Disturbance of the human gut microbiota in patients with Myotonic Dystrophy type 1

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ABSTRACT

Myotonic dystrophy type 1 (DM1) is a rare autosomal dominant genetic disorder. Although DM1 is primarily characterized by progressive muscular weakness, it exhibits many multisystemic manifestations, such as cognitive deficits, cardiac conduction abnormalities, and cataracts, as well as endocrine and reproductive issues. Additionally, the gastrointestinal (GI) tract is frequently affected, encompassing the entire digestive tract. However, the underlying causes of these GI symptoms remain uncertain, whether it is biomechanical problems of the intestine, involvement of bacterial communities, or both. The primary objective of this study is to investigate the structural changes in the gut microbiome of DM1 patients. To achieve this purpose, 35 patients with DM1 were recruited from the DM-Scope registry of the neuromuscular clinic in the Saguenay–Lac-St-Jean region of the province of Québec, Canada. Stool samples from these 35 patients, including 15 paired samples with family members living with them as controls, were collected. Subsequently, these samples were sequenced by 16S MiSeq and were analyzed with DADA2 to generate taxonomic signatures. Our analysis revealed that the DM1 status correlated with changes in gut bacterial community. Notably, there were differences in the relative abundance of Bacteroidota, Euryarchaeota, Fusobacteriota, and Cyanobacteria Phyla compared to healthy controls. However, no significant shift in gut microbiome community structure was observed between DM1 phenotypes. These findings provide valuable insights into how the gut bacterial community, in conjunction with biomechanical factors, could potentially influence the gastrointestinal tract of DM1 patients.

1. Introduction

Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disorder associated with multisystem involvement [1]. DM1 is characterized by progressive muscle weakness, atrophy, and myotonia, along with a wide range of clinical features including heart conduction defects and gastrointestinal alterations [2–4]. It is also highly variable in symptoms severity, age of onset, and clinical presentation [5]. DM1 is caused by a CTG triplet repeat expansion (> 50 repeats) in the 3′ noncoding region of *DMPK*, the gene encoding the DM protein kinase for a myosin kinase expressed [6]. The *DMPK* gene is located on chromosome 19q13.3 [7].

The core pathogenic feature of DM1 is the intra-nuclear sequestration of RNA-binding proteins with the toxic RNA repeat [8,9]. Specifically, the transcription of the CTG repeat to a CUG$^{(n)}$ repeat in the RNA, leads to a reduction in the number of circulating RNA-binding proteins, including muscle blind like splicing regulator 1 (MBNL1) [10,11]. This repeat disrupts the precursor mRNA splicing mechanism, resulting in the production of a wide array of non-complete and nonfunctional proteins [12]. Most of the clinical manifestations in DM1 patients are directly linked to some of these abnormal splicing events. For instance, the loss of insulin receptors contributes to insulin resistance [13] while the loss

of chloride channels is associated with myotonia [14].

In clinical terms, DM1 exhibits a wide range of highly variable manifestations [15]. These clinical features involve multiple organs, including cognitive deficits, cardiac conduction abnormalities, and cataracts, as well as endocrine, hypothyroidism, and reproductive problems [16,17]. Furthermore, impairment of the gastrointestinal (GI) tract is frequent and may affect any part of the digestive system from the pharynx to the anal sphincter [18–20]. Based on the age of onset of these clinical manifestations and CTG repeats, patients with DM1 can present five different phenotypes on initial examination. These phenotypes are classified as congenital, childhood, juvenile, adult, and late onset, with the adult phenotype being the most prevalent [21,22].

Among the manifestations of DM1, GI symptoms, including constipation, diarrhea, cramping, abdominal pain, or bloating, affect over one third of DM1 patients [23,24]. Approximately 25% of patients with DM1 complain that GI symptoms are their most bothersome clinical manifestation [20,25]. Furthermore, there are signs of slowed GI motility as shown by late gastric emptying, or gastroparesis [26] and a slow intestinal transit time in patients with DM1 [25]. Other reported signs include gastric acid reflux, fluctuations between diarrhea and constipation, fecal incontinence [27,28], as well as gallbladder problems with a high frequency of cholecystectomy, and liver issues [29]. These symptoms share similarities with irritable bowel syndrome, even though it is more likely correlated to smooth muscle myotonia of the GI system [12].

The origin of GI manifestations in DM1 patients is likely multifactorial. They may be due to alterations in hormonal secretion [30,31] myoelectric activity [31,32], and smooth muscle function [33]. As of now, there is no clear guideline for the treatment of GI symptoms in DM1 patients [18,29]. Bellini et al. (2006) suggested that GI involvement in DM1 patients can be related to the disease's duration but not the severity of skeletal muscle involvement [18]. Additionally, a small study [34] found a correlation between CTG repeat size and gastrointestinal symptoms of abdominal pain and constipation, but larger studies did not find a significant correlation [35,36]. Recent reports suggested that Small Intestinal Bacterial Overgrowth (SIBO) might be a contributing factor of GI symptoms in DM1 patients [24,37–40]. Consequently, we hypothesized that some of the GI symptoms experienced by patients with DM1 could be associated with a dysbiosis in their gut microbiota structure. The GI symptoms in DM1 patients can significantly impact their quality of life, and a better understanding of these gastrointestinal issues is essential to help alleviate these discomforts.

To explore whether the GI symptoms of DM1 patients arise from changes in the gut bacterial composition, we conducted 16S rRNA gene sequencing on stool samples collected from a total of 35 DM1 patients. From this group of 35 DM1 patients, a sub-group of 15 DM1 patients were paired with 15 of their close family members as healthy controls. Additionally, we included 20 non-paired DM1 patients in the study. For the non-paired DM1 patients, we further categorized them into two subgroups: "Pediatrics", comprising childhood and juvenile-onset cases, and "Adult", including adult and late-onset cases. DNA sequencing was used to identify bacterial populations exhibiting significant differences in relative abundance between DM1 patients and the control group. This approach allowed us to explore potential associations between gut bacterial composition and GI symptoms in DM1 patients, providing valuable insights into the role of the gut microbiota in this disorder.

2. Methods

2.1. Participants and stool sample collection

The participants were selected from the DM-Scope registry of the Saguenay–Lac-St-Jean neuromuscular clinic using a convenience sampling method. The samples comprised 35 patients with DM1 where a sub-group of 15 DM1 were paired with a control living in the same house to minimize diet and lifestyle differences. Inclusion criteria for DM1

participant consisted of 1) a confirmed genetic diagnosis of DM1; 2) age over 18 years; 3) body mass index < 30; and 4) the ability to provide informed consent. Exclusion criteria included: 1) the presence of any known chronic pathology other than DM1; 2) a history of typhoid fever; 3) digestive disorders, including acute or chronic diarrhea, within the last 3 months unrelated to DM1; 4) recent antibiotic therapy; 5) travel to an intertropical zone within the last 3 months; 6) extended stays in an intertropical zone for several years; and 7) hospitalizations abroad lasting more than 24 h within the last 12 months. For the control, the inclusion criteria were: 1) age over 18 years; 2) the ability to provide informed consent. Exclusion criteria were the same as for the DM1 participant. Sample collection occurred from January 2020 to May 2022 (COVID delay) using the OMNIgene•Gut OM-200 collection tube from DNA Genotek (Canada). In the next step, the samples were transported to the laboratory on ice and stored at − 80 °C till processing. After DNA extraction from fecal samples, 16S rRNA gene amplicon sequencing was performed at the RNomics Platform of the University of Sherbrooke (https://rnomics.med.usherbrooke.ca/). Information on sex, age, type, and severity of GI symptoms, as well as family background were collected from each subject through the International collaboration on incontinence questionnaire-Bowel module (ICIQ-B) [3,41–43]. For assessment of GI symptoms and its impact on Quality of Life (QoL), affected individuals were contacted and assessed at home or at the neuromuscular clinic with this self-report condition-specific questionnaire. The ICIQ-B includes 21 main items, 17 of which address three factors: Bowel Pattern, Bowel Control, and Impact on QoL. A higher score in ICIQ-B means higher negative impacts.

2.2. DNA library preparation and 16S rRNA gene sequencing

16S amplicon libraries were prepared according to our previous works [44,45]. Fresh stool was collected using the OMNIgene•GUT OMR-200 collection tube (Genotek) and stored at − 80 °C. Extractions of microbial DNA from the stool samples were performed using the QIAamp DNA stool mini kit (Qiagen) with some modifications [46]. Briefly, 200 µl of stool samples were centrifuged and resuspended in 500 µl of ASL Buffer (Qiagen) with 250 µl of 0.1 mm glass beads. Samples were homogenized for 3 min at 6500 rpm (Precellys 24) and then incubated at 95 °C for 15 min. Another round of homogenization was performed for 3 min at 6500 rpm and then samples were centrifuged at 13 000 rpm for 10 min at room temperature. The supernatants were transferred to 1/10 vol 3 M Sodium Acetate (pH5.2), 1 mL of 100 % EtOH and incubated at − 80 °C for 15 min. Samples were centrifuged for 20 min at 4 °C and pellets were resuspended in 200 µl Tris 10 mM pH7.5 and EDTA 1 mM. 2 µl for DNAse-Free RNase (10 mg/mL) was added and incubated at 37 °C for 30 min followed by the addition of 15 µl of proteinase K and 200 µl of AL Buffer (Qiagen) incubated at 70 °C for 30 min 200 µl of 100 % EtOH was added and transferred to a QIAamp column. The next step was done according to the manufacturer's instructions and the DNA samples were stored at − 80 °C. For the amplification of the V4 region of the bacterial 16SrRNA gene, the 515 F (5′-GTGC CAGCMGCCGCGGTAA-3′) and 806 R (5′-GGACTACHVGGGTWT CTAAT-3′) paired primers were used [47]. Lastly, the pooled and indexed libraries were sequenced in paired-end modus on an Illumina MiSeq [48] at the RNomics Platform of the University of Sherbrooke (https://rnomics.med.usherbrooke.ca/).

2.3. Microbiome analyses

2.3.1. Generating taxonomic signature

In this work, microbiome analyses were performed in two different assessment purposes, one for comparing 15 paired samples of DM1 patients with their 15 control samples and the other one for subgroup analysis of all 35 DM1 samples containing the remaining 20 non-paired DM1 patients based on their phenotypes in the two groups of "Pediatrics" and "Adult". "Pediatrics" included childhood, and juvenile-onset,

while adult and late-onset were classified as "Adult". Sequences were checked for quality, trimmed, merged, and checked for chimeras using the DADA2 v1.22.0 pipelines [49] for 16S library and phyloseq v.1.38.00 [50] as packages for R (R Development Core Team; http://www.R-project.org) in RStudio v.4.1.0 [51]. Bacterial community matrices were built from the resulting unique Amplicon Sequence Variant (ASV) based on the SILVA v.138 bacterial databases [52]. All these steps were performed according to the complete workflow tutorials which can be found at https://benjjneb.github.io/dada2/tutorial.html.

2.3.2. Community-level analyses

We studied the alpha- and beta-diversity and the relative abundance of bacterial ASVs. To estimate alpha-diversity, the vegan [53] and phyloseq [50] package developed for the R environment were used [51]. We calculated different alpha-diversity metrics to evaluate samples' bacterial diversity such as Shannon index [54] and Simpson's index [55], which are widely used metrics within-sample diversity as well as Chao1 [56] estimating the number of species in a community. Principal coordinate analysis (PCoA) plots were built to visualize the main differences in bacterial community structure [57]. We also compared bacterial distribution across samples to determine if there was a difference in the richness or evenness of samples between both statuses. We used Wilcoxon rank sum exact test to compare the alpha-diversity measures between DM1 participants and controls.

Beta-diversity was quantified by Bray-Curtis and both weighted and unweighted UniFrac distance matrices [58], which together capture both the relative abundance distributions across samples and the phylogenetic relationships. An unweighted UniFrac distance counts only the presence and absence of species and considers the fraction of branch length unique to each community. By contrast, a weighted UniFrac distance uses the relative abundance of species and influences the branch length with abundance difference. Significant statistical changes in gut bacterial community structure based on UniFrac distances were obtained using PERMANOVA [59] through the vegan package. PERM-DISP [60,61], a resemblance-based permutation test, was also performed for comparisons focusing strictly on the null hypothesis of homogeneity of multivariate dispersions. This test can identify if the dispersion of the group data from the centroids drives the significant difference of the PERMANOVA test or if it is the centroids of the group data themselves. Finally, VENN analyses [62] were also conducted for the relative abundance of various taxa in the samples.

2.3.3. Differential abundance analysis

Differently abundant taxa between DM1 participants and controls were identified by ANCOM-BC (Analysis of Communities of the Microbiome), a novel methodology based on the compositional log-ratios [63]. ANCOM-BC uses relative abundances to infer absolute abundances based on Aitchison's methodology [64]. We performed ANCOM-BC analyses for various taxonomic levels at a $P = 0.05$ with Benjamini–Hochberg FDR correction. We also used ANCOM-BC Volcano plots for visualization and detection of statistically significant genera from two different experimental conditions (Control vs. DM1) in terms of log fold change (X-axis) and negative log10 of P-value (Y-axis).

2.3.4. Functional prediction analysis

PICRUSt2 software was used to obtain enzyme commission numbers and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways from the biom file [65]. This method can predict the abundance of gene families and related functional pathways of microbial communities based on ASVs marker gene sequences. Further analyzing and interpreting on the output from PICRUSt2 was performed using ggpicrust2 [66], an R package for visualization and analysis of PICRUSt2 calculations. Various Differential Abundance (DA) methods were applied on the data including ALDEx2 [67], Maaslin2 [68], DESeq2 [69], LinDA [70], Lefser [71], metagenomeSeq [72], and edgeR [73]. The P-values were

then adjusted by Benjamini and Hochberg (BH) method [74].

3. Results

3.1. Characteristics of the study population

In this study, a total of 35 DM1 participants ranging between 26 and 76 years old (mean age = 45.6 years) were recruited. Among the participants, 19 were women and 16 were men. The age of onset for the DM1 participants varied, with 15 individuals presented with juvenile onset, 10 with childhood onset, 5 with adult onset, and the remaining 5 having late onset. As for the 15 DM1 participants with the paired control samples, 10 individuals were juvenile, 3 individuals were adult, and 2 from individuals with childhood onset. The paired healthy controls consisted of 15 individuals in total, including 10 men and 5 women, with ages ranging from 37 to 89 years old (mean age = 55.7 years). The healthy control participants lived in the same house with the DM1 patients. Of the 15 healthy control participants, 10 were spouses and not genetically related (10 out of 15 participants = 67 %), and 5 were genetically related (33 %). A summary of the study population details was provided in Tables 1 and 2. As for age, DM1 patients were significantly younger than healthy control participants ($P = 0.005$) indicating that age could have a confounder effect on the results of gut microbiome analysis. In contrast, sex distribution was not different between Controls and DM1 patients ($P = 0.147$). In terms of GI problems, our results didn't show a significant difference in bowel pattern score, bowel control score, diarrhea and constipation between groups ($P \geq 0.05$) while there was a difference in Quality of life score ($P = 0.01$). However, DM1 patients might have more diarrhea problems compared to patients although it did not reach significance threshold because of the limited number of samples (24 cases of diarrhea in DM1 patients compared to

Table 1
Detailed data of population recruited at the study.

Characteristics	Total (n = 50)	Controls (n = 15)	DM1 participants (n = 35)	P value[1]
Age, y				0.005
Mean (SD)	47.3 (14.4)	54.5 (14.6)	44.3 (13.3)	
[min-max]	25-86	35-86	25-75	
Sex, n (%)				0.147
Women	26[52]	10[67]	16[46]	
Men	24[48]	5[33]	19[54]	
CTG repeat length in blood				-
mean (SD)	-	-	637 (488)	
[min-max]	-	-	74-2000	
Phenotype, n (%)				-
Childhood	-	-	10[29]	
Juvenile	-	-	15[43]	
Adult	-	-	5[14]	
Late-onset	-	-	5[14]	
Bowel pattern score				0.316
Mean (SD)	5.3 (2.4)	5.0 (1.6)	5.5 (2.6)	
[min-max]	[2–13]	[2–7]	[2–13]	
Bowel control score				0.712
Mean (SD)	6.4 (5.7)	7.1 (4.6)	6.3 (6.9)	
[min-max]	[0–19]	[2–14]	[0–19]	
Quality of life score				**0.01**
Mean (SD)	8.1 (7.1)	4.0 (3.7)	8.9 (7.3)	
[min-max]	[0–23]	[0–10]	[0–23]	
Constipation, n (%)				0.493
Absent	19[30]	3[2]	16[46]	
Present	24[48]	5[33]	19[54]	
Unknown	7[14]	7[47]	0	
Diarrhea, n				**0.11**
Absent	16[32]	5[33]	11[31]	
Present	27[54]	3[2]	24[69]	
Unknown	7[14]	7[47]	0	

[1]p value were assessed between DM1 participants and controls subjects using a Student T or Fisher tests.

Table 2

The detailed data from Differential Abundance (DA) analysis of functional gene content of various pathways in DM1 patients compared to healthy controls performed by PICRUSt2.

Functional genetic pathways	Adjusted P value[1]
Amino acid metabolism	
D-glutamine and D-glutamate metabolism	0.714009392
D-Alanine metabolism	0.396564457
Alanine, aspartate, glutamate metabolism	0.714009392
D-Arginine and D-ornithine metabolism	0.714009392
Selenocompound metabolism	0.714009392
Histidine metabolism	0.621328054
Cysteine and methionine metabolism	0.716462608
Taurine and hypotaurine metabolism	0.714009392
Valine, leucine, and isoleucine degradation	0.514575416
Phosphonate and phosphinate metabolism	0.466319984
Lysine degradation	0.842327573
Energy metabolism	
Carbon fixation in photosynthetic organisms	0.738779192
Carbon fixation in prokaryotes	0.212982542
Sulfur metabolism	0.452652881
Oxidative phosphorylation	0.954629181
Methan metabolism	0.44977559
Nitrogen metabolism	0.714009392
Carbohydrate metabolism	
Pentose phosphate pathway	0.419799368
Fructose and mannose metabolism	0.368324617
Amino sugar and nucleotide sugar metabolism	0.566683392
Pentose and glucoronate interconversion	0.368324617
TCA cycle	0.409996967
Galactose metabolism	0.507628722
Ascorbate and aldarate metabolism	0.389198715
Lipid metabolism	
Butanoate metabolism	0.207617934
Glycerolipid metabolism	0.62829694
Linoleic acid metabolism	0.372102773
Inositol phosphate metabolism	0.714009392

[1]p values were calculated between genetic contents of DM1 patients and control samples by various DA methods. The data in the table represents Wilcoxon rank test in AlDEx2 [66]. Other methods like Maaslin2 [67], DESeq2 [68], LinDA [69], Lefser [70], metagenomeSeq [71], and edgeR [72] were also tested. The adjusted method for p values was Benjamini and Hochberg (BH).

three cases in healthy controls, *P* = 0.11).

3.2. DNA Sequencing and Filtering

A total of 1281,064 raw reads were obtained by high-throughput sequencing of 16S rDNA gene of bacteria (V4 region) from 30 fecal samples in the paired samples analysis. After filtering low-quality reads as well as singletons and doubleton sequences, 1115,961 clean reads were left with a median read length of 253 bp (~12.88 % of removed raw data). The average number of ASVs per sample was 3580. As for the subgroup analysis of DM1 participants, the number of raw reads and clean reads, were 1631,683 and 1146,381 respectively. The lengths of merged sequences all fall within the average range of 256 bp containing 4290 ASVs. Technical controls were added to our samples to confirm the quality of our sequencing data.

3.3. Bacterial composition of stool samples

Our study found a total of 10 bacterial phyla, 57 family, and 195 genera within the 15 paired DM1 samples and their 15 healthy controls. Taxonomic profiles were generated for each sample at phylum and genus levels and organized based on the status and phenotypes of DM1 in two sets: "Pediatrics" and "Adult" (Fig. 1). Among all groups, the dominant phyla consisted of Firmicutes, accounting for more than 50 % in controls and DM1 participants, mostly in the "Pediatrics". Bacteroidota represented about 15 % in DM1 participants, particularly in the "Adult" group. Actinobacteria constituted around 20 % of the gut

Fig. 1. Bacterial relative abundance at the phylum and genus level in all 35 DM1 patients and 15 controls. a) At the phylum level based on their status. b) At the phylum level for various phenotypes of DM1. c) At the genus level based on their status. d) At the genus level for various phenotypes of DM1. "Pediatrics" includes congenital, childhood and juvenile onset, while "Adult", and late onset were classified as "Adult".

microbiota in DM1 participants, with equal abundance in both "Pediatrics" and "Adult" groups. The prevalence of bacteria at the phylum level in the gut microbiome of DM1 patients compared to the control samples has been illustrated in Fig. 2. ANCOM-BC analysis indicated that the relative abundance of Bacteroidota, Euryarchaeota, Fusobacteriota, and Cyanobacteria Phyla changed significantly between different DM1 status. At the genus level, the most relatively abundant genera was *Blautia* (14 %), *Bacteroides* (10 %), *Collinsella* (10 %), *Prevotella-9* (14 %), *Bifdidobacterium* (8 %), and *Akkermansia* (14 %). The "Other" category represented ~50 % of the gut bacteria in controls,

showing a high diversity of genera. In contrast, the most prevalent genera in DM1 participants were *Blautia* (24 %) and *Bacteroides* (14 %), especially among the "Pediatrics" (Fig. 1).

3.4. Bacterial alpha- and beta-diversity analyses

In this study, we employed three different metrics of alpha-diversity (Chao1, Shannon, and Simpson) to compare the richness (number of taxonomic groups) or evenness (distribution of abundances of the groups) between statuses. Each estimator calculates the number of
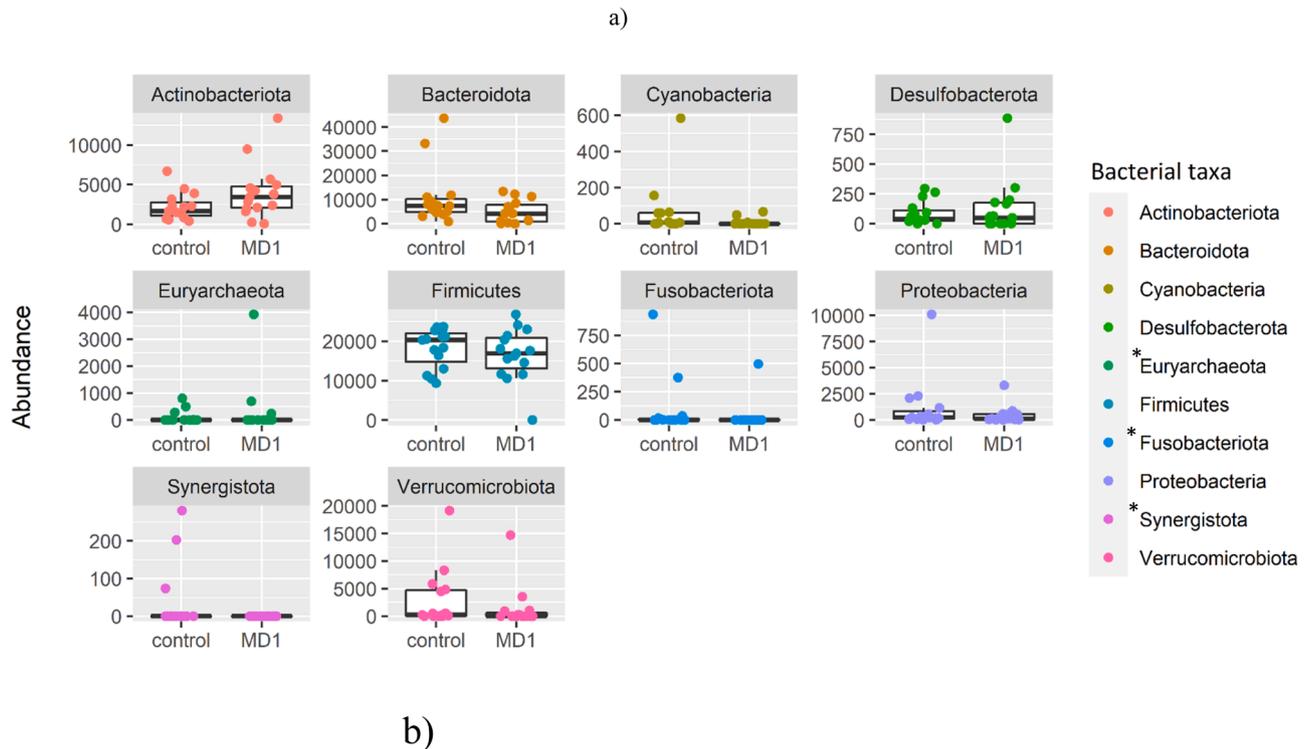
Phyla	StatusWT (*p* value)
Firmicutes	0.97
Verrocumicrobiota	0.40
Actinobecteriota	0.16
Bacteroidota	0.05
Proteobacteria	0.88
Euryarchaeota	0.00[*]
Desulfobacterota	1.00
Fusobacteriota	0.00[*]
Cyanobacteria	0.00[*]
Synergistota	0.12

Fig. 2. a) The prevalence of bacteria at the phylum level in the gut microbiome of paired DM1 patients compared to their control samples. (* *P* value of lower than 0.05). b) *P* value of different taxa calculated by ANCOM-BC analysis in the paired samples analysis.

different species per ASVs, with Chao1 specifically considering the importance of rare ASVs in estimating diversity [75]. Our results showed that there are no significant differences between DM1 patients and their paired controls for bacterial alpha-diversity using the three alpha-diversity measures including Shannon, Simpson, and Chao1 ($P \geq 0.05$) (Fig. 3). Similarly, as for the functional analysis using PICRUSt2, we also didn't find a significant difference in the control and DM1 samples. The details of these analysis were summarized in four groups of amino acid, energy, carbohydrate, and lipid metabolisms (Table-2).

In the subgroup analysis, the beta-diversity, assessed using Bray-Curtis and Unifrac metrics, was found to be statistically insignificant ($P \geq 0.05$). The differences in gut bacterial community structure across paired samples was plotted using a PCoA based on weighted and unweighted UniFrac distances (Fig. 4). The PERMANOVA analysis revealed a variation across the paired samples ($P \leq 0.05$, $R^2 = 0.080$), demonstrating a significant difference between samples from the DM1 participants and those from the control group. Further analysis indicated that approximately 8 % of the variation in bacterial community structure could be attributed to the DM1 disease status ($P = 0.037$). Additionally, the PERMDISP analysis indicated no significant dispersion between the samples ($P \geq 0.05$), which suggest the PERMANOVA results are not affected by differences in dispersion across phenotypes. This indicates that the observed changes in bacterial community structure are not simply due to variations in the dispersion of data points but are indicative of true differences in the gut bacterial community between DM1 participants and controls.

3.5. Taxa with significantly different abundance between DM1 patients and controls

VENN analyses showed that out of the identified ASVs, 442 were shared across paired samples, while other ASVs were unique to the status of DM1, with 410 ASVs exclusive to the controls and 280 ASVs exclusive to the paired DM1 participants (Fig. 5). To further identify the taxa that were variably spread between the paired samples, ANCOM-BC was used following alpha- and beta-diversity analyses (Fig. 6). This software estimates then compares the absolute abundance of bacteria based on the Log2-transformed fold change (Log2FC) of ASV tables at the genus level in DM1 patients compared to paired controls. This analysis identified 24 significant genera, among which 8 decreased in DM1 participants compared to control samples, while the remaining 16 genera increased in DM1 groups (Fig. 6b). These findings indicated that the top taxa with significantly higher abundance in healthy controls were mostly from the Firmicutes phylum, including *Megasphaera*, *Lactobacillus*, *Hungatella*, *Tyzzerella*, *Clostridium innocuum*, *Eisenbergiella*, *Eubacterium nodatum*, *Lachnospiraceae UCG-009*, and *Angelakisella*. In addition, the taxa that were considerably higher in the paired DM1 participants were attributed to *Clostridium CAG-352*, *Eubacterium ruminantium*, and *Eu. fissicatena* in the Firmicutes phylum. Finally, an increase in the phyla of DM1 participants was observed in *Paraprevotella*, *Prevotella-9*, *Prevotella* and *Coprobacter* in the Bacteroidetes phylum, as well as *Fusobacterium* and *Methanobrevibacter* from Fusobacteriota and Euryarchaeota phyla respectively. As mentioned previously, at the phylum level, the significant differences were found in Bacteroidota, Euryarchaeota, Fusobacteriota, and Cyanobacteria phyla (Fig. 2). These specific taxa identified through ANCOM-BC analysis provide a more detailed understanding of the differences in the gut bacterial community composition between DM1 participants and controls.

4. Discussion

We characterized the gut bacterial community structure and diversity of DM1 participants and controls, covering various DM1 phenotypes from a population living in the region of Saguenay–Lac-St-Jean in Canada. Our investigation combined diversity and differential

abundance analyses as well as functional analysis to find potential alterations of gut microbiome enzymatic capacity and putative functional pathways. Our findings revealed differences in the beta-diversity of the gut bacterial composition between DM1 and healthy control participants. However, when focusing on alpha-diversity, we did not observe significant differences between patients and controls (Fig. 3; $P \geq 0.05$). These results suggest that the DM1 status is not associated with changes in gut bacterial alpha-diversity. Similarly, as for functional analysis, ggpicrust2 did not report any statistically significant features in four groups of amino acid, energy, carbohydrate, and lipid metabolisms (Table-2). The results indicated the resemblance of gut microbiome enzymatic capacity and functional pathways in the paired samples between control and DM1 participants. It might be stemmed from the low sample size of our study and the large inter-individual variation within microbial communities making it hard to detect differences between groups. On the other hand, when exploring beta-diversity analysis, we discovered that DM1 status explains 8 % of the variation in gut bacterial community when comparing paired samples DM1 vs. healthy controls ($P \leq 0.05$). This difference was confirmed at both phylum and genus levels with ANCOM-BC differential abundance analysis. However, in subgroups, the beta-diversity was not significantly different between the various phenotypes. In other words, the alteration of gut microbiome from pediatric to adult onset was not explained by the results of present study. As such, it appears that the phenotype of DM1 based on the CTG repeat size is not related to the differences observed between participants. From these findings, we cannot conclude that there is a clear link between the CTG repeat size and the alteration of the gut bacterial profile in various phenotypes of DM1 patients. This may suggest that other factors beyond the CTG repeat size play a more significant role in shaping the gut microbiome in DM1 phenotypes. On the other hand, the absence of statistical significance in this study could also be due to the difficult access to samples, thus reducing sample size. Yet, the findings of the present study confirmed that gut microbiome variations in DM1 patients are observed when compared to healthy participants, which suggests an interesting avenue of research to identify potential mechanisms of interactions between gut bacteria and GI tract symptoms in DM1 patients.

The human gut microbiota is established from birth and then continuously shaped by various factors such as age and environmental factors including disease and diet. [76]. As a result, the gut microbiota has been proposed to be closely associated with the overall health status of their host. For instance, in inflammatory bowel disease (IBD), there is a notable dysbiosis of the gut microbiota, characterized by a reduced alpha-diversity and shifts in bacterial composition, including a decrease in the bacterial phylum Firmicutes as well as an increase in the phyla Proteobacteria and Bacteroidetes [77]. Furthermore, patients with nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), or cirrhosis exhibit a significant increase in the phylum Proteobacteria in their stool samples compared to the healthy controls. A healthy gut microbiota consists of five major phyla, namely Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia, with Bacteroidetes and Firmicutes representing the dominant phyla comprising around 90 % of the gut microbiota [78]. The Firmicutes phylum encompasses more than 200 different genera, including *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminicoccus*. Notably, the *Clostridium* genus alone accounts for 95 % of this phylum [79]. In contrast, the Bacteroidetes phylum is characterized by two main genera, *Bacteroides* and *Prevotella* [79]. The Actinobacteria phylum is comparably less abundant and is mainly characterized by the *Bifidobacterium* genus [78]. Findings from our current work indicated that paired DM1 participants seems to have a higher abundance of Firmicutes compared to the controls, while the relative abundance of the Bacteroidetes phylum was significantly higher in paired DM1 participants. This suggests a shift in the ratios of Firmicutes/Bacteroidetes (F/B) in DM1 participants, which has been previously positively associated with loose stools [80]. This observation is consistent with Hollister *et al.*, which
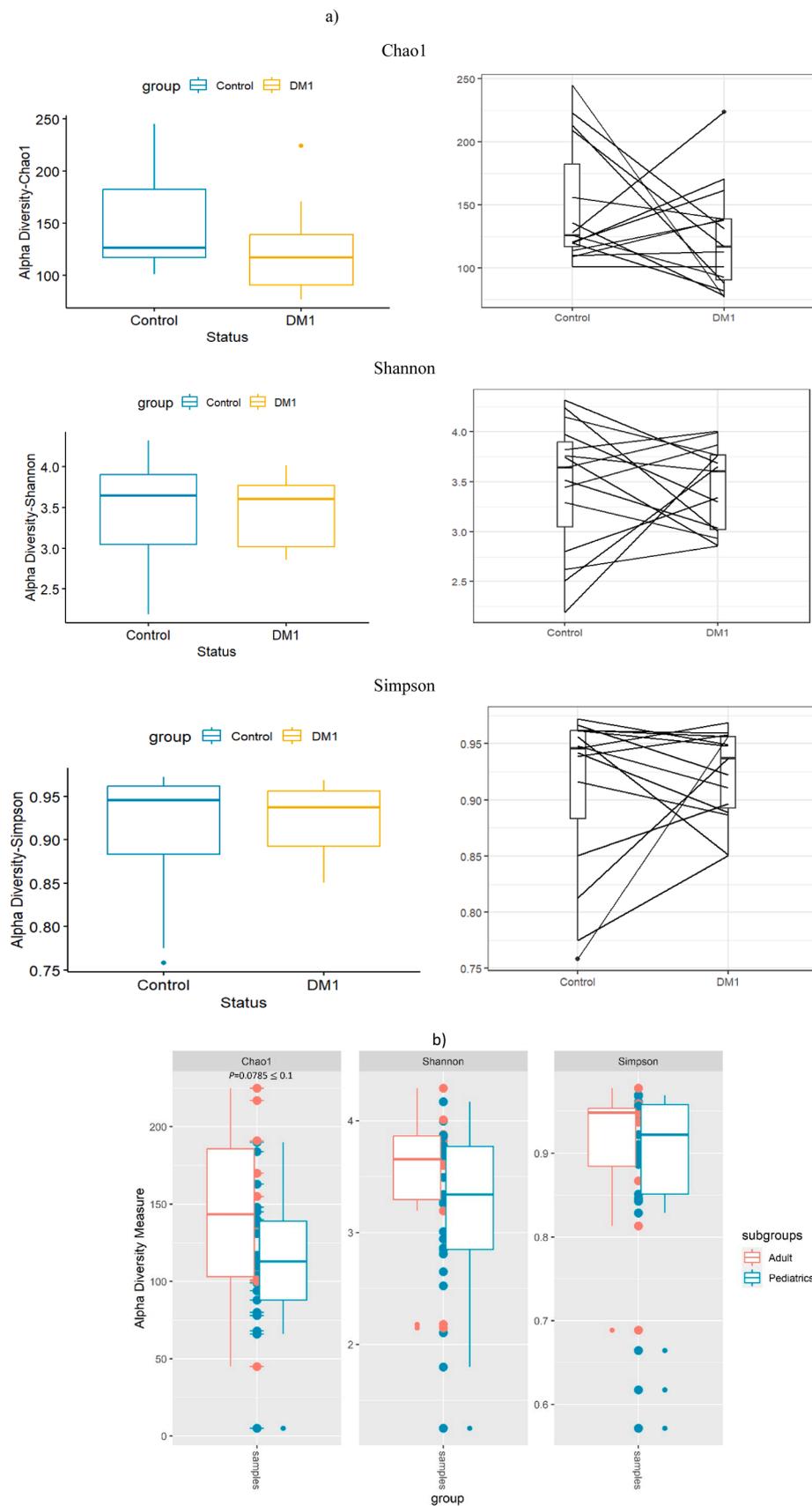
Fig. 3. Alpha-diversity analysis (Shannon, Simpson, and Chao1) in this study. a) Comparing the alpha diversity of all 15 DM1 samples with 15 healthy controls using Paired-sample Wilcoxon test ($P \geq 0.05$). On the right-hand side, each line is related to each paired sample including one DM1 patient and one control. In blue, controls and in yellow, patients with DM1. b) phenotypic subgroups of all 35 DM1 patients. In blue, Pediatrics and in red, Adults.

a)
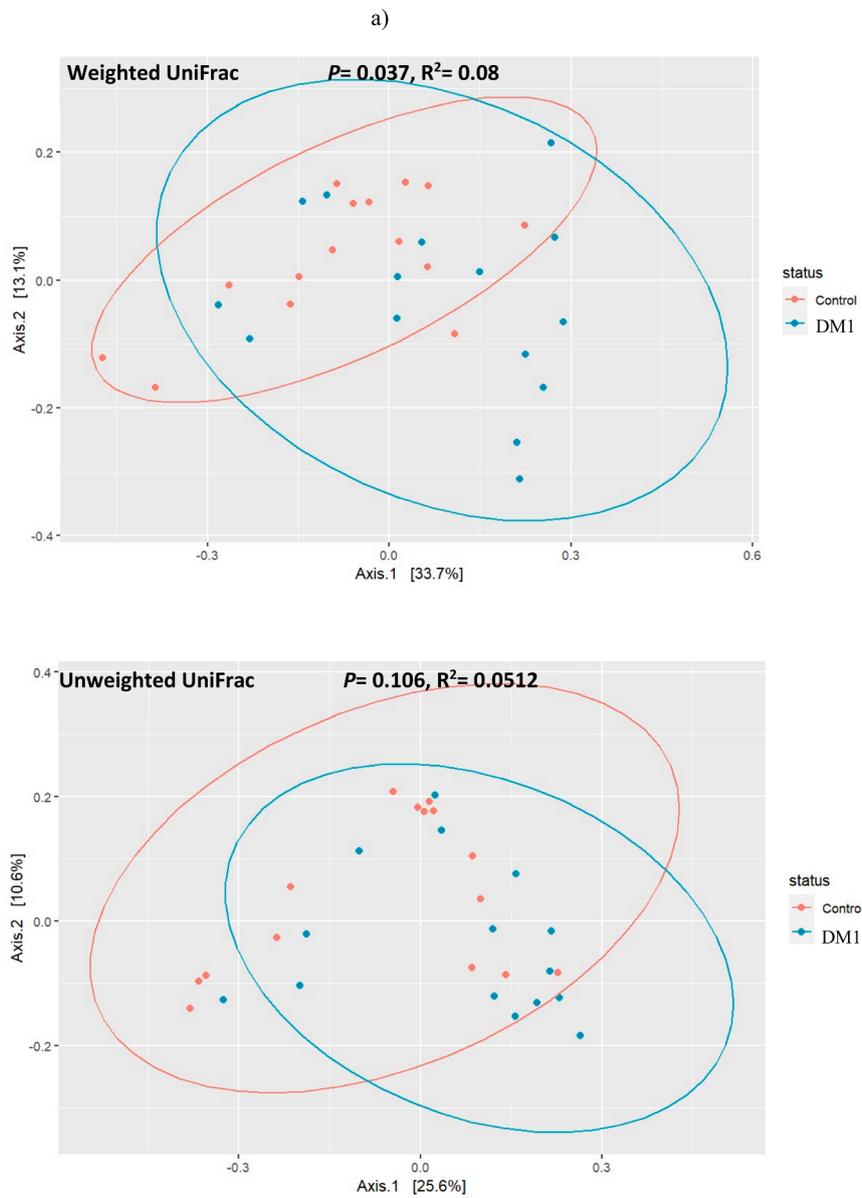
Fig. 4. PCoAs of beta-diversity between paired samples in the study representing the significance difference for DM1 patients and controls. (a) PCoA based on weighted UniFrac ($P \leq 0.05$, $R^2 = 0.08$) and (b) unweighted UniFrac. In red, controls and in blue, patients with DM1.
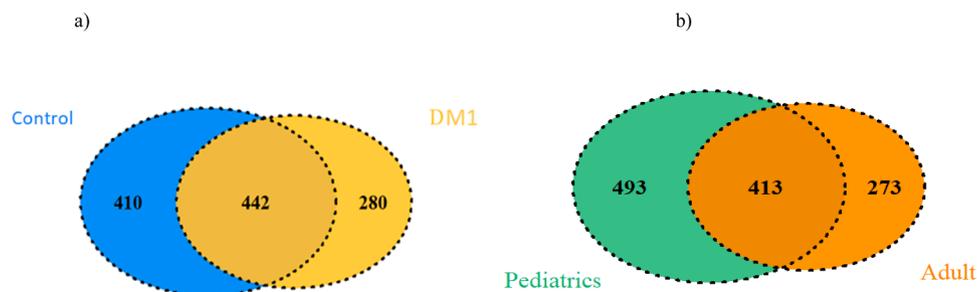
Fig. 5. Shared and unique ASVs among both status of samples (a) as well as the various phenotypes of DM1 compared to controls(b).

confirmed that the alteration of microbial diversity and F/B ratio are linked to GI symptoms in persons with irritable bowel syndrome (IBS) [80]. In addition, change over the F/B ratio have been correlated with inflammation-associated diseases, such as cancer, obesity, and diabetes [81]. The level of interleukin 6, a proinflammatory cytokine, in the serum of DM1 patients correlates with muscle weakness and operational capacity limitations [82]. Indeed, this pro-inflammatory signature is a common feature in different DM1 tissues [83] probably suggesting that gut microbiota changes like F/B ratio variation can have a potential role in the presence of inflammation in DM1 patients. Furthermore, our

a)

b)
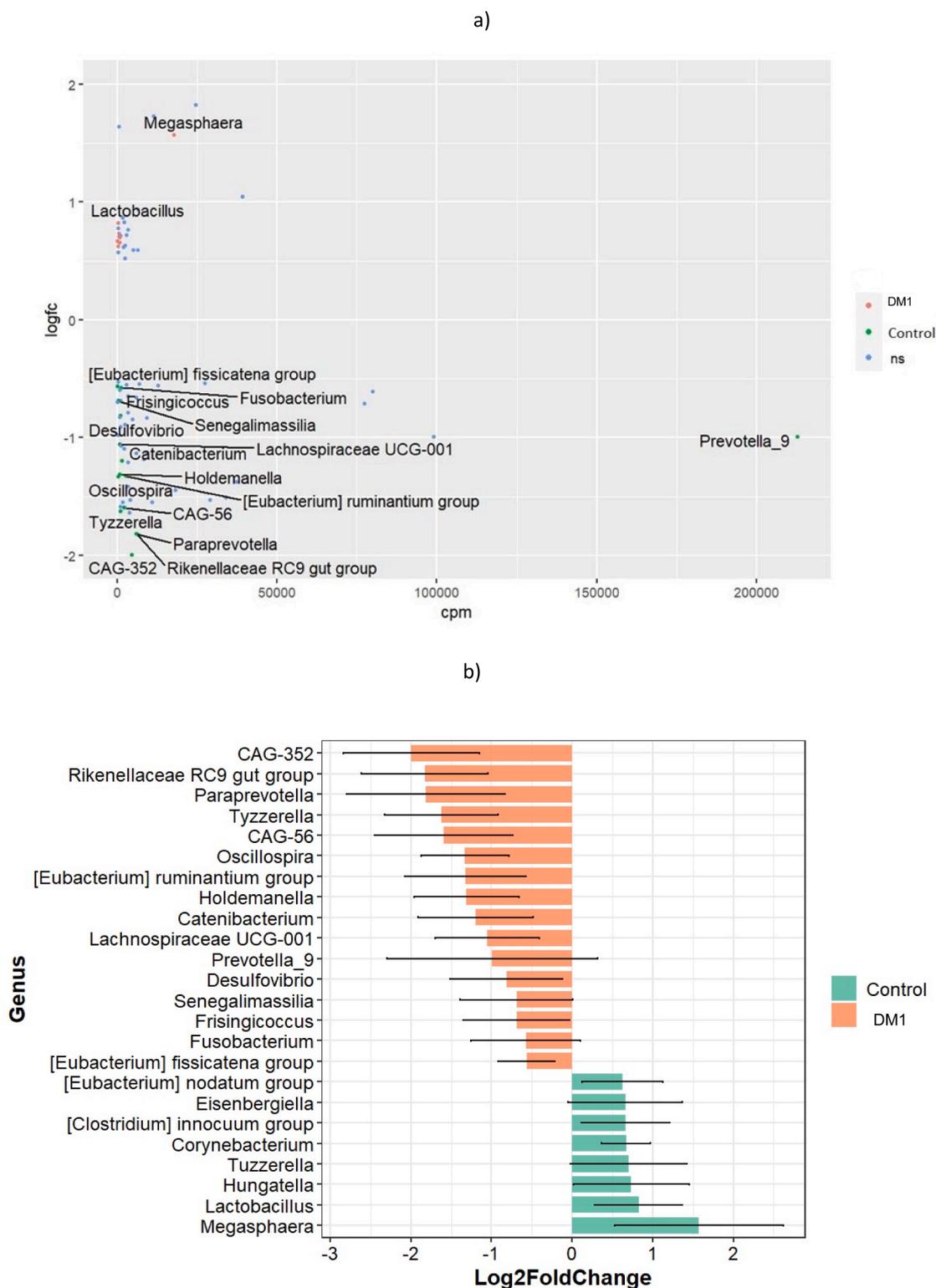
Fig. 6. ANCOM differential abundance analysis. a) ANCOM volcano plot. Volcano plot representing the Log2-transformed fold change (Log2FC, x-axis) of ASV table at the genus level in DM1 patients compared to paired controls in relation to their p value (neglogQval, y-axis). A positive x-axis means a species is abundant in DM1 groups and a negative x-axis value means a species is abundant in control samples. b) Bar graph demonstrating the most significantly differed genera with higher and lower abundance in DM1 and healthy control population.

current study also revealed lower relative abundance of Actinobacteria phylum in control groups compared to the paired DM1 participants, including *Corynebacterium, Rothia,* and *DNF00809,* indicating another type of changes in the microbial signature of gut microbiome in DM1 disease.

In this study, we observed no significant differences at the phylum level apart from Bacteroidota, Euryarchaeota, Fusobacteriota, and Cyanobacteria (Fig. 2). However, significant differences were detected at the genus level. According to our findings, the abundance of *Bacteroidetes* and *Fusobacterium* was higher in the paired DM1 samples

compared to controls. These observation align with previous studies in colon adenocarcinoma (COAD), where tumor samples exhibited significantly higher levels of *Bacteroidetes*, Bacteroidales, and *Fusobacterium* compared to their normal counterparts [84]. Additionally, a global analysis of microbiome signatures in four major types of GI cancer including stomach adenocarcinoma (STAD) reported amplified relative abundance of Firmicutes in tumor samples and a significant drop in Proteobacteria compared to normal samples [84]. Our results also showed a higher relative abundance of *clostridium* CAG-352 from the Firmicutes phylum in paired DM1 participants compared to the controls. Moreover, other studies also have reported higher relative abundance of *Lachospiraceae* [85,86] and *Prevotella* [87] in gastric cancer patients. Our data also suggests that there is a higher relative abundance of *Lachospiraceae* and *Prevotella* in DM1 samples. While our results are limited to a very small cohort and used only amplicon sequencing (which can at best illustrate the active, dormant, and inactive bacterial taxa at the genus level), our findings show changes in the gut microbiota composition in DM1 patients that are in line with bacterial communities that have been detected in patients with cancer. Furthermore, many research works have suggested that patients with DM1 are at increased risk of cancer progression in various locations, including the colon [88], endometrium, ovary, brain [89], and possibly cutaneous melanoma and thyroid cancer [90–92]. Future studies in animal models testing the causal relationships between DM1-like microbiota and the development of gut cancer are needed to confirm a mechanistic link.

Another research study [93] also explored the correlation between gut microbiome composition and subjective IBS symptoms, such as abdominal pain, which had a positive correlation with the genera *Prevotella* (family Erysipelotrichaceae). Notably, we observed a higher relative abundance of this genus in the gut microbiome of paired DM1 participants. Additionally, we noted a decrease in the presence of *Lactobacillus*, a beneficial probiotic bacterium, in the gut microbiome of DM1 patients, providing possible evidence for gut microbiome dysbiosis in DM1 patients. *Lactobacillus* is known to inhibit both the growth and adherence of pathogenic bacteria, promoting their removal [94]. This might reduce diarrhea, bloating and cramps caused by these harmful bacteria [95]. Moreover, several Firmicutes families (Rikenellaceae, Christensenellaceae, Dehalobabacteriaceae, Oscillospiraceae, Mogibacteriaceae, Ruminococcaceae, Sutterellaceae, Desulfovibrionaceae, and Erysipelotrichaceae) were associated with lower extra-intestinal pain severity in a previous study [80], and interestingly, they were found to be in high relative abundance in the paired DM1 participants in our study. Since these changes can create various complications such as gastric cancer, colorectal cancer, and IBS symptoms, our findings suggest a potential connection between the gut microbiome and the GI symptoms observed in DM1 participants. Again, because of the small cohort size and of the known limitations of amplicon sequencing, it is worth noting that these significant changes in the bacterial population of DM1 patients compared to controls should be evaluated further either in animal models or in larger cohorts with more precise microbiological and genomic techniques. Nonetheless, our research shows that there is a detectable shift in the structure of gut microbiome for DM1 participants, both in terms of their relative abundance and bacterial community composition.

In general, several aspects of our research should be considered when analyzing the results. First, it is important to not overlook the effect of other factors on the gut microbiome, such as age, sex, method of sampling, diet, and other random drivers and the restrictions of the microbiome analysis. Indeed, the comprehensive understanding of the dietary habits of all patients influencing the gut microbiota structure was lacking. This includes whether they were fasted or fed during sample collection, the timing of the last meal consumed, and the percentage of fiber in their diet. One additional limitation is the documentation of the GI symptoms, solely depending on a generic patient-reported outcome measure, restricts our understanding of the real GI tract involvement including gastric and small intestine symptoms in DM1. In addition,

because of other obvious individual differences, the results of the study should be regarded with caution. Second, this research is an observational study with only 35 DM1 samples, relying on correlations from relative abundance data, which does not provide information on absolute abundances in the gut and differences in the genetic content of functional pathways. Furthermore, the use of convenience sampling from a single registry may limit the generalizability of the findings. Third, 16S rRNA gene sequencing only provides data on live, dormant, and inactive microbes. This study only focused on bacteria, whereas the gut is colonized by a complex community. Excluding other components of the gut microbiome such as fungi, viruses, and protozoan eukaryotes may ignore important aspects influencing GI symptoms in DM1. Fourth, feces represent the microbial content at the end of the gastrointestinal tract, far from the small intestine where crucial interactions with immune cells occur. As a result, the gut microbiota composition and function in the small intestine, which might be critical in understanding certain aspects of DM1, remain beyond the scope of our investigation. In future works, shotgun metagenomics sequencing or whole genome sequencing instead of 16S rRNA gene sequencing could provide more information through identifying bacteria at species level, in addition to functional analysis and collecting data from all domains of life including fungi, viruses, and protozoan eukaryotes with their genetic content in DM1 patients.

5. Conclusion

To the best of our knowledge, our study stands as a unique exploration of gut bacterial communities of DM1 patients using a 16S ribosomal RNA gene sequencing approach. Our results indicated that gut bacterial genera diversity of DM1 participants significantly changes compared to control samples. These results could serve as the basis for studying the gut microbiome of DM1 patients, aiming to understand how we can restore their microbiota to a healthier status and alleviate their gastrointestinal difficulties. In addition, our results provide a valuable reference for analyzing microbiome variations in the gastrointestinal tract of DM1 patients. It is worth noting that drawing direct comparisons between 16S rRNA gene sequencing studies can be challenging because of the variance introduced by DNA extraction, sequencing, and taxonomic assignment approaches. Further functional studies are essential to gain a deeper understanding of the complex interplay between gut microbiota and DM1.

CRediT authorship contribution statement

Karine Prévost: Methodology, Resources. **Isabelle Fisette-Paul Hus:** Methodology, Writing – review & editing, Investigation. **Philippe Balthazar:** Data curation, Methodology, Software. **Nicolas Dumont:** Investigation, Methodology. **Élise Duchesne:** Investigation, Methodology. **Cynthia Gagnon:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Valérie Gagné-Ouellet:** Investigation, Methodology, Resources. **Eric Massé:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Laforest-Lapointe Isabelle:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing, Software. **Manijeh Mahdavi Mazdeh:** Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

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