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Original article

Associations between micronutrient intakes and gut microbiota in a group of adults with cystic fibrosis

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SUMMARY

Background: Cystic fibrosis (CF) involves chronic inflammation and oxidative stress affecting mainly the respiratory and digestive systems. Survival rates for CF have improved with advances in treatment including nutritional interventions such as micronutrient supplementation. Diet can modulate gut microbiota in the general population with consequences on local and systemic immunity, and inflammation. The gut microbiota appears disrupted and may associate with pulmonary status in CF. This study investigated associations between micronutrient intakes and gut microbiota variations in a group of adults with CF.

Methods: Faecal microbiota of sixteen free-living adults with CF was profiled by 16ss rDNA sequencing on the GS-FLX platform. Associations were tested between UniFrac distances of faecal microbiota and time-corresponding micronutrient intakes. Associations between relative abundances of bacterial taxa and micronutrient intakes (those showing significant associations with UniFrac distances) were examined by Spearman correlation.

Results: Unweighted UniFrac distances were associated with intakes of potassium and antioxidant vitamins C, E and beta-carotene equivalents, whereas weighted UniFrac distances were associated with antioxidant vitamins riboflavin, niacin equivalents, beta-carotene equivalents and vitamin A equivalents. Intakes of beta-carotene equivalents, vitamin C, vitamin E, niacin equivalents and riboflavin correlated negatively with *Bacteroides* and/or its corresponding higher level taxa. Intakes of beta-carotene equivalents and vitamin E also positively correlated with *Firmicutes* and specific taxa belonging to *Firmicutes*.

Conclusion: Some micronutrients, particularly antioxidant vitamins, correlated with gut microbiota variations in the studied cohort. Further research is required to clarify whether antioxidant vitamin intakes can influence CF gut microbiota and potential clinical/therapeutic implications in CF.

1. Introduction

Cystic fibrosis (CF), caused by absence of functional cystic fibrosis transmembrane regulator (CFTR) Cl^- channels vital for electrolyte and water exchanges at the epithelium due to mutations in the corresponding *cftr* gene, leads to an array of abnormalities such as aberrant mucus secretion and inflammation in multiple organ systems including the respiratory and digestive systems [1,2]. Digestive dysfunctions such as maldigestion and malabsorption in CF highlight the importance of nutrition and consequently diet [2]. It is plausible that the altered dynamics of the CF gut and the characteristics of therapeutic diets could impact on the gut microbiota [3]. Indeed, accumulating evidence suggests an altered gut microbiota in both children and adults with CF [4,5]. Potentially pathogenic *Proteobacteria* taxa seemed to be elevated while beneficial taxa of *Bifidobacterium*, *Firmicutes* and *Bacteroidetes* were suppressed [4,5]. Indirect evidence *in vitro* and from animal models and the non-CF population has emphasised the role of gut microbiota and its metabolites in chronic inflammation, immune

Non-standard abbreviations: 16ss rDNA, 16 small subunit ribosomal DNA; CD, Crohn's disease; CFRD, cystic fibrosis-related diabetes; CFTR, cystic fibrosis transmembrane regulator; CVD, cardiovascular disease; ENaC, epithelial Na^+ channel; FDR, false discovery rate; GI, gastrointestinal; NHE, Na^+/H^+ exchanger; SCFA, short chain fatty acid.

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regulation, metabolic and energy homeostasis [6–11]. These four aspects are key components of the pathophysiology and management of CF lung disease and comorbidities such as gut inflammation, CF-related diabetes (CFRD) and low bone density [1,12]. The association of nutritional status with lung function, low bone density and survival in CF [12,13] supports the potential role of gut microbiota in CF management [3] via regulation of metabolism and energy homeostasis [7]. This is further highlighted by the link between longitudinal changes in gut bacterial colonisation and airway events in CF infants [14].

Preliminary studies on probiotic interventions provide evidence that manipulation of the gut microbiota may help reduce respiratory exacerbations [15,16] and gut inflammation [4,17] in children and adults with CF. Variations of dietary constituents have been demonstrated to modulate gut microbiota in the general population [18,19]. It is thus plausible that gut microbiota variations could also be shaped by variations in dietary constituents in the CF population. While associations between gut microbiota and dietary constituents have been demonstrated in the general population [20], data in CF are lacking.

Given the frequent use of micronutrient supplements in CF [21], the purpose of the present study was to explore the relationship between micronutrient intakes and the gut microbiota overall and specific bacterial taxa and identify potential micronutrient candidates to manipulate the gut microbiota in CF to help develop nutritional therapies to complement current CF treatment by modifying gut microbiota composition and/or functions. It should be noted that the results presented are part of a broader study on associations between dietary intakes, clinical variables, and the gut microbiota in CF.

2. Methods

2.1. Participant recruitment and ethics

Eighteen free-living adults with stable CF and signed informed consent were recruited with help from local CF community support organisations from the Sydney and Brisbane areas. The University Human Research Ethics Committee (Ref No: PBH/39/11/HREC) approved the study. Recruitment criteria were modified from Wu et al. [20] without excluding those on medications common in CF treatment such as antibiotics [21]. The intention was to minimise participation burden and not to alter their treatment or intervene their dietary intakes including the consumption of probiotic products. For the same intention, serum nutrient status was not assessed, neither was colonoscopy taken to examine mucosal microbiota.

2.2. Data and faecal sample collection

All information and instruments or methods used for collection are summarised in Table S1. Data and faecal samples were collected from April to September, 2013. Instructions and equipment for faecal sample collection were posted to participants prior to study commencement. Explanations to record a 3-day food diary using household measures, and a template and an example for such a diary were also posted. The three days consecutively covered two weekdays and one weekend day. Participants provided faecal samples on either the second or third day of the 3-day diary period or the day after the 3-day period. Participants were instructed to store the fresh faecal samples in domestic freezers immediately after collection. The diary and the frozen faecal sample were transported in an insulated bag containing frozen ice packs to a face-to-face appointment where completeness and clarity of the food diary was checked with the participant.

One participant discontinued prior to commencement of the 3-day food diary due to other unspecified commitments.

2.3. Faecal sample DNA extraction, amplification and sequencing

Frozen faecal samples were processed at the Australian Genome Research Facility (AGRF Ltd, Brisbane, Australia). From each sample, DNA was extracted using the PowerLyzer® PowerSoil® DNA Isolation Kit in conjunction with the PowerLyzer® 24 homogenizer (both MO BIO Laboratories, Inc.; Carlsbad, CA, USA). Bacterial DNA was then sequenced using 454 pyrosequencing on the GS-FLX platform using XLR70 chemistry (Roche, Australia) as per manufacturer's manuals, targeting the 16 small subunit (ss) hypervariable regions V1V3 (Table S2).

One sample failed the quality control step prior to sequencing, leaving n = 16 for downstream analyses.

2.4. Bioinformatics and statistical analyses

The composition of the collected microbial samples, the community diversity and unweighted and weighted UniFrac distances [22] were analysed using the Quantitative Insights Into Microbial Ecology (QIIME) software package version 1.8 (http://qiime.org/) [23]. Reverse primers and chimera were removed. Open-reference OTUs (operational taxonomic unit) were picked against the Greengenes database (May 2013 version) following the default procedure with default settings.

Dietary intake data in 3-day food diaries were entered and summarised in FoodWorks 7 (Xyris Software, Australia) [24] based on the AUSNUT 2007 food composition database [25]. All micronutrients for subsequent analyses are displayed in Table S3.

Associations of the overall variations of gut microbiota among the participants based on weighted and unweighted UniFrac distances with micronutrient intakes were assessed using the statistical method adonis [26] separately for each micronutrient and corrected for multiple testings at a false discovery rate (FDR) = 0.3 [27]. For micronutrients identified as being associated with microbial community by adonis, their correlations with the relative abundances of specific taxa were tested using Spearman's correlation test [28] at an FDR = 0.3 (at ranks genus, family, order, class and phylum). Given the exploratory nature of this study, FDR = 0.3 was chosen to not omit any potential taxa that may be associated with any of the tested micronutrients. All relative abundances of taxa and micronutrient intake values were standardised among the samples with a mean = 0 and standard deviation = 1. Intakes of macronutrients and the relationship with gut microbiota variations in this group of free-living adults with CF will be reported separately. The test power of adonis depends on factors including sample size [29]. Because of the small sample size, associations between intakes of micronutrients and gut microbiota variations controlled for clinical characteristics and macronutrient intakes were not conducted. The effects of these parameters on the relationship between intakes of micronutrients and gut microbiota in CF requires exploration by studies with a larger sample size and perhaps targeted parameters of interest for cost-effectiveness.

3. Results

3.1. Participant characteristics, dietary intakes, and gut microbiota composition

Micronutrient intakes as reported in a 3-day food diary using household measures and participants' clinical characteristics are summarised in the Supplementary Tables S3–S4. Relationships between these clinical characteristics and gut microbiota variations

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