The Gut Microbiome of Adults With Type 1 Diabetes and Its Association With the Host Glycemic Control

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Smadar Shilo[1,2,3], Anastasia Godneva[1,2], Marianna Rachmiel[4,5],Tal Korem[1,2,6],Yuval Bussi[1,2] ,Dmitry Kolobkov[1,2], Tal Karady[1,2], Noam Bar[1,2], Bat Chen Wolf[1,2], Yitav Glantz-Gashai[3], Michal Cohen[3,7], Nehama Zuckerman Levin[3,7], Naim Shehadeh[3,7], Noah Gruber[5,8], Neriya Levran[8,9], Shlomit Koren[5,10], Adina Weinberger[1,2], Orit Pinhas-Hamiel[5,8†], Eran Segal[1,2†]

[†] Corresponding authors

[1] Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel.
[2] Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel.
[3] Pediatric Diabetes Clinic, Institute of Diabetes, Endocrinology and Metabolism, Rambam Health Care Campus, Haifa, Israel.
[4] Pediatric Endocrinology Unit, Shamir Medical Center, Zerifin, Israel.
[5] Sackler School of Medicine, Tel-Aviv University, Tel Aviv, Israel.
[6] Department of Systems Biology, Columbia University, New York, USA
[7] Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel
[8] Pediatric Endocrine and Diabetes unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Ramat-Gan, Israel.
[9] Robert H Smith faculty of agriculture, food and environment, the Hebrew University of Jerusalem, Rehovot, Israel.
[10]Diabetes Unit, Shamir Medical Center, Zerifin, Israel

Corresponding author:

Prof. Eran Segal, Department of Molecular Cell Biology and Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel, Tel: 972-8-934-3540, Fax: 972-8-934-4122, Email:eran.segal@weizmann.ac.il

Prof. Orit Pinhas-Hamiel, Pediatric Endocrinology and Diabetes Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Ramat-Gan, Israel, Tel: 972-3-530-5015, Fax: 972-3-5305055

Email: Orit.Hamiel@sheba.health.gov.il

Abstract

OBJECTIVE Previous studies have demonstrated an association between gut microbiota composition and Type 1 diabetes (T1D) pathogenesis. However, little is known about the composition and function of the gut microbiome in adults with longstanding T1D or its association with host glycemic control.

RESEARCH DESIGN AND METHODS We performed a metagenomic analysis of the gut microbiome obtained from fecal samples of 74 adults with T1D, 14.6 ± 9.6 years following diagnosis, and compared their microbial composition and function to 296 age-matched healthy controls (1:4 ratio). We further analysed the association between microbial taxa and indices of glycemic control derived from continuous glucose monitoring measurements and blood tests and constructed a prediction model which solely takes microbiome features as input to evaluate the discriminative power of microbial composition for distinguishing individuals with T1D from controls.

RESULTS Adults with T1D had a distinct microbial signature that separated them from controls when employing prediction algorithms on held-out subjects (auAUC=0.89±0.03). Linear discriminant analysis showed several bacterial species with significantly higher scores in T1D, including *Prevotella copri* and *Eubacterium siraeum,* and species with higher scores in controls, including *Firmicutes bacterium* and *Faecalibacterium prausnitzii* ($p < 0.05$, FDR corrected for all). On the functional level, several metabolic pathways were significantly lower in adults with T1D. Several bacterial taxa and metabolic pathways were associated with the host's glycemic control.

CONCLUSIONS We identified a distinct gut microbial signature in adults with longstanding T1D and associations between microbial taxa, metabolic pathways, and glycemic control indices. Additional mechanistic studies are needed to identify the role of these bacteria for potential therapeutic strategies.

Introduction

Type 1 diabetes (T1D) is a common chronic disease in children and adolescents. The incidence of T1D has been rapidly rising in the past decade, especially in young children (1). While a genetic predisposition for T1D exists, this rapid increase in the prevalence of the disease and the fact that less than 10% of genetically susceptible individuals will eventually develop T1D are suggestive of a large contribution of environmental factors to disease pathogenesis. These may include viral infections and nutritional factors (2). Gut microbiota composition has also been highlighted as a possible risk factor, with several studies in humans and animal models implicating its potential role in disease pathogenesis (3–5). These observations have further led to the 'balanced signal' hypothesis, stating that microbiome composition may promote or inhibit T1D development (6). Several suggested mechanisms for the possible influence of the gut microbiome on T1D pathogenesis include immunological deregulation mediated by gut dysbiosis, as there is evidence that the microbiome plays an important role in the development and maturation of the immune system (7) and gut leakiness, as structural mucosal alterations and gut dysfunction was observed in both human and animal studies on T1D (8).

In recent years, a rapidly growing number of studies have investigated the role of the gut microbiome in T1D (9). However, most studies focused on disease pathogenesis while only a few studies thus far have investigated the microbiome composition of individuals with a longstanding diagnosis, and those were mostly conducted on small cohorts and used a variety of computational analysis methods (10,11). In addition, while evidence on the regulating roles of the microbiome in normal and impaired glycemic response is accumulating in both animal models and humans (12), little is known on the role of the microbiome in glycemic control in individuals with longstanding T1D. Here, we analysed microbial composition and function in a cohort of individuals with T1D who are at least one year following diagnosis and the associations between microbial taxa, functional pathways, and glycemic indices in individuals with T1D.

Research design and methods

Study design

We conducted a prospective clinical cohort originally designed in order to study the postprandial glycemic responses (PPGRs) of individuals with T1D. Full details on recruitment and the study protocols are specified in a companion paper by Shilo et al (13), focused exclusively on modeling the PPGR in individuals with T1D. In brief, on the first day of the study, participants were invited to a study initiation meeting at the medical center. In this meeting, a physician authorized participation and acquired informed consent, anthropometric measurements were obtained, and blood tests were drawn and analysed in the hospital's laboratories. Health and lifestyle questionnaires were filled by the participants. Throughout the two weeks of study participation, participants used a proprietary smartphone App (www.personalnutrition.org), to log, in real-time, food intake, sleep times, physical activity, and medication intake with the exception of insulin which was recorded in the CSII devices. Participants were asked to follow their normal routine and dietary habits, with the exception of seven standardized meals. Participants were asked to provide one microbiome sample collected during the two weeks of study participation.

Participant Recruitment

Enrollment and recruitment were conducted in three medical centers in Israel between March 2017 and April 2019 (Fig. 1). The inclusion criteria for the study included age between 3-70 years old (13). However, as previous studies demonstrated that the interpersonal variation in the composition of the bacterial communities is significantly greater among children (14) and as a large variation exists in clinical phenotypes between children and adults (15) , we choose to include only adults (18-70 years old) in the analyses presented here. Additional inclusion criteria were more than one year following T1D diagnosis, using continuous glucose monitoring (CGM) and continuous subcutaneous insulin infusion (CSII) devices simultaneously and a capability to work with a mobile phone app on a daily basis for the recording of the dietary intake. Exclusion criteria included an active inflammatory or neoplastic disease, pregnancy and antibiotic usage 3 months prior to participation in the study. Participants who reported a diagnosis of celiac disease were excluded from all microbiome analyses since it was previously shown in several studies that celiac disease is correlated with a change in gut microbial composition (16).

Study population

Overall, 142 individuals with T1D were recruited to the study, and 124 participants provided a stool sample. Seven participants reported a diagnosis of celiac disease and were therefore excluded from microbiome analyses, resulting in 117 individuals. From them, 74 were older than 18 years and were included in the analyses (Fig. 1). The average age was 32.3 ±14.4 years (median (IQR) 26 (21-43) years) and average disease duration was 14.6 ± 9.6 years (median (IQR) 12 (7.8-18.3) years). Mean HbA1c% level was 7.3±1% (56.3±10.9 mmol/mol) (see Table S1 for mean values of all blood test results at study initiation). The mean BMI value was 25.1 ± 4 (kg/m^2). Of the 74 participants, 33 (44.6%) had at least one additional comorbidity. The most common comorbidities were hypothyroidism (12 participants, 16.22%) and hyperlipidemia (10 participants, 13.51%). Thirty-nine participants (52.7%) consumed additional medications apart from insulin during the study. The most common medications were Levothyroxine (12 participants, 16.22%), oral contraceptives (8 participants, 10.21%), and antilipidemic drugs (8 participants, 10.21%) (see Table S2 for a full list of medical conditions and medications consumed by the participants during the study). Cohort characteristics are presented in Table 1. Of the 74 individuals, 73 logged meals in real-time during the two weeks of study participation (see a companion paper by Shilo et al. (13)). Total energy intake was 1,666,610 kcal (22,521±7,951 per person). Average carbohydrate, fat and protein consumption were 43±1%, 38±7% and 17±4% from the total energy respectively.

Cohort matching

In order to compare between the composition of the microbiome in adults with T1D and healthy adults, we used gut metagenomic profiles obtained from Israeli adults (17). Out of 35,304 Israeli adults who submitted their sample between 13/01/2017 and 01/05/2021, 14,012 were excluded due to a different sequencing method, and 13,295 were excluded due to the presence of one of the following metabolic, gastrointestinal or systemic diseases: type 2 diabetes, T1D, gestational diabetes, pre-diabetes, impaired glucose tolerance or impaired fasting glucose, metabolic syndrome, fatty liver disease, morbid obesity, inflammatory bowel disease, Crohn's disease, ulcerative colitis, undetermined colitis, pancreatic diseases, celiac disease, irritable bowel syndrome, diverticulosis, hepatitis or other liver diseases, cholangitis or other bile-

related diseases, HIV, autoimmune diseases and cancer. Next, individuals with T1D were matched by age to healthy controls in a 1:4 ratio (1 adult with T1D to 4 healthy controls), resulting in 296 healthy individuals who were included as controls (Fig. 1, Table 1). Comparison between individuals with T1D and healthy controls were done using the Linear discriminant analysis (LDA) method (18). FDR corrected p-values were computed following the Benjamini Hochberg procedure and were employed at the rate of 0.1.

Stool sample collection and Genomic DNA Extraction

Participants entering the study received a verbal explanation from the study coordinators and detailed printed instructions for stool collection. Microbiome sampling was done using a swab and an OMNIgene-GUT (OMR-200; DNA Genotek) stool collection kit. Each participant was requested to collect stool via one swab and one separate OMNIIgene-GUT kit. However, only samples collected by OMNIIgene-GUT kits were sequenced and analysed since it has the advantage of maintaining DNA integrity in typical ambient temperature fluctuations and since samples of the control group were collected only by the OMNIIgene-GUT kits. Collected samples were immediately stored in a home freezer (−18°C) and transferred in a provided cooler to our facilities where it was stored at −80°C (−20°C for OMNIIgene-GUT kits) until DNA extraction. Samples from adults with T1D were sequenced between 04/2019 and 08/2019, and samples from healthy controls were sequenced between 04/2019 and 05/2021. All samples analysed in this study were sequenced using the same sequencing methods including sequencing protocols of DNA extraction, library preparation and sequencing machine. Control samples demonstrated that performing the process on different days had no effect on the results when the sequencing protocols are kept the same.

Metagenomic DNA was purified using MagAttract PowerSoil DNA extraction kit (Qiagen) optimized for the Tecan automated platform. Next-Generation Sequencing (NGS) libraries were prepared using Nextera DNA library prep (Illumina) and sequenced on a NovaSeq sequencing platform (Illumina). Sequencing was performed with 100bp single end reads with a depth of 10 million reads per sample. We filtered metagenomic reads containing Illumina adapters, filtered low-quality reads, and trimmed low-quality read edges. We detected host DNA by mapping with bowtie 2 (19) to the Human genome with inclusive parameters and removed those reads. Bacterial relative abundance (RA) estimation was performed by mapping bacterial reads to species-level genome bins (SGB) representative genomes (20). We selected all SGB representatives with at least five genomes in a group, and for these representative genomes kept unique regions as a reference data set. Mapping was performed using bowtie 2 (19), and abundance was estimated by calculating the mean coverage of unique genomic regions across the 50 percent most densely covered areas as previously described (21). Feature names include the lowest taxonomy level identified. In addition, we also estimated the RA of bacterial groups, such as *Akkermansia*, *Alistipes*, *Roseburia*, *Eubacterium*, and *Faecalibacterium prausnitzii* as a summation of the abundances of SGBs belonging to the relevant species by NCBI classification.

Microbial Biodiversity indices and functional analysis

Microbiome alpha diversity was calculated by Shannon's diversity index. Richness was calculated as a number of species in the sample detected with an abundance of at least 1e-4. Comparison between microbial indices and RA of microbial taxa were performed using Mann-Whitney U test. HUMAnN2 v2.8.2 (22) was used to integrate taxonomic information with functional profiles.

Associations with clinical phenotypes

To analyse the association between clinical and microbial features and measures of glycemic control we used several indices. These included fasting glucose, HbA1c level, and lipids measured by a blood test at study initiation and indices calculated based on CGM measurements during the two weeks of study participation, available for 73 participants. CGM derived features included the percentage of the time spent in hypoglycemia and hyperglycemia defined as glucose values below 70 mg/dl (3.9 mmol/L) and above 180 mg/dl (10 mmol/L) respectively, time in range, defined as time spent in glucose values between 70-180 mg/dl (3.9-10 mmol/L) (23) and coefficient of variation (CV) as a measure of glucose variability (24). For participants who also logged meals throughout the study period (73 individuals), PPGRs were calculated (see in a companion paper by Shilo et al. (13)). Pearson correlations between the clinical phenotypes, RA converted to a log space of microbial taxa, and metabolic pathways were calculated.

T1D prediction model based on microbial features

To evaluate the discriminative power of microbial composition for T1D, we constructed a prediction model based on Xgboost (25), which solely takes microbiome features as inputs. This model can capture nonlinear interactions between bacteria and was previously shown to outperform other methods for the classification of human microbiome data (26). The mean and standard deviation of the ROC curve were computed by using the curves that were generated in 5-fold cross-validation. In addition, we verified that when randomly swapping the target labels, the performances reflected a random prediction, hence an AUC very close to 0.5, as an additional control. We analysed feature attributes using SHAP (SHapley Additive exPlanation) to explore model interpretability. SHAP values represent the average change in the model's output upon conditioning on a specific feature (27).

Ethical approval

The study was approved by Rambam Medical Center Institutional Review Board (IRB); Tel Hashomer Hospital IRB, Shamir Medical Center IRB; and Weizmann Institute of Science IRB. All participants signed written informed consent forms. All identifying details of the participants were removed prior to the computational analysis. Trial was registredted in http://clinicaltrials.gov/, NCT: NCT02919839.

Results

Correlations between microbial strains, functional pathways, and clinical phenotypes

We first sought to explore the associations between microbial features, functional pathways, and clinical parameters (Fig. 2). Several bacterial taxa were significantly associated with glycemic indices including a negative correlation between the relative abundance of *Prevotellaceae* species SGB592 and SGB1340 and HBA1c level ($r = -0.35$) and a positive correlation between *Enterobacterales* species (SGB2483) and glucose average ($r = 0.41$) ($p<0.05$, FDR corrected for all). Species from the *Clostridiaceae* family (SGB1422) were positively correlated with time in range ($r = 0.38$). Several associations between microbial taxa and lipids were also observed: *Faecalibacterium prausnitzii* species (SGB15339) were negatively correlated with total cholesterol levels ($r=-0.41$), and species from the *Clostridiales* order, and *Firmicutes* class (SGB1421 and 1451) were negatively correlated with triglyceride levels ($r=-0.4$). In addition, several metabolic pathways were significantly associated with

glucose average, including pathways relating to aromatic acid biosynthesis (COMPLETE-ARO-PWY, r=0.42), chorismate biosynthesis from 3-dehydroquinate (PWY-6163, r=0.39), and chorismate biosynthesis I (ARO-PWY, r=0.42). In contrast, an inverse correlation was observed between pyrimidine nucleobases' salvage pathway (PWY-7208, r=-0.41, Fig. S1) and glucose average ($p<0.05$, FDR corrected for all). No statistically significant associations were found between nutritional parameters and bacterial taxa.

Microbiome composition in individuals with T1D

To improve our understanding of the composition of the gut microbiome in T1D, we compared individuals with T1D to healthy controls (1:4 matching by age, see Methods). Overall, 74 adults with T1D were compared to 296 healthy adults. There were no statistically significant differences in sex, weight, or BMI between groups. As expected, healthy adults had significantly lower levels of HbA1c (Table 1). Microbial alpha diversity was not significantly different between the groups (Fig 3C), aligned with previous studies (11,28) but in contrast with others reporting a lower diversity in individuals with T1D (29,30). In addition, species richness and the ratio of *Firmicutes/Bacteroidetes* of taxonomic profiles were not significantly different between the groups (Table S3). Linear discriminant analysis showed a total of 17 bacterial taxa with significantly higher LDA scores in individuals with T1D and 15 bacterial taxa with significantly higher LDA scores in healthy adults (Fig. 3A, 3D, Table S4). Bacterial species with significantly higher scores in individuals with T1D included *Prevotella copri, Eubacterium siraeum,* and *Alistipes inops* and several species with a higher score in healthy adults, including *Firmicutes bacterium, Alistipes putredinis, Faecalibacterium prausnitzii,* and *Ruminococcus gnavus* ($p <0.05$, FDR corrected). Dimensionality reduction techniques, including Principal Component Analysis (PCA), in which the principal coordinate combination with the greatest contribution rate was PC1 = 7.7 %, PC2 = 4.1 %, and t-distributed Stochastic Neighbor Embedding (t-SNE), did not reveal visually distinctive differences between individuals with T1D and controls (Fig. S2). On the functional level, when comparing metabolic pathways, several metabolic pathways, including L-glutamate and L-glutamine biosynthesis, L-ornithine de novo biosynthesis, and superpathway of hexuronide and hexuronate degradation, were significantly lower in adults with T1D ($p <0.05$, FDR corrected).

Classification of individuals with T1D by microbial features

We next analysed our ability to distinguish individuals with T1D from controls based solely on microbiome features. We constructed a prediction model based solely on microbial features and used cross-validation schemes for validation of the model (see Methods). The discrimination performance of the model had an area under the receiver operating curve (auROC) of 0.89±0.03, permutations p-value<0.001(Fig. 3B). The most impactful microbial taxa for the prediction were *Prevotella copri,* which impacted the model toward the prediction of T1D, and *Ruminococcus,* which impacted the model toward the prediction of a healthy state (Fig. S3).

Discussion

In this study, we profiled the gut microbiome composition in adults with longstanding T1D and identified several associations between bacterial taxa, metabolic pathways and the glycemic control of the host. While a growing body of evidence, mainly originating from studies on animal models, suggests that gut microbiota has a causal impact on host glycemic control (31) that may be mediated by mechanisms such as modulation of incretin secretion, short-chain fatty

acid production, metabolism of bile acid, and regulation of adipose tissue (32), data regarding the role of the microbiome in the glycemic control of individuals with T1D are still sparse.

Here, bacterial taxa and metabolic pathways that were significantly associated with glycemic indices of the host included *Enterobacterales* species and pathways relating to aromatic acid and chorismate biosynthesis, which were correlated with glucose average, *Prevotellaceae* species that were inversely correlated with HBA1c level. and pyrimidine nucleobases' salvage pathways that were inversely correlated with glucose average ($p<0.05$, FDR corrected for all). Several small-scale studies have previously shown different associations, including a study on 12 Chinese subjects with T1D (33) that demonstrated an inverse correlation of the abundance of *Faecalibacterium* and HbA1c levels, a study conducted in Brazil that included 20 individuals with T1D and demonstrated a correlation between the relative abundances of *Bacteroidetes, Lactobacillales,* and *Bacteroides dorei* and HbA1c levels (34). Importantly, the correlations observed in this study were not strong, and further studies integrating multi-omic data, including metagenomic, metatranscriptomic and metaproteomic, along with high-quality clinical and nutritional data, are needed to in order to identify the potential role of these bacteria and metabolic pathways and their influence on the host's glycemic control.

We identified a distinct gut microbial signature in adults with longstanding T1D compared to healthy adults. By utilizing an expanded reference set (20) for the first time in individuals with T1D, as well as a relatively large control group, we show a total of 17 bacterial taxa with significantly higher LDA scores in T1D and 15 bacterial taxa with significantly higher LDA scores in controls (Fig. 3). Although dimensionality reduction analyses did not reveal visually distinctive differences (Fig. S2) and the diversity and richness were not statistically different between groups, we were able to devise a model that accurately distinguishes between adults with T1D and healthy controls using only microbiome features (auAUC=0.89±0.03,.Fig. 3B). Interestingly, the most impactful microbial taxa for the prediction were *Prevotella copri,* which impacted the model toward the prediction of T1D, and *Ruminococcus,* which impacted the model toward the prediction of a healthy state (Fig. S2), aligned with the results of the LDA analysis, showing higher scores for *Prevotella copri* in *T1D* and *Ruminococcus gnavus* in healthy adults (Fig. 3).

Previous studies (9) reported various results regarding the taxonomic composition of the gut microbiome in individuals with T1D compared to healthy controls and their interpretation is challenging due to a large heterogeneity in both study population and analytic approaches. It is also worthy to note that it was also previously shown that gut microbiota in type 1 diabetes differs at taxonomic and functional levels not only in comparison with healthy subjects but also compared to non-autoimmune diabetes (35). The most common findings in individuals with T1D included alterations in the following bacterial species: *Bacteroides, Streptococcus, Clostridium, Bifidobacterium, Prevotella, Staphylococcus, Blautia, Faecalibacterium, Roseburia, and Lactobacillus (36)*. In the largest human cohort to date, no particular taxa was associated with the development of T1D development, but the microbiome of control children was found to contain more genes related to fermentation and biosynthesis of SCFA compared to children who eventually developed T1D (4). An additional study also reported a decrease in SCFA producers in individuals with longstanding T1D. Moreover, it was previously shown that feeding non-obese diabetic (NOD) mice with an SCFA-rich (butyrate and acetate) diets had substantial effects on their immune system and a protective effect from the development of diabetes (37). In this cohort, when comparing metabolic pathways, we found several metabolic pathways, including L-glutamate and L-glutamine biosynthesis, L-

ornithine de novo biosynthesis, and superpathway of hexuronide and hexuronate degradation, that were significantly lower in adults with T1D ($p < 0.05$, FDR corrected). To the best of our knowledge, these findings were not previously described in individuals with T1D and their role should be further explored in future work.

The strength of our study includes a relatively large sample size compared to previous studies, the integration of data on glucose measurements obtained from CGM devices, and the expanded reference set we used. The greatest limitation of our study is its observational nature. Further studies are needed in order to attribute causality to the gut microbiome alterations we describe as currently whether these taxa are a cause or an effect of the disease remains unclear. In addition, although the sample size of the cohort is relatively large, it may still be insufficient to reach robust associations with clinical phenotyping. Finally, several additional factors may influence the composition of the gut microbiome. For example, nutritional habits may differ between individuals with T1D compared to healthy individuals. While we did not have detailed nutritional data on our control group, macronutrient distribution in the T1D cohort was very similar to healthy adults in Israel as measured in a previous study performed by our group (38). In this study, healthy individuals logged meals during one week and consumed an average of 46±8% carbohydrate, 36±7% fat, and 15±3% protein from the total energy, compared to an average of 43±1% carbohydrate, 38±7% fat and 17±4% protein consumed by the T1D cohort. Moreover, in the group of individuals with T1D, no associations were found between nutritional parameters and bacterial taxa. Medication consumption may also influence microbial composition and we therefore excluded individuals with antibiotic usage three months prior to participation. While other types of medications, such as proton pump inhibitors, may also have an effect (39), they were only consumed by a very small percentage of our cohort (Table S2). Family kindred may also have a pronounced effect on the structural and functional composition of the gut microbiome (40). However, none of the adults with T1D included in this study were family members sharing the same household. Microbiome composition is also heavily influenced by geographic location (14), and therefore additional studies are needed in order to determine if our findings can be generalized to non-Israeli populations.

In conclusion, our study highlights a distinct gut microbial composition in individuals with longstanding T1D compared to healthy individuals.We identified unknown associations between microbial taxa, metabolic pathways, and clinical phenotypes and note the importance of expanding the gut microbiome reference set, as it allows us to also identify associations with unclassified bacterial strains that may play a part in disease pathogenesis. Our findings provide a foundation for additional large-scale analyses of the gut microbiome in individuals with T1D in order to identify host–microbe interactions and to identify the causal role of these bacterial taxa for the development of novel therapeutic strategies in T1D.

Article Information

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Duality of Interest

Eran Segal is a paid consultant for DayTwo. No other potential conflicts of interest relevant to this article were reported. No pharmaceutical manufacturers or companies from the industry contributed to the planning, design, or conduct of the trial.

Authors contribution

SS and AG conceived the project, designed and conducted the analyses, interpreted the results and wrote the manuscript. MR provided data and interpreted the results. TK conceived the project, designed the analysis and interpreted the results,YB designed the analysis and interpreted the results DK and TK designed and conducted the analyses, MC, NZL, NS, NG, NL, SKS provided data and interpreted the results. NB designed the analysis and interpreted the results. BCW and YGG coordinated and designed data collection. A.W. conceived the project and directed sample sequencing. OPH and ES conceived the project, designed and conducted the analyses, interpreted the results and supervised the project and analyses. All authors reviewed and approved the manuscript and vouch for the accuracy and completeness of the data. E.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data availability statement

Metagenomic sequencing data that support the findings of this study are available. Clinical data cannot be shared due to restrictions by informed consent. Dataset is available at https://data.mendeley.com/datasets/bcz47mhvc3/1

Code availability statement

Analysis code is available at https://github.com/Nastyagodneva/T1D_Microbiome.

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Tables

Mean (SD)	T1D Adults	Healthy Adults	p-value
N	74	296	
Age (years)	32.3 (14.4)	32.8 (13.9)	0.37
Time from T1D diagnosis (years)	14.6 (9.6)		
Sex - Male (%)	28 (37%)	80 (27%)	0.06
Weight (kg)	71 (12)	72 (15)	0.63
BMI (kg/m^2)	25 (4)	26 (4)	0.38
HbA1c (%), (mmol/mol)	7.3 (1.0), 56.3 (10.9)	5.1 (0.4), 32.2 (4.4)	0.005>

Table 1: Cohort characteristics Comparison between individuals with T1D and healthy controls were computed using the Mann-Whitney U test.

Figure legends

Figure 1: Cohort selection. *participants were excluded due to the presence of one of the following metabolic, gastrointestinal or systemic diseases: type 2 diabetes, T1D, gestational diabetes, pre diabetes, impaired glucose tolerance or impaired fasting glucose, metabolic syndrome, fatty liver disease, morbid obesity, inflammatory bowel disease, crohn's disease, ulcerative colitis, undetermined colitis, pancreatic diseases, celiac disease, irritable bowel syndrome, diverticulosis, hepatitis or other liver disease, cholangitis or other bile-related disease, HIV, Autoimmune disease and cancer..

Figure 2: Correlations between microbial strains, functional pathways, and clinical phenotypes. values of Pearson correlation between phenotypes and bacterial species are presented ($p<0.05$, FDR corrected). Average glucose is calculated from the glucose values recorded in continuous glucose monitoring (CGM) devices during the study. CGM % of time in good range is defined as the percentage of time spent in glucose values between 70-180 mg/dl (3.9-10 mmol/L)

Figure 3: Microbiome composition in adults (A) LDA score (log 10) of microbial features that are differential between adults with T1D and healthy controls. Red- higher score in T1D, Green- higher score in healthy controls (HC), ranked by the effect size. g-genus, s-strain, f-

family (B) Prediction model for distinguishing individuals with T1D from healthy controls: ROC curve of a prediction model based solely on microbiome features is presented (blue) (C) Shannon diversity index of individuals with T1D and healthy controls (D) Cladogram showing a taxonomic representation of the differences between healthy and individuals with T1D. Red -more common in T1D. Green - more common in healthy controls