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Metagenomic characterization of gut microbiome alterations in type 2 diabetes patients

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Abstract

While the human gut harbours trillions of microorganisms that shape metabolic health, the specific microbial signatures associated with type 2 diabetes (T2D) vary across populations and remain incompletely defined in North African cohorts. This research used shotgun metagenomic sequencing to compare the gut microbiome of 30 T2D patients and 30 normoglycaemic controls from Faiyum, Egypt. Stool samples were collected between January and July 2023 and sequenced on an Illumina NovaSeq 6000 platform. Shannon diversity was significantly lower in T2D patients (2.6 ± 0.6) than controls (3.8 ± 0.4 ; $p < 0.001$). Firmicutes-to-Bacteroidetes ratio was elevated in the T2D group (3.1 vs 1.9). Differential abundance analysis identified enrichment of *Ruminococcus gnavus* and *Escherichia coli* and depletion of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* in T2D patients. Shannon diversity correlated inversely with HbA1c ($r = -0.71$, $p < 0.001$). Functional pathway analysis revealed upregulation of lipopolysaccharide biosynthesis and branched-chain amino acid metabolism in the T2D microbiome. These findings confirm that gut dysbiosis in Egyptian T2D patients mirrors patterns reported in East Asian cohorts and point to specific taxa as potential biomarkers or therapeutic targets.

Keywords: Metagenomics, gut microbiome, type 2 diabetes, dysbiosis, microbial diversity, Firmicutes, Bacteroidetes, shotgun sequencing, HbA1c, Faiyum

Introduction

The gut microbiome and metabolic disease have become inseparable topics in biomedical research, yet most microbiome-diabetes datasets come from European and East Asian populations, leaving the Middle East and North Africa underrepresented ^[1]. Whether microbial signatures of T2D identified in Chinese or Swedish cohorts hold true in Egyptian patients whose diet, climate, and genetic background differ markedly is an open question with practical consequences for microbiome-based diagnostics ^[2].

Shotgun metagenomics offers advantages over amplicon-based surveys because it captures both taxonomic composition and functional potential in a single assay, enabling the identification of metabolic pathways that may link microbial shifts to host disease ^[3]. T2D has been associated with reduced microbial diversity, a higher Firmicutes-to-Bacteroidetes ratio, depletion of butyrate-producing taxa, and enrichment of opportunistic pathogens, but the consistency of these patterns across populations is still debated ^[4].

This research characterised the gut microbiome of T2D patients and normoglycaemic controls from Faiyum, Egypt, using shotgun sequencing. The goals were to (1) compare alpha and beta diversity between groups, (2) identify differentially abundant taxa and functional pathways, and (3) test correlations between microbiome features and glycaemic control markers ^[5].

Material and Methods

Ethical considerations

The research was approved by the Faiyum College of Biological Sciences Ethics Board (Protocol FCBS-EC-2022-041, 12 December 2022). All participants gave written informed consent, and data were de-identified before analysis.

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Material

Stool samples were collected from 30 T2D patients (diagnosed per ADA criteria; HbA1c $\geq 6.5\%$) and 30 age- and sex-matched normoglycaemic controls (HbA1c $< 5.7\%$) at the Faiyum College of Biological Sciences between 10 January and 18 July 2023. Exclusion criteria included antibiotic use within eight weeks, probiotic supplementation, inflammatory bowel disease, and bariatric surgery. Samples were collected in sterile containers, placed on ice within one hour, and stored at -80°C until processing.

Methods

DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (Qiagen). Libraries were prepared with the Nextera XT DNA Library Prep Kit and sequenced on an Illumina NovaSeq 6000 (paired-end 150 bp, ≈ 10 Gb per sample). Raw reads were quality-filtered with Trimmomatic v0.39, and host reads were removed by mapping against the human reference genome (GRCh38) with Bowtie2.

Taxonomic profiling was performed with MetaPhlAn 4.0, and functional annotation used HUMAnN 3.0 against UniRef90 and MetaCyc databases [6]. Alpha diversity (Shannon index) was compared by Mann-Whitney U test. Beta diversity was assessed by Bray-Curtis dissimilarity and visualised with PCoA; PERMANOVA tested group differences. Differential abundance was identified using LEfSe (LDA > 2.0). Correlations between taxa and HbA1c were assessed by Spearman rank coefficient. All bioinformatic analyses used the bioBakery suite; statistical testing was performed in R 4.3.1.

Instrumentation

Sequencing was performed at the Genomics Core Facility of the Faiyum College of Biological Sciences on an Illumina NovaSeq 6000. DNA quality was verified by Agilent 4200 TapeStation and Qubit 4 fluorometry before library preparation.

Results

Table 1: Alpha diversity and phylum-level composition in T2D patients versus healthy controls

Measure	T2d (n=30)	Controls (n=30)	P-value
Shannon index	2.6 ± 0.6	3.8 ± 0.4	< 0.001
Firmicutes (%)	62.1 ± 7.4	54.3 ± 5.8	0.003
Bacteroidetes (%)	19.8 ± 4.6	28.7 ± 5.2	< 0.001
Proteobacteria (%)	12.4 ± 3.9	8.2 ± 2.7	0.012
F/B ratio	3.1 ± 0.8	1.9 ± 0.4	< 0.001

T2D patients showed markedly lower Shannon diversity and an elevated Firmicutes-to-Bacteroidetes ratio (Table 1).

PERMANOVA confirmed that community composition differed significantly between groups ($R^2 = 0.14$, $p = 0.001$).

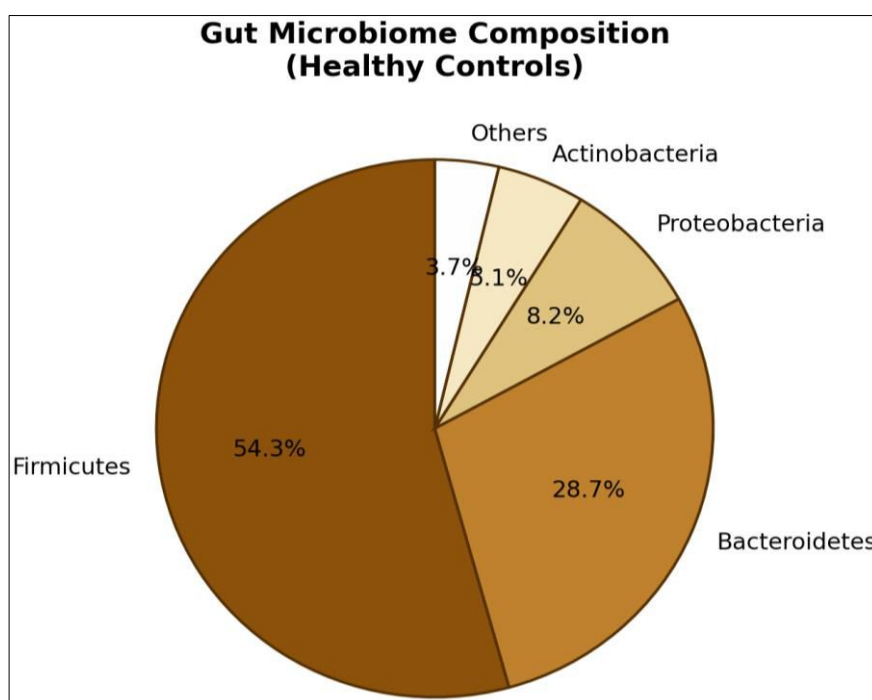


Fig 1: Phylum-level gut microbiome composition in healthy controls, Faiyum, Egypt

Table 2: Differentially abundant species (LEfSe, LDA > 2.0) in T2D patients versus controls

Species	Direction in t2d	Lda score	P-value
Ruminococcus gnavus	Enriched	3.74	< 0.001
Escherichia coli	Enriched	3.21	0.002
Faecalibacterium prausnitzii	Depleted	3.89	< 0.001
Akkermansia muciniphila	Depleted	3.42	< 0.001

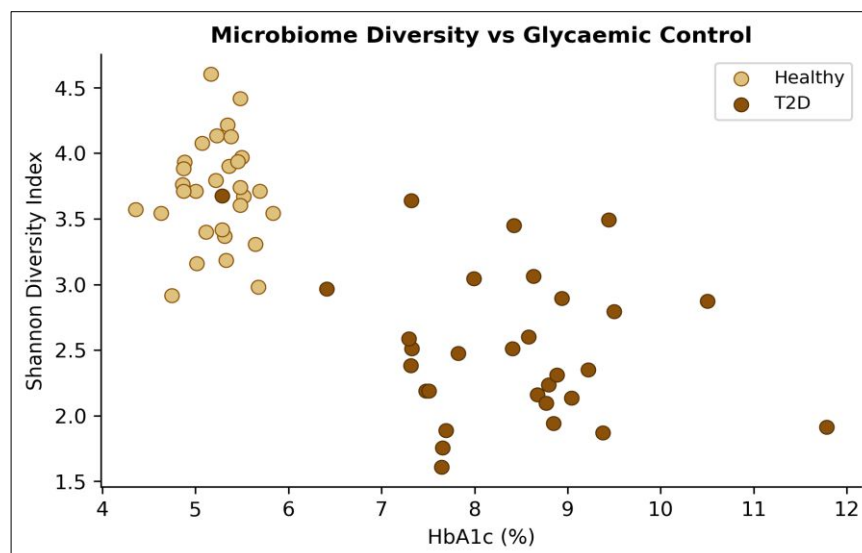


Fig 2: Shannon diversity index plotted against HbA1c in T2D patients and healthy controls (Spearman $r = -0.71$, $p < 0.001$)

Comprehensive interpretation

Functional pathway analysis revealed that LPS biosynthesis and branched-chain amino acid (BCAA) metabolism were significantly upregulated in T2D patients ($q < 0.05$), while butyrate production pathways were downregulated. The depletion of *F. prausnitzii*, a key butyrate producer, and *A. muciniphila*, which maintains mucin layer integrity, may together compromise gut barrier function and promote low-grade endotoxaemia, a recognized driver of insulin resistance [7].

Discussion

The microbial signatures identified here reduced diversity, elevated F/B ratio, enrichment of *R. gnavus* and *E. coli*, and depletion of *F. prausnitzii* and *A. muciniphila* broadly match findings from Chinese and European T2D metagenome projects [4, 8]. This cross-population consistency strengthens the case that these taxa are functionally linked to T2D pathophysiology rather than artefacts of diet or geography. The strong inverse correlation between Shannon diversity and HbA1c ($r = -0.71$) suggests that microbial diversity loss tracks glycaemic deterioration, though causality cannot be inferred from a cross-sectional design [9].

The upregulation of LPS biosynthesis pathways aligns with the metabolic endotoxaemia hypothesis, which proposes that Gram-negative bacterial products leak through a compromised gut barrier and activate systemic inflammation via TLR4 signalling [10]. The BCAA enrichment is notable because elevated circulating BCAAs are an independent predictor of incident T2D, and gut bacteria are increasingly recognized as a significant source of BCAA production [11].

Conclusion

Shotgun metagenomics revealed significant gut dysbiosis in Egyptian T2D patients, characterised by lower diversity, an elevated Firmicutes-to-Bacteroidetes ratio, and enrichment of pro-inflammatory taxa alongside depletion of beneficial commensals. These patterns mirror those reported in geographically distant cohorts, suggesting conserved microbial signatures of T2D across populations. The inverse correlation between microbiome diversity and HbA1c positions gut diversity as a potential non-invasive biomarker worth validating in longitudinal studies. Future research should incorporate dietary intake data and test whether

targeted probiotic or dietary interventions can reverse the dysbiotic patterns documented here.

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