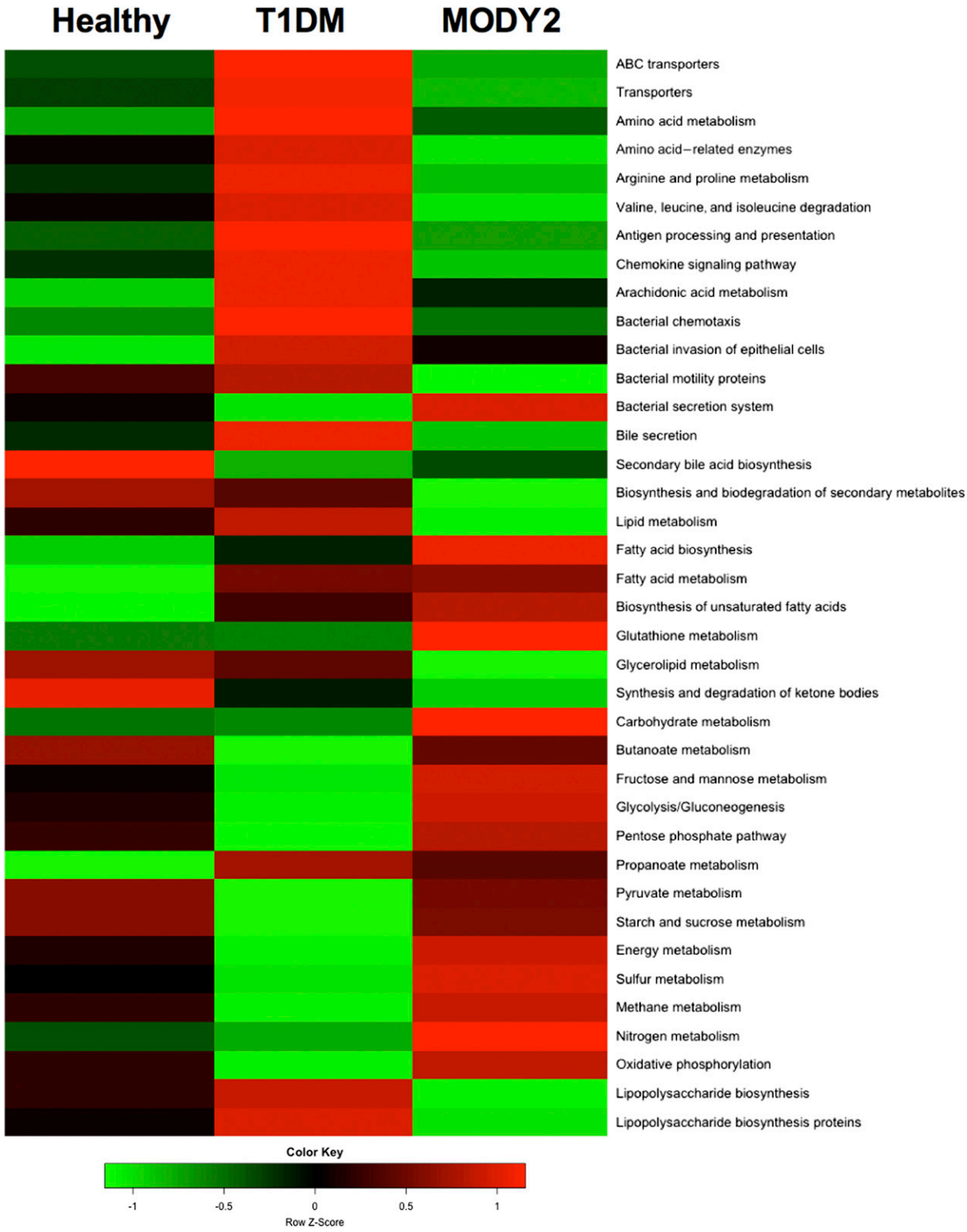

Figure 3—Predicted functional composition of metagenomes based on 16S rRNA gene sequencing data of type 1 diabetes (T1DM), MODY2, and healthy control subjects. Heat map of differentially abundant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways identified in the three study groups. The values of color in the heat map represent the normalized relative abundance of KEGG pathways.

Prevotella abundance and a significant decrease in *Ruminococcus* and *Bacteroides*, when compared with type 1 diabetes and healthy control subjects. Despite the great variability of intestinal microbiota in type 1 diabetes (regardless of ethnicity, age, and geography), most published studies (including the present one) identify *Bacteroides* (acetate- and propionate-producing bacteria) as the main genus leading to type 1 diabetes–associated dysbiosis (21,22).

Bacteroides, a Gram-negative bacterium, could contribute to chronic inflammation by the impairment of the barrier

function of the epithelial cell layer (23). In our study, subjects with type 1 diabetes, when compared with MODY2 and healthy control subjects, presented significantly higher levels of LPS and proinflammatory cytokines IL-1β, IL-6, and TNF-α along with a significant decrease in anti-inflamatory citokines IL-10 and IL-13. Moreover, the significant correlations found between gut microbiota and host serum levels of LPS and cytokines indicate that the significant depletion of *Faecalibacterium*, *Roseburia*, and *Bifidobacterium*, reported to exert anti-inflammatory effects and strengthen gut barrier function (through cytokine production modulation and butyrate production, respectively) (24,25), together with the significant increase of *Veillonella* (a lactate-utilizing and propionate-producing bacteria with proinflammatory capacity) and *Bacteroides*, could raise paracellular permeability and low-grade inflammation in type 1 diabetes (26,27). This situation could allow luminal antigens to escape from the gut and promote islet-directed autoimmune responses. Therefore, the lower abundance of anti-inflammatory bacteria unable to regulate epithelial integrity could be associated with the intestinal immune activation in type 1 diabetes (28,29).

On the other hand, we have found a significant increase in the relative abundance of *Prevotella* in MODY2 when compared with type 1 diabetes and healthy control subjects. Given that *Prevotella* is an important succinate producer and mucin degrader bacteria, this could suggest a lack of mucin on the intestinal epithelial layer of MODY2 subjects, disturbing the protection of the host mucosal surfaces. Moreover, *Prevotella*-produced succinate is a bacterial metabolite that leads to inhibition of hepatic glucose output and improves glycemic control and energy metabolism through the activation of intestinal gluconeogenesis (30). Accordingly, Spégel et al. (13) suggested that in MODY2, despite the shift in insulin secretion, metabolic control remains intact probably due to the existence of compensatory mechanisms external to β-cells. Therefore, we suggest that the significant increase in the abundance of *Prevotella* found in MODY2 patients could be related to the maintenance of glycemic control.

In addition, we have found in type 1 diabetes a significant positive correlation between zonulin levels and the relative abundance of *Bacteroides* and *Veillonella*, and a significant negative correlation with *Faecalibacterium* and *Roseburia*, as well as a significant positive correlation between zonulin and *Prevotella* in patients with MODY2. Zonulin regulates intestinal permeability by modulating intercellular tight junctions. A possible mechanism is based on bacterial antigens and microorganism toxins that may be sensed by molecules related to epithelial cell tight junctions like zonulin, altering their activity and consequently increasing gut permeability and bacterial translocation (21,31). However, zonulin levels alone do not show impaired epithelial integrity, and in accordance with other authors, the impaired epithelial integrity found in the patients with type 1 diabetes might be caused by the binding of *Veillonella* (a lactate-utilizing bacteria) to immature cells in colonic crypts able to ferment glucose to lactate, which are pushed to the luminal surface and form poor tight junctions (32). Also, in type 1 diabetes, there is an increased intestinal permeability that may affect absorption of antigens capable of attacking and damaging pancreatic β-cells (33–35). Thus, the significant increase in gut permeability may be an important player in the development of type 1 diabetes. Moreover, some authors have stated that a leaky gut could be involved in the development of type 1 diabetes complications, as high serum LPS activity has been associated with features of metabolic syndrome, visceral fat mass, and the progression of kidney disease in type 1 diabetes (36–38).

On the other hand, we have shown that the relative abundance of *Blautia* and the Firmicutes-to-Bacteroidetes ratio were positively correlated with HbA_{1c} in type 1 diabetes, and in MODY2, HbA_{1c} levels were negatively correlated with the abundance of *Ruminococcus*. Butyrate-producing intestinal bacteria such as *Ruminococcus* and *Blautia* could play an important role in blood glucose regulation and lipid metabolism, as shown by fecal transplantation studies (39). Some authors have reported that *Blautia* is positively correlated with serum glucose, HbA_{1c} levels, and the number of type 1 diabetes autoantibodies, suggesting that *Blautia* might influence the development of type 1 diabetes through the regulation of T-cell differentiation (20,40).

Also, we report a significantly lower Firmicutes-to-Bacteroidetes ratio in type 1 diabetes and a negative correlation between this ratio and HbA_{1c}. This has been previously demonstrated by our group (8) and by other authors who have reported a decline in Firmicutes and an increase in Bacteroidetes abundance over time in the gut microbiome until the development of type 1 diabetes (41).

The PICRUSt analysis demonstrated that several microbial functions were significantly over- or underrepresented between study groups, due to important differences in bacteria composition. Thus, when compared with healthy control subjects and MODY2, gut microbiota in type 1 diabetes showed a depleted abundance of genes involved in metabolic pathways such as carbohydrate and energy metabolism. Conversely, there was an increase in genes related to lipid and amino acid metabolism, ABC transport, LPS biosynthesis, arachidonic acid metabolism, antigen processing and presentation, and chemokine signaling pathways related to inflammation and immune response. The relative abundance of genes associated with a given pathway may indicate an increased metabolic capacity of the gut microbiota with regard to this pathway. Interestingly, a higher level of arachidonic acid metabolism (inflammatory intermediate) in type 1 diabetes gut microbiota might be the result of a growth of proinflammatory pathobionts in the gut (42).

Our study has certain limitations but also some important strengths. The limitations include the relatively small sample size (mainly caused by the very low prevalence of MODY2 in children), which may not be enough for detecting differences between low-abundance microbes that may be of relevance or to assess overall differences between type 1 diabetes and MODY2. Another limitation is the inherent nature of the study, a cross-sectional design, where only an association and not a cause can be inferred, and where potential changes of the gut microbioma from a healthy state to a gut pattern potentially boosting autoimmunity in type 1 diabetes were not evaluated. Finally, although PICRUSt gives functional information of potential importance, it is a limitation compared with shotgun metagenomics analysis. On the other hand, the strengths of our study lie in the careful design, the inclusion of patients

with MODY2, the well-matched cohorts (including age, mode of delivery, breastfeeding time, antibiotics use, BMI, and glycemic levels), and the next-generation sequencing of the microbiome.

In conclusion, our data suggest that gut microbiota in type 1 diabetes not only differs at the taxonomic level regarding MODY2 and healthy subjects but also at the functional level, involving different metabolic pathways.

Type 1 diabetes was characterized by a less diverse gut microbiota profile, with Bacteroidetes dominating at the phylum level and an increased proportion of proinflammatory bacteria. Therefore, the type 1 diabetes gut microbiota profile was associated with impaired epithelial integrity, low-grade inflammation, and autoimmune response. On the other hand, gut microbiota in MODY2 was characterized by a dominance of succinate-producer and mucin-degrading bacteria, potentially modulating glucose metabolism in the intestine of the host and influencing systemic energy homeostasis.

Our results provide evidence of a different microbiota profile and functionality in children with type 1 diabetes, not only in comparison with healthy subjects but fundamentally with regard to a model of nonautoimmune diabetes, suggesting a potential role of gut microbiota in the autoimmune process involved in type 1 diabetes. Given that most studies to date have shown that intestinal microbiota, rather than being involved in the initiation of the disease process of type 1 diabetes, might be involved in the progression from β-cell autoimmunity to the clinical disease (43), future longitudinal studies should be aimed at evaluating if the modulation of gut microbiota in patients with a high risk of type 1 diabetes could modify the natural history of this autoimmune disease.

Acknowledgments. The research group belongs to the Centro de Investigación Biomédica en Red (CIBER, CB06/03/0018) of the Instituto de Salud Carlos III.

Funding. This study was supported by the Ministerio de Educación, Cultura y Deporte (FPU13/04211 to D.C.-C.), the Miguel Servet Type I program from the Instituto de Salud Carlos III (cofounded by the Fondo Europeo de Desarrollo Regional) (CP16/00163 to I.M.-I. and CP13/00065 to M.I.Q.-O.), and the Servicio Andaluz de Salud (research contract B-0033-2014 to J.C.F.-G.).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. I.L.-G. conceived the study, developed the experimental design, acquired and selected all samples used in this study, compiled the database, performed statistical analysis and data interpretation, and wrote the manuscript and provided critical revision. L.S.-A. performed laboratory assays and wrote the manuscript. B.M.-T. acquired and selected all samples used in this study, compiled the database, performed statistical analysis and data interpretation, and wrote the manuscript and provided critical revision. D.C.-C. performed laboratory assays, compiled the database, and performed statistical analysis and data interpretation. I.M.-I. performed laboratory assays, compiled the database, performed statistical analysis and data interpretation, and wrote the manuscript. A.U.C. acquired and selected all samples used in this study, compiled the database, and performed statistical analysis and data interpretation. F.J.T. conceived the study, developed the experimental design, and wrote the manuscript and provided critical revision. J.C.F.-G. conceived the study, developed the experimental design, compiled the database, performed statistical analysis and data interpretation, and wrote the manuscript and provided critical revision. M.I.Q.-O. conceived the study, developed the experimental design, acquired and selected all samples used in this study, performed laboratory assays, compiled the database, performed statistical analysis and data interpretation, and wrote the manuscript and provided critical revision. All authors read and approved the final manuscript. J.C.F.-G. and M.I.Q.-O. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Khashan AS, Kenny LC, Lundholm C, Kearney PM, Gong T, Almqvist C. Mode of obstetrical delivery and type 1 diabetes: a sibling design study. Pediatrics 2014;134:e806–e813
2. Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. Lancet 2016;387:2340–2348
3. Hansen CH, Krych L, Nielsen DS, et al. Early life treatment with vancomycin propagates *Akkermansia muciniphila* and reduces diabetes incidence in the NOD mouse. Diabetologia 2012;55:2285–2294
4. Roesch LF, Lorca GL, Casella G, et al. Culture-independent identification of gut bacteria correlated with the onset of diabetes in a rat model. ISME J 2009;3:536–548
5. Kriegel MA, Sefik E, Hill JA, Wu HJ, Benoist C, Mathis D. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc Natl Acad Sci U S A 2011;108:11548–11553
6. Vaarala O. Is the origin of type 1 diabetes in the gut? Immunol Cell Biol 2012;90:271–276
7. He C, Shan Y, Song W. Targeting gut microbiota as a possible therapy for diabetes. Nutr Res 2015;35:361–367
8. Murri M, Leiva I, Gomez-Zumaquero JM, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC Med 2013;11:46
9. Vaarala O. Leaking gut in type 1 diabetes. Curr Opin Gastroenterol 2008;24:701–706
10. Bosi E, Molteni L, Radaelli MG, et al. Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia 2006;49:2824–2827
11. Mathis D, Benoist C. The influence of the microbiota on type-1 diabetes: on the threshold of a leap forward in our understanding. Immunol Rev 2012;245:239–249
12. Zak-Gołąb A, Kocełak P, Aptekorz M, et al. Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects. Int J Endocrinol 2013;2013:674106
13. Spégel P, Ekholm E, Tuomi T, Groop L, Mulder H, Filipsson K. Metabolite profiling reveals normal metabolic control in carriers of mutations in the glucokinase gene (MODY2). Diabetes 2013;62:653–661
14. Nyunt O, Wu JY, McGown IN, et al. Investigating maturity onset diabetes of the young. Clin Biochem Rev 2009;30:67–74
15. Fajans SS, Bell GI. MODY: history, genetics, pathophysiology, and clinical decision making. Diabetes Care 2011;34:1878–1884
16. American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In *Standards of Medical Care in Diabetes—2017*. Diabetes Care 2017;40(Suppl. 1):S11–S24
17. Sanchez-Alcoholado L, Castellano-Castillo D, Jordán-Martínez L, et al. Role of gut microbiota on cardio-metabolic parameters and immunity in coronary artery disease patients with and without type-2 diabetes mellitus. Front Microbiol 2017;8:1936
18. Bhute S, Pande P, Shetty SA, et al. Molecular characterization and meta-analysis of gut microbial communities illustrate enrichment of Prevotella and Megasphaera in Indian subjects. Front Microbiol 2016;7:660
19. de Goffau MC, Luopajärvi K, Knip M, et al. Fecal microbiota composition differs between children with β-cell autoimmunity and those without. Diabetes 2013;62:1238–1244
20. Kostic AD, Gevers D, Siljander H, et al.; DIABIMMUNE Study Group. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell Host Microbe 2015;17:260–273
21. Mejía-León ME, Petrosino JF, Ajami NJ, Domínguez-Bello MG, de la Barca AM. Fecal microbiota imbalance in Mexican children with type 1 diabetes. Sci Rep 2014;4:3814
22. Brown CT, Davis-Richardson AG, Giongo A, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One 2011;6:e25792
23. Tlaskalová-Hogenová H, Stěpánková R, Kozáková H, et al. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cell Mol Immunol 2011;8:110–120
24. Sokol H, Pigneur B, Watterlot L, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 2008;105:16731–16736
25. Tamanai-Shacoori Z, Smida I, Bousarghin L, et al. *Roseburia* spp.: a marker of health? Future Microbiol 2017;12:157–170
26. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability–a new target for disease

prevention and therapy. BMC Gastroenterol 2014;14:189
27. Burger-van Paassen N, Vincent A, Puiman PJ, et al. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. Biochem J 2009;420:211–219
28. Li N, Hatch M, Wasserfall CH, et al. Butyrate and type 1 diabetes mellitus: can we fix the intestinal leak? J Pediatr Gastroenterol Nutr 2010;51:414–417
29. Vaarala O. Gut microbiota and type 1 diabetes. Rev Diabet Stud 2012;9:251–259
30. De Vadder F, Kovatcheva-Datchary P, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. Cell Metab 2016; 24:151–157
31. Vaarala O. Human intestinal microbiota and type 1 diabetes. Curr Diab Rep 2013;13:601–607
32. Lopez CA, Miller BM, Rivera-Chávez F, et al. Virulence factors enhance *Citrobacter rodentium* expansion through aerobic respiration. Science 2016;353:1249–1253
33. Watts T, Berti I, Sapone A, et al. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. Proc Natl Acad Sci U S A 2005;102: 2916–2921
34. Gülden E, Wong FS, Wen L. The gut microbiota and type 1 diabetes. Clin Immunol 2015; 159:143–153
35. Lee AS, Gibson DL, Zhang Y, Sham HP, Vallance BA, Dutz JP. Gut barrier disruption by an enteric bacterial pathogen accelerates insulitis in NOD mice. Diabetologia 2010;53:741–748
36. Nymark M, Pussinen PJ, Tuomainen AM, Forsblom C, Groop PH, Lehto M; FinnDiane Study Group. Serum lipopolysaccharide activity is associated with the progression of kidney disease in Finnish patients with type 1 diabetes. Diabetes Care 2009;32:1689–1693
37. Lassenius MI, Pietiläinen KH, Kaartinen K, et al.; FinnDiane Study Group. Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. Diabetes Care 2011;34:1809–1815
38. Lassenius MI, Ahola AJ, Harjutsalo V, Forsblom C, Groop PH, Lehto M. Endotoxins are associated with visceral fat mass in type 1 diabetes. Sci Rep 2016;6:38887
39. Puddu A, Sanguineti R, Montecucco F, Viviani GL. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. Mediators Inflamm 2014;2014:162021
40. Qi CJ, Zhang Q, Yu M, et al. Imbalance of fecal microbiota at newly diagnosed type 1 diabetes in Chinese children. Chin Med J (Engl) 2016;129:1298–1304
41. Giongo A, Gano KA, Crabb DB, et al. Toward defining the autoimmune microbiome for type 1 diabetes. ISME J 2011;5:82–91
42. Candela M, Biagi E, Soverini M, et al. Modulation of gut microbiota dysbioses in type 2 diabetic patients by macrobiotic Ma-Pi 2 diet. Br J Nutr 2016;116:80–93
43. Knip M, Siljander H. The role of the intestinal microbiota in type 1 diabetes mellitus. Nat Rev Endocrinol 2016;12:154–167