Review

Understanding Patterns of the Gut Microbiome May Contribute to an Early Detection and Prevention of Type 2 Diabetes Mellitus

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Review

Understanding Patterns of the Gut Microbiome May Contribute to an Early Detection and Prevention of Type 2 Diabetes Mellitus

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Abstract: The rising burden of type 2 diabetes mellitus (T2DM) is a growing global public health problem, particularly prominent in developing countries. Early detection of T2DM and prediabetes is vital for reversing the outcome of disease, allowing early intervention. In the past decade various microbiome-metabolome studies attempted to address the question whether there are any common microbial patterns which would indicate either prediabetic or diabetic gut microbial signatures. Because current studies have a high methodological heterogeneity and risk of bias, we have selected studies which adhered to similar design and methodology. We have performed a systematic review to assess if there are any common changes in microbiome belonging to diabetic, prediabetic and healthy individuals. The presented here cross-sectional studies collectively covered a population of 65754 people, with 1800 in 2TD group, 2770 in prediabetic group and 61184 in control group. The overall microbial diversity scores were lower in T2D and prediabetes cohort in 86% of analysed herein studies. Re-programming microbiome is potentially one of the safest and long-lasting ways to eliminate diabetes in its early stages. The differences in abundance of certain microbial species could serve as an early warning for a dysbiotic gut environment and could be easily modified before the onset of disease by changes in lifestyle, through taking probiotics, introducing diet modifications, or stimulating vagal nerve. This review shows how metagenomic studies already had and will continue uncovering novel therapeutic targets (probiotics, prebiotics or targets for elimination from flora). This work clearly shows that gut microbiome intervention studies, if performed according to standard operating protocols using predefined analytic framework (e.g. STORMS), could be combined with other similar studies allowing broader conclusions from collating all global cohort studies efforts and eliminating the effect-size statistical insufficiency of a single study.

Keywords: gut microbiome; diabetes; hyperglycaemia; prediabetes; microflora; type 2 diabetes mellitus; gut flora; kynerunine; *Akkermansia muciniphila*; metformin; GLP-1

1. Introduction

It has been estimated that globally the prevalence of T2D mellitus will increase to 7079 individuals per 100,000 by 2030 [1]. In the USA around 1 in 14 adults are estimated to have prediabetes, whilst in the UK 1 in 9 adults is affected, often without being aware of it [2,3] Current preventative and treatment strategies for prediabetes and diabetes is limited and do not always bring desired results [4]. Since diabetes starts with chronic mild hyperglycaemia, so called prediabetes (defined as fasting plasma glucose of 6.1–7.0 mmol/l or HbA_{1c} of 42–48 mmol/mol [6.0–6.5%]) [5] it is beneficial to capture this early phase. Change in microbiome, lifestyle and diet are the least invasive ways to stop glucose desensitization and to cease further onset of diabetes.

The gut microbiome consists of communities of metabolically active microorganisms, which growth is either promoted or limited by the host´s diet (including prebiotics), lifestyle, and health

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factors. Each person's microbial patterns differ in abundance and composition – therefore making it a unique fingerprint [6]. However, during the recent years of microbiome field research, common patterns of microbes have been distinguished and associated with eubiosis (which reflects a healthy state) or dysbiosis (when the microbiome affects the host negatively) [7]. Thus, gut microbiome represents one of the greatest, still undiscovered field of possible markers and early predictors showing an association of bacteria with metabolic diseases, cancers, inflammatory responses, and cognitive abilities. The microbiome can be described at several levels for instance the taxonomic level resolution/granularity, from phylum, via class, order, genus and species, or through their biological functions, such as producers of short chained fatty acids, or bioactive molecules (like kynurenine pathway), or general pathogenic or opportunistic characteristics. So far, most taxonomic studies in humans have focused on microbiome analysis at the genera or phylum levels. However, it is now clear that some strains from within certain phyla are more beneficial than others, prompting researchers to zoom into lower taxonomic levels and investigate microbial intricacies at the species level.

The taxonomic patterns clearly correspond with complex metabolic interactions between the host and their microbial communities; therefore, the interplay is more complicated than previously thought [8]. For instance, the type and abundance of bacterial species producing short chain fatty acids (such as acetate, propionate and butyrate) from nutrients in the large intestine of humans, thrive in the presence of prebiotics and dietary fibre [9]. Therefore, it is possible to stimulate microbial gut flora through specific diet and lifestyle and derby prevent development of dysbacteriosis.

Gut Microbiome and Hyperglycaemia

Gut microbiome strongly affect glucose metabolism but also the type of sugars and fats being consumed by the host have an impact on the type of bacterial species human gut is colonized with [10]. There is a reciprocal relationship between diet and gut microbiome composition along with molecules produced by microflora which pass into the host´s circulation. The eubiotic gut epithelial barrier is maintained by a healthy, diverse microbiome composed primarily of 4 phyla: *Bacteriodetes, Firmicutes, Actinobacteria* and *Proteobacteria.* Phylogenetically related groups of bacteria, namely *Proteobacteria* and *Enterobacteriaceae* were previously associated with poor glycemic control and with negative metabolic syndromes including obesity, insulin resistance and impaired lipid profile [11,12]. The combined data from the past decade's cohort studies, strongly support the hypothesis of prediabetes-associated microbial pattern that differs from healthy microflora[13–15]. Gathered here research data comparing healthy versus diabetic gut microflora unequivocally confirm there are statistically significant differences in microbiome between these groups. Butyrate producers are significantly reduced in prediabetes and type 2 diabetes suggesting a clear metabolic deficit in these individuals [16,17]. Bacteria producing butyrate showed strong anti-inflammatory properties, hence promoting healthy intestinal barrier [17]. Short chain fatty acid (SCFA) producers break down non-absorbed carbohydrates and convert them into beneficial by-products used by host cells. Butyrate producers make up a functional group rather than taxonomic group and are either Gram positive or Gram-negative [17]. Short chain fatty acids, especially butyrate showed to improve ion absorption, intestinal barrier function, cell differentiation, motility, immune regulation at the intestinal level [18]. Additionally, SCFAs significantly improve electrolyte balance due to their non-ionic diffusion and SCFAs transporters. Since SCFAs are utilizing HCO3- exchange to enter the colonic cells, they contribute to reduction of intracellular oxidative stress and stimulation of Sodium Chloride uptake [19]. Recent findings also confirm importance of Tryptophan (Trp) metabolic pathway in multiple diseases inclusive of T2DM. Metabolites of Tryptophan such as kynurenine, kynurenate, xanthurenate and quinolinate and L-tryptophan have been increased in circulation of insulin resistant individuals [20,21] whilst indole propionate was negatively associated with T2DM [21]. Indole-propionate associated bacteria span three phyla *Firmicutes, Actinobacteria and Bacteroidetes* and are majorly SCFAs producers, utilising complex carbohydrates[21]. Therefore, when analysing microbiome, it is insufficient to only describe the microbiome at the phylum level but to dive into the lower taxonomic level to ensure more precise conclusions and higher specificity. The lower

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taxonomic information would allow design of tailored microbial therapy to treat these metabolic inefficiencies.

The aim of our study was to gather already published information on which bacterial taxa was associated with development of T2DM. We have selected the articles on the base of study design similarity, namely same research question, Illumina sequencing method, similar analytic protocols. Based on collected information we could not however rule out if the recorded changes in microbiome occurred prior to diabetes or due to developing diabetes, therefore we prompt the reader to interpret the association cautiously without linking to causation of T2DM.

2. Materials and Methods

A systematic review of the literature describing clinical cohort studies was performed on Ovid MEDLINE, Ovid Embase, PubMed, Google Scholar adhering to PRISMA guidelines [22] . Data was extracted from each published cohort study by structured survey. The search terms used for the search included: '' gut microbiome AND T2DM AND Illumina '' , ''gut microbiome AND Type 2 Diabetes Mellitus AND Illumina'' with limits to human cohort studies published between year 2010-2024. The information was indexed using controlled vocabulary and key words (NVivo). Exclusion criteria was applied to studies in which sequencing methods differed substantially, and studies where only targeted quantitative PCR has been undertaken. Further exclusion criteria applied to duodenal microbiome, with limitation to gut microbiome sampled by stool analysis only. Studies evaluating treatment groups were included only if they had T2D untreated and non-diabetic control groups. Data was extracted from each published cohort study by structured survey.

We have retrieved 10932 original articles and review papers published during 2010-2024. We have excluded articles in which 2TDM treatment has been solely evaluated. Our study yielded 10932 unique articles, from which 20 observational cohort studies were included in our analysis and 1 machine learning association study. The presented here cross-sectional studies collectively covered a population of 66022 people, with 1800 in 2TD group, 2904 in prediabetic group and 61318 in control group. The selected studies covered a diverse population from various geographical locations and various ethnicities.

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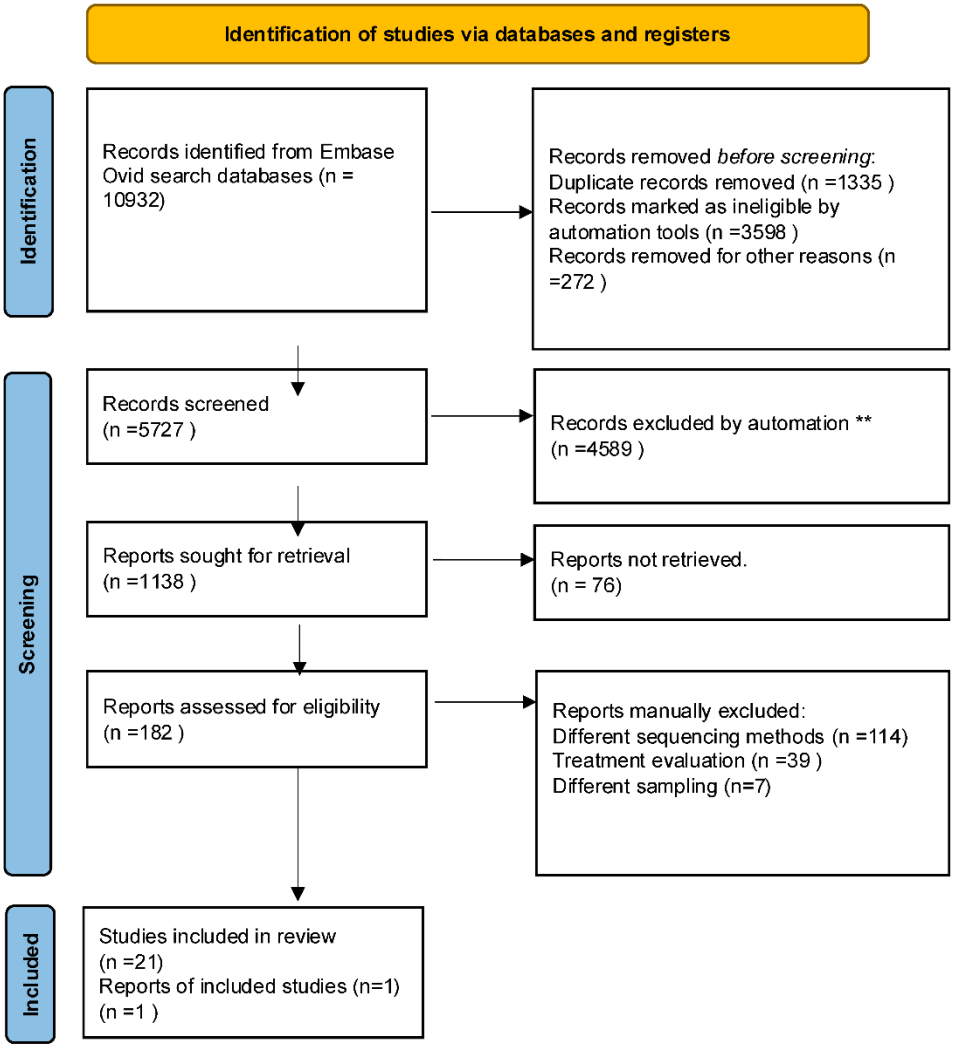

Figure 1. PRISMA diagram of studies identification and selection.

3. Results

Most of studies (86%) analysed here confirm that Type 2 Diabetes and prediabetes groups had significantly lower alpha diversity score measured either by Shannon index score or Chao index. In general, all studies consistently reported that healthy microflora differed significantly from patients with T2D by certain species depletion and enrichment. The lower taxonomic analysis across all studies showed consistency in findings of Type 2 diabetes negatively correlated bacteria, shown in Figure 2a. Depletion of *Akkermansia spp., Ruminococcae, Faecalibacterium spp, Clostridiaceae, Bifidobacterium spp, Bacteroides spp.* were all found negatively correlated with T2DM across the highest number of cohorts included in this study. Consistently across all cohort studies *Shigella*, *Escherichia* and *Ruminococci* were positively associated with the diabetic state, where increase in abundance of those species was strongly linked to T2D and T2D diseases progression (longitudinal cohort studies). Out of 20 cohorts, 7 reported increases in *Lactobacillae* and *Dorea*, and one study reported increase in *Lachnospiracea* (to which *Dorea* belongs at a higher taxonomic level). Additionally, 4 cohorts reported increase in *Proteobacteria* phylum without lower taxonomic details (Figure 1b).Only one study out of 20 analysed here reported decrease in abundance of *Blautia* and *Anaerostipes* in T2D participants, hence reporting those as positive species associated negatively with T2D progression [23]. Diener et.al study found that higher levels of *Blautia* and *Anaerostipes* were associated with lower areas under the glucose curve and normal beta cell function (FDR adjusted LRT $p < 0.05$); however, these results weren't confirmed by other studies analysed here. Two studies

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which qualified under our selection of cohort design and methodology, reported the contrary: *Anaerostipes and Blautia* as genera which increased abundance levels correlated positively with T2D [24,25]. The discrepancy might be due to use of metformin treatment in Diener et.al study, which is widely known to modify microbiome. The increase in abundance of bacteria from the genus *Butyricimonas* was also reported to counteract T2DM, which when zoomed into family level, showed prevalence of *Christensenellaceae and Rikenellaceae*. All the cohort studies analysed in this manuscript are consistent with the hypothesis of butyrate-producers abundance having a negative correlation with T2D onset, suggesting that ethnical or geographic differences don't interfere with the metagenomic markers. However, it has also been reported that gut microbiome differs across geographic regions, with multiple species being good predictors for T2DM in some studies whilst showing no predictive value in other locations (e.g., China vs Sweden [13,26]. Some studies , like the Finnish cross-populational study, zoomed into lowest taxonomic levels revealing four consistently associated with T2D bacterial species: *Clostridium citroniae* (hazard ratio [HR] 1.21; 95% CI 1.04–1.42), *C. bolteae* (HR 1.20; 95% CI 1.04–1.39), *Tyzzerella nexilis* (HR 1.17; 95% CI 1.01–1.36), and *Ruminococcus gnavus* (HR 1.17; 95% CI 1.01–1.36) [24]. Whilst the same study showed that at the higher taxonomic level bacteria belonging to genera such as *Ruminococci, Blautia, Egghertella* are correlated with the incidence of T2D [23]. This review shows how scarce is the whole genome sequencing data and the information on microbiome at the species level taxonomic resolution, underlying the need for such studies to drive innovation in this field.Common discrepancy across studies presented here included *Faecalibacterium* which in some studies analysed here (3 out of 21) was positively associated with T2D whilst in 6 other large cohorts it was found negatively associated with T2D and prediabetes. This discrepancy in findings might be due to insufficient taxonomic analysis at the species level. For example *Faecalibacterium prausnitzii is a* Gram-negative, spore former and a butyrate producer which abundance has been negatively associated with Inflammatory bowel disease [27–29]. In our analyses of 21 studies, we had found bacteria reported as positive and negative in different studies (See supplementary Table 1).

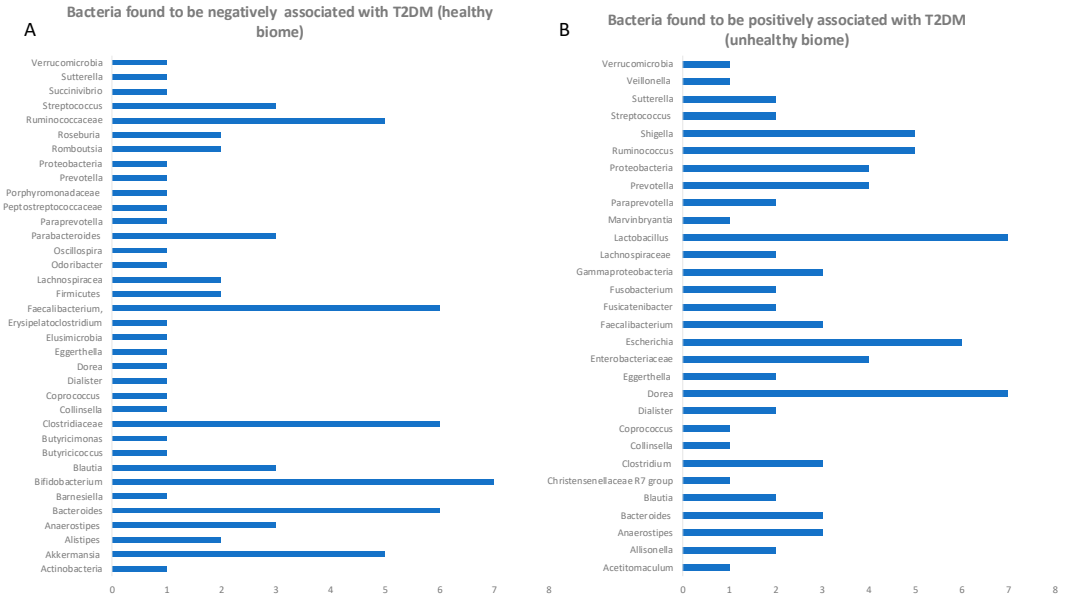

Figure 2. Cumulative list of bacterial taxa reported in the publications reviewed here**.** (a) A number of published studies describing bacteria negatively associated with type 2 diabetes mellitus. Panel (b) number of studies in which bacterial taxa were found negatively associated with type 2 diabetes.

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4. Discussion

In the past decade there were numerous cohort clinical observational studies aimed at revealing gut microbiome differences between diabetic versus healthy subjects. However just few of these studies involved enough large population sample to draw general conclusions. Many studies of gut microbiome showed that gut microbial flora might differ due to diet, geographical location, ethnic origin, and many more confounding factors, therefore this systematic review takes a unique cross-sectional approach to gather the data from multi-ethnical, similarly designed studies. We have selected the studies with similar methodological design, namely metagenome sequencing method using similar primers or whole genome, to ensure comparability. The combined results represent a cohort of 65754 global population, covering all ethnicities and multiple geographic locations. Here, we confirmed specific microbial patterns of gut microbes which could indicate prediabetes or type 2 diabetes. The most recent metadata analysis by Gurung et.al. 2020 summarized 42 human studies in which type 2 diabetes has been investigated in terms of microbiome. According to these results genera negatively associated with T2D were reported as follows: *Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia and Roseburia* (increase in these species would associate with lower T2D risk)[30]. Our results consider the most recent cohort studies over the past decade, showing that lower taxonomic classification is more informative and could help in selecting some taxa as predictive markers. Additionally, we would like to emphasize the need for larger amplicon sequencing or whole genome sequencing approach to allow more precise readouts with highest resolution towards species. The results of our analysis confirm protective capabilities of taxon *Anaerostipes* which are butyrate producers. This is in line with previous findings that butyrate content in the lumen is associated with lower Peroxisome proliferator-activated receptor gamma activity (it's a protein that regulates genes involved in energy metabolism), increased glycolysis and lower oxygen consumption [31]. The analysis of herein referenced cohort studies also confirm positive effect of *Faecalibacterium*, however it might be worth investigating what is the optimal abundance of this genera as few other studies determined it as negative species. Other short-chain fatty acids producers from the *Clostridiacea* class were also confirmed in our study to be negatively correlated with type 2 diabetes. The higher abundance of this succinate-consuming species was earlier correlated with increased levels of propionate and butyrate production in gut [32]. The causal link of diabetes and the decrease in *Clostridiacea* class could be explained by the decrease in production of SCFAs, leading to a depletion of glucagon-like peptide 1 (GLP-1) and insulin production, since these SCFAs can lead to the secretion of GLP-1 by binding to G-protein coupled receptors (GPCRs) from L-cells; GLP-1 promotes insulin secretion and beta-cell proliferation [33].

An important multi-omic study by Zhao et.al. 2017 investigated metabolic profiles using Liquid Chromatography/Mass Spectrometry and Gas Chromatography/Mass Spectrometry method for faecal metabolome, revealed that the concentration of SCFAs were predominantly reduced in the T2D group (Kruskal-Wallis H-test, $P< 0.01$, BMI and age adjusted $P <0.01$)[34]. In addition, the same study found levels of lysophosphatidylcholine (LPC), cholic acid and palmitoyl carnitine to be higher in T2D group by 4.51 to 13.84 fold change (Kruskal-Wallis H test, $P< 0.001$). LPC is known for its demyelination properties *in vivo*, but also could be found in circulation at various levels as it is essential component of normal human brain development.

Interestingly, in line with taxonomic analysis, another gut microbiome metabolomics study revealed that bacterial genes highly associated with T2D encoded tyrosine degradation enzymes, pentose phosphate pathway proteins, lactose, galactose and butyrate production proteins [35]. These findings support the hypothesis of specific bacterial composition which might differ healthy gut microbiome from T2D one, further allowing analysis of diet and its effect on microflora and metabolic by-products. Steps towards improvement of gut microbiome have already been taken via probiotic and pre-biotic therapy, however there are multiple novel strains discovered in the past decade with proven efficiency *in vitro* mouse models awaiting clinical trials. Particularly administration of *Akkermanisa muciniphila* has been confirmed by several studies as microbial probiotic able to improve glucose tolerance and insulin resistance in mice[36,37]. In all the cohort studies taken under analysis for the purpose of this review, *Akkermansia muciniphila* proved to be consistently increased.

A.muciniphila has been recently discovered to be of major importance in gut health, contributing to reduction of obesity and decrease of metabolic disorders in diabetic mouse models [38]. *A.muciniphila* can restore the mucosal layer in the gut and is considered to reduce gut permeability, hence acting as anti-inflammatory probiotic species[39]. Overall, consistently in all studies published so far abundance of *Akkermansia spp*. has been inversely correlated with diabetes, Body Mass Index (BMI), Inflammatory Bowel Disease (IBD)and autism spectrum [40–42]. The bacteria is also commercially available on the market in the UK and Europe- suggesting its large therapeutic potential in the field of microbial sciences. Our study confirms that the gut microbiota represents an important modifiable factor to consider when developing precision medicine approaches for the prevention and/or delay of T2D.The future work to continue this study would be a stratified metanalysis, gathering the raw sequencing data from reviewed here studies which could potentially reveal more information and allow more sensitive statistical evaluation. For that reason we strongly support repositories with open access such as European Genome-Phenome Archive [43] or NIH Human Microbiome Project, which would allow further discovery in this exciting field.

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Conflicts of Interest: The authors declare no conflicts of interest.

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