Fecal microbiota transplantation as tool to study the interrelation between microbiota composition and miRNA expression

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ABSTRACT

The intestinal gut microbiota is important for human metabolism and immunity and can be influenced by many host factors. A recently emerged host factor is secreted microRNA (miRNA). Previously, it has been shown that secreted miRNAs can influence the growth of certain bacteria and conversely, that shifts in the microbiota can alter the composition of secreted miRNAs. Here, we sought to further investigate the interaction between the gut microbiota and secreted miRNAs by the use of fecal microbiota transplantation (FMT). Subjects with the metabolic syndrome received either an autologous (n = 4) or allogenic (n = 14) FMT. Fecal samples were collected at baseline and 6 weeks after FMT, from which the microbiome and miRNA composition were determined *via* 16S rRNA sequencing and miRNA sequencing, respectively. We observed a significant correlation between the fecal miRNA expression and microbiota composition, both before and after FMT. Our results suggest that the FMT-induced shift in microbiota altered the fecal miRNA profile, indicated by correlations between differentially abundant microbes and miRNAs. This idea of a shift in miRNA composition driven by changes in the microbiota was further strengthened by the absence of a direct effect of specific miRNAs on the growth of specific bacterial strains.

1. Introduction

In past decades, the importance of the intestinal microbiota in human metabolism and immunity has become evident (Cani, 2017). There are several known factors through which the host can influence the intestinal microbiota, such as secretory IgA, antimicrobial peptides and mucins (Chang and Kao, 2019; Hasan and Yang, 2019). A more specific host factor that has only recently emerged are secreted microRNAs (miRNA) (Li et al., 2020b). MicroRNAs are non-coding RNAs, comprising 18–23 nucleotides, which regulate gene expression at the post-transcriptional level (Sarshar et al., 2020). Studies concerning miRNAs have mainly focused on their role within eukaryotic cells and replication of eukaryotic viruses (Hanna et al., 2019; Layton et al., 2020). However, miRNAs are present extracellularly and circulate in body fluids (Freedman et al., 2016), including the intestinal lumen (Ayyadurai et al., 2014). In line, miRNAs in human feces have been identified as potential biomarkers for intestinal diseases (Ahmed et al., 2009; Link et al., 2012; Rojas-Feria et al., 2018).

Recently, studies have found that miRNAs secreted by the hosts intestinal epithelial cells can alter the intestinal bacterial composition (Liu et al., 2016, 2019) and that conversely the gut microbiota can influence the expression of miRNAs, mainly through metabolites (Moloney et al., 2018; Peck et al., 2017; Virtue et al., 2019; Zhu et al., 2020). In addition, plant-derived exosome-like particles containing miRNAs have been shown to influence bacterial growth, localization and production of microbial metabolites (Sundaram et al., 2019; Teng et al., 2018). While

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the molecular mechanisms *via* which eukaryotic miRNAs affect prokaryotes remain to be further elucidated, these studies suggest that miRNAs can play a role in interspecies communication. Conversely, the microbiota can alter the expression and secretion of fecal miRNAs by IECs, thereby promoting proliferation and regulating permeability of IECs (Nakata et al., 2017; Peck et al., 2017). In line, it has been shown that bacteria can influence colorectal inflammation and cancer through regulation of miRNAs that enhance the intestinal barrier function (Rodríguez-Nogales et al., 2017).

To further study the relation between the intestinal microbiota and intestinal miRNA expression, we used fecal microbiota transplantation (FMT) as a tool. During an FMT, the fecal microbiota from a healthy, thoroughly screened donor is administered to a recipient *via* an upper or lower gastrointestinal route, thereby inducing a shift in the recipients microbiota to restore a balanced microbiota composition and function (Cammarota et al., 2017; Khoruts and Sadowsky, 2016). Currently, FMT is used as an (experimental) treatment for a plethora of diseases, providing an interesting opportunity to determine the contribution of the intestinal microbiota to disease pathology and thereby leading to important mechanistic insight (Hanssen et al., 2021; Wortelboer et al., 2019). In the past years, we have performed two randomized controlled clinical trials in which subjects with the metabolic syndrome (MetS) were treated with either an allogenic or autologous FMT (Kootte et al., 2017; Vrieze et al., 2012). Here, we studied whether an FMT altered the fecal miRNA expression in a subpopulation of the most recent clinical trial (Kootte et al., 2017). Next, we investigated whether observed changes in miRNA composition were correlated with the intestinal microbiota composition. Finally, we tested whether the associated miRNAs could influence the growth of the specific microbes in an *in vitro* model.

2. Materials and methods

2.1. Participants and fecal samples

Human fecal samples were collected from subjects who underwent an FMT (Kootte et al., 2017). In short, subjects were Caucasian males, who had obesity (body mass index (BMI) $\geq 30 \, kg/m^2$), fulfilled at least 3 out of 5 criteria for metabolic syndrome (NCEP criteria (Grundy et al., 2005): fasting plasma glucose ≥ 5.6 mmol/l, triglycerides ≥ 1.7 mmol/l, waist-circumference >102 cm, high-density lipoprotein (HDL) cholesterol <1.03 mmol/l, blood pressure $\geq 130/85$ mmHg) and were treatment naïve. Main exclusion criteria were a history of cardiovascular events, cholecystectomy and use of probiotics or medication.

Subjects were randomized to receive either allogenic (feces from lean healthy donor) or autologous (control = own feces) FMT *via* a nasoduodenal tube. Subjects and feces donors collected fresh feces on the morning of FMT and after 6 weeks subjects collected a follow-up fecal

sample. Samples were directly stored at −80 °C. Feces from 18 subjects (n = 14 allogenic, n = 4 autologous) and 5 lean feces donors was used for 16S rRNA gene amplicon sequencing and miRNA sequencing. Characteristics of these groups are depicted in Table 1. Study procedures were in compliance with the principles of the declaration of Helsinki and approved by the Academic Medical Center ethics committee. All subjects provided written informed consent.

2.2. 16S rRNA sequencing

DNA was extracted from fecal samples using a repeated bead beating protocol and subsequently purified using the Maxwell RSC Whole Blood DNA kit (Costea et al., 2017). Next, 16S rRNA gene amplicons spanning the V3-4 region were generated using a single step PCR protocol using universal primers B341 F and B806R. Amplicon libraries were purified using Ampure XP beads and pooled equimolarly (Kozich et al., 2013). An Illumina MiSeq platform using v3 chemistry with 2 × 251 cycles was used to sequence the library.

Forward and reverse reads, truncated to 240 and 210 bases respectively, were merged using USEARCH (Edgar, 2010). Merged reads were removed if they did not pass the Illumina chastity filter, had an expected error rate higher than 2, or were shorter than 380 bases. Amplicon sequence variants (ASVs) were inferred for each sample individually using UNOISE3 and ASV abundances were determined by mapping unfiltered reads against the joint ASV set (Edgar, 2010). Taxonomy was assigned to ASVs using the RDP classifier (Wang et al., 2007) and SILVA 16S ribosomal database V132 (Quast et al., 2013).

2.3. miRNA profiling

Total RNA was extracted from fecal material using the Qiagen RNeasy PowerMicrobiome kit. RNA concentration and integrity were assessed by Nanodrop and Bioanalyzer, respectively. Thereafter, small RNA fragments were separated from large RNA fragments. To monitor the size distribution and to normalize data between samples, synthetic RNA spike-ins were added as described previously (Locati et al., 2015). Small RNA libraries were prepared using the Ion Total RNA-seq kit v2 and barcoded with IonXpress RNA-Seq BC01-BC16 according to the manufacturer's protocols. Library templates where clonally amplified on Ion Sphere particles using the Ion PI Template OT2 200 Kit on an Ion OneTouch 2 Instrument, followed by enrichment of template-positive Ion Sphere Particles using the Ion OneTouch ES. Libraries were sequenced on the Ion Proton system using the Ion PI Sequencing 200 kit and Ion PI Chip v2.

Reads were first mapped to the synthetic spike-in sequences, after which remaining reads were categorized based on length (small <15 nucleotides (nt), medium 15−45 nt and large >45 nt). Next, reads were aligned to human miRNA sequences from miRbase version 21

Table 1
Characteristics of MetS subjects and lean donors.

	MetSyn						Healthy donor (N = 5)		P-value
	Allogenic (N = 14)		Autologous (N = 4)		P-value	Combined (N = 18)			
Age (years)	54	(6)	53	(8)	*0.865*	**53**	(7)	**29**	(6)	***0.000***
Male sex (n (%))	14	(100)	4	(100)	*1.000*	**18**	(100)	**5**	(100)	*1.000*
BMI (kg/m²)	35.0	(2.9)	33.9	(3.7)	*0.539*	**34.8**	(3.0)	**22.3**	(1.5)	***0.000***
Waist circ. (cm)	120.0	(7.8)	118.4	(7.7)	*0.722*	119.6	(7.5)			
Syst. BP (mmHg)	139	(14)	147	(17)	*0.357*	141	(15)			
Diasyst. BP (mmHg)	87	(9)	89	(14)	*0.699*	87	(10)			
Glucose (mmol/L)	6.4	(1.2)	6.1	(0.7)	*1.000**	**6.4**	(1.1)	**5.2**	(0.3)	***0.000****
Triglycerides (mmol/L)	1.73	(1.07)	1.70	(0.71)	*0.798**	**1.72**	(0.98)	**0.86**	(0.25)	***0.046****
HDL (mmol/L)	1.14	(0.19)	1.20	(0.18)	*0.605*	**1.16**	(0.18)	**1.63**	(0.36)	***0.040***

Unless otherwise specified, data are reported as mean (SD) and statistical testing is performed by independent *t*-test. *Data not normally distributed; p-value calculated by independent Mann-Whitney *U* test. MetS = metabolic syndrome; Waist circ. = waist circumference; syst. BP = systolic blood pressure; diasyst. BP = diasystolic blood pressure; HDL = high-density lipoprotein cholesterol.

(Griffiths-Jones, 2004; Griffiths-Jones et al., 2008, 2006; Kozomara and Griffiths-Jones, 2014, 2011) and mapped reads were counted. Finally, IsomiR analysis was performed to identify miRNA sequence variants with respect to the reference sequence. Only the miRNAs found within the medium size fragments were used for further analysis, since miRNAs are generally ~18–23 nucleotides in length.

2.4. Bioinformatics and statistical analysis

Microbiome and miRNA data were analyzed in R Studio 4.0.5. Distance matrices were calculated using the clr transformed count tables and Euclidean distance. Compositional differences were tested using subjected stratified permanova as implemented in vegan, while Procrustes was used to test compositional correlations (Oksanen et al., 2019). All permanova and Procrustes analysis were performed with 999

permutations. Compositional shifts were visualized using inter-individual variance corrected multilevel PCA (Rohart et al., 2017). Then, for the subjects who received an allogenic FMT, ASV and miRNA deltas were normalized and a univariate Spearman correlation matrix was built for the 250 most abundant ASVs and miRNAs. False discovery rate (FDR) was used to correct for multiple comparisons.

Finally, we investigated whether the identified miRNAs could impact the growth of the associated bacteria *in vitro*, which has been shown in previous studies (Liu et al., 2016, 2019; Teng et al., 2018). Therefore, we first identified whether there was any overlap between the genome of the identified ASV and the miRNA. In addition, we focused on correlations with a high significance and a negative slope, since growth inhibition by host secreted miRNAs would be biologically more likely. The methods and results of this *in vitro* validation model are described in the supplementary material.
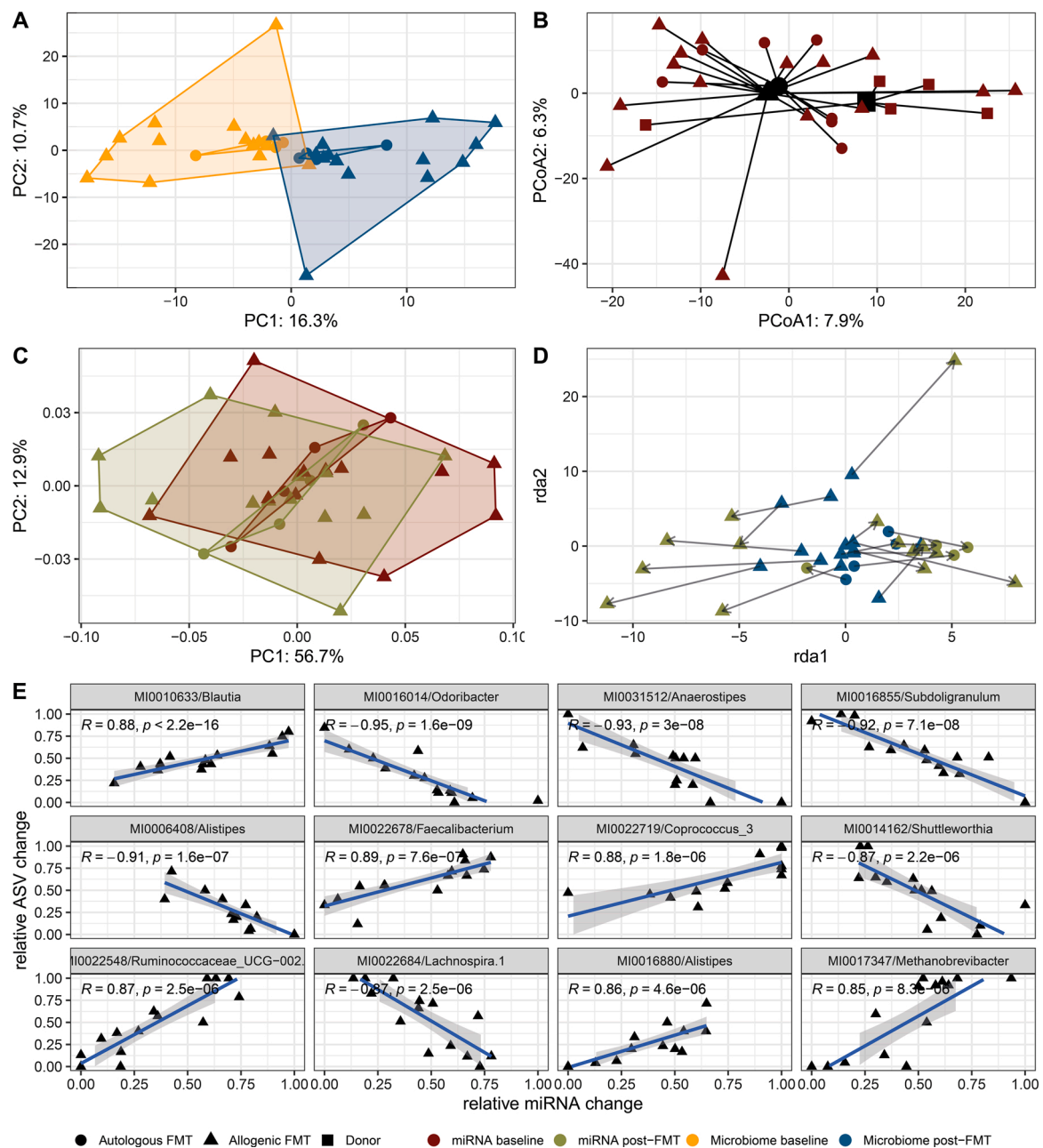
Fig. 1. (**A**) Multi-level PCA of the intestinal microbiota composition before and after FMT; (**B**) Ordination of the fecal miRNA composition of MetS subjects at baseline and healthy donors; (**C**) Multi-level PCA of the miRNA composition before and after FMT; (**D**) Procrustes rotation of the post FMT multilevel ordinations; (**E**) Correlation plots between the normalized ASV and miRNA abundance (top 12 based on the strongest Spearman correlation coefficients and highest significance).

3. Results

16S rRNA gene amplicon sequencing resulted in a total of 3411 ASVs, of which 554 were present in more than 12 subjects in at least one sample. FMT induced a significant shift in intestinal microbiota composition (P = 0.005), which was apparent for both the allogenic and autologous FMT groups (Fig. 1A).

Ion Proton sequencing resulted in at least a million reads per sample. Since most of the small RNA molecules were of bacterial origin, merely 7128 reads on average (range: 2049–14425 reads) could be assigned as miRNAs. Nevertheless, this resulted in a total of 3753 identified miRNAs, of which 2813 were annotated as mature and 940 as putative mature. Of these identified miRNAs, 1286 were present in more than 12 subjects in at least one of the timepoints. No obvious difference in miRNA composition at baseline was identified between MetS subjects and healthy donors (Fig. 1B). However, this could be a power issue due to the low number of healthy donors (n = 5). The miRNA profiles were subject specific (P = 0.001) and 53 % of the variance was inter-individual. Unlike the changes in gut microbiota, shifts in the miRNA composition were not significant (P = 0.085; Fig. 1C). Using a Procrustes correlation analysis, we did observe a significant correlation between ordinations of the microbiome and miRNA expression on both time points (P = 0.004, P = 0.001 respectively). This indicates that subjects that share similar microbiota also share similar miRNA profiles (Fig. 1D). Furthermore, Procrustes analysis of the multi-level PCA indicated that changes in microbiome did coincide with changes in miRNA expression (p = 0.007).

Thus, we next investigated whether there were any direct correlations between changes in microbe and miRNA abundance. Fig. 1E shows the 12 correlations with the strongest Spearman correlation coefficients between differentially abundant fecal ASVs and miRNAs. Within this selection, *Blautia* and *Faecalibacterium* showed a strong positive correlation with hsa-miR-2114-5p and hsa-miR-6833-5p, respectively, which remained significant after correction for multiple testing. Conversely, *Odoribacter*, *Anaerostipes*, *Subdoligranulum* and *Alistipes* showed a strong negative correlation with hsa-miR-3622b-5p, putative hsa-miR-3648-2-3p, putative hsa-miR-4493-5p and hsa-miR-1272-5p, respectively. Table S1 describes the 12 univariate correlations and summarizes the known literature on the biological role of the identified miRNAs.

Interestingly, we found hsa-miR-3622b-5p aligned with the DNA/mRNA for the DNA polymerase III subunit alpha of *Odoribacter splanchnicus.* To test whether this miRNA could impact the growth of *O. splanchnicus,* we co-cultured them in an *in vitro* experiment (see supplementary material). However, we did not observe a direct effect on the growth of the bacterium, as depicted in figure S1. Similarly, we did not observe an effect of putative hsa-miR-4493-5p on the growth of *Subdoligranulum variabile.* The lack of discernable effect of the miRNAs on growth of these specific bacteria make it more likely that the changes in the microbiome induce the differences in miRNA expression.

4. Discussion

Accumulating evidence suggests that the gut microbiota can influence the expression of (circulating) miRNAs, suggesting a new route of communication between microbiota and host (Peck et al., 2017). In addition, studies have reported changes in the gut microbiota influenced by secreted host miRNAs (Liu et al., 2016, 2019) and plant-derived miRNAs (Sundaram et al., 2019; Teng et al., 2018). FMT is an interesting approach to assess the interrelation between the gut microbiota and miRNA composition. Previously, our group observed an improvement in insulin resistance in MetS subjects who received FMT from a lean healthy donor (Kootte et al., 2017; Vrieze et al., 2012). This improvement associated with changes in both duodenal and fecal microbiota composition. In the present study, intestinal miRNA profiles correlated with microbiota profiles. Although FMT did not induce a significant global shift in the miRNA profiles, compositional changes

within the microbiome could be correlated to changes in miRNA profiles. Furthermore, changes in specific microbe abundance could be correlated to changes in miRNA abundance.

Fecal miRNAs have been characterized in human feces previously and have been identified as biomarkers for several diseases (Ahmed et al., 2009; Link et al., 2012; Rojas-Feria et al., 2018). In addition, shifts in fecal miRNAs as a result of microbiota perturbation have been observed in mouse models (Moloney et al., 2018). Furthermore, it is known that the intestinal microbiota composition differs between healthy and obese subjects, which could drive a different miRNA expression (Virtue et al., 2019). However, in present study we did not observe a significant difference in miRNA composition between healthy and MetS subjects. This is most probably explained by a low statistical power due to the low number of healthy donors.

The introduction and engraftment of new donor-derived microbiota could drive the shift in miRNA excretion by the host, although the specific mechanisms remain poorly understood (Li et al., 2020b). One example is the metabolite butyrate, which has been shown to alter the miRNA expression in colorectal cancer cells and thereby reduce the cell proliferation (Hu et al., 2015, 2011; Humphreys et al., 2013). In addition, bacterial endotoxins such as lipopolysaccharide (LPS) and flagella have been shown to influence miRNA expression, thereby maintaining intestinal homeostasis and influencing inflammation (Anzola et al., 2018; Xue et al., 2011). Another example is found in colibactin-producing *E.coli*, which can induce the expression of miR-20a-5p, leading to an increased secretion of growth factors and ultimately promoting colon tumor growth (Dalmasso et al., 2014). Within our study population, it would be interesting to assess whether microbial metabolism could affect the miRNA expression. However, we felt that using an inferred proxy for microbial metabolism in combination with the limited statistical power of the study would not result in any reliable associations. Therefore, the detailed mechanisms *via* which the intestinal microbiota influence the miRNA expression warrant further investigation, preferably by directly measuring metabolites of interest.

In two small studies, associations between an altered microbiota of subjects with obesity or type 2 diabetes and circulating miRNAs in plasma were found (Assmann et al., 2020; Li et al., 2020a). However, since these are cross-sectional studies, this does not prove any causality. Using our univariate regression model, we identified several correlations between differentially abundant microbes and miRNAs at baseline and 6 weeks after FMT. First of all, we found a strong positive correlation between *Blautia* and hsa-miR-2114-5p. This miRNA was first identified in epithelial ovarian cancer and thereafter shown to be downregulated in pancreatic cancer, while being upregulated in gastric cancer (Liu et al., 2014; Müller et al., 2015; Wyman et al., 2009). Next, we identified a negative correlation between *Anaerostipes* and putative hsa-miR-3648-2-3p. Previous research reported that expression of this miRNA in macrophages can be induced by LPS and inhibits the NFκB pathway (Baillie et al., 2017). Moreover, hsa-miR-3648 has been found to downregulate tumor suppressor Adenomatous polyposis coli 2 (APC2), leading to an increased cell proliferation (Rashid et al., 2017; Xing, 2019). Finally, we found a strong inverse correlation between *Subdoligranulum* and putative hsa-miR-4493-5p. Identified in 2010 from malignant human B cells (Jima et al., 2010), hsa-miR-4493 has been shown to have a protective function against proliferating glioma cells (Zhang et al., 2019).

To date, no direct relation between the microbiome and the above described miRNAs has been reported in literature. The intestinal epithelial cells (IECs) and some hopx-expressing cells are the main sources of fecal miRNAs (Liu and Weiner, 2016). Previously, it has been observed that miRNA profiles differ between IEC subtypes and that commensal microbes can influence the miRNA expression in IECs, promoting proliferation and regulating permeability of IECs (Nakata et al., 2017; Peck et al., 2017). In addition, it has been shown that bacteria can influence colorectal inflammation and cancer through regulation of

miRNAs that enhance the intestinal barrier function (Rodríguez-Nogales et al., 2017). In line, the three miRNAs identified here are associated with cell proliferation and could play a role in intestinal barrier function. However, the current study only permits us to speculate about the potential role of the fecal miRNAs and the precise function has to be further investigated.

More recently, studies have found that miRNAs secreted by IECs can directly impact bacterial growth and subsequently alter the microbiota composition (Liu et al., 2019, 2016). Further strengthened by evidence that exogenous diet-derived miRNAs can influence intestinal bacterial growth and metabolites (Sundaram et al., 2019; Teng et al., 2018), these studies suggest that miRNAs mediate in interspecies communication. Within our top 12 correlations we identified one ASV-miRNA pair in which there was overlap between the genome and miRNA sequence, namely *Odoribacter splanchnicus* and hsa-miR-3622b-5p. This miRNA was first identified from a collection of cervical tumors in 2010 (Witten et al., 2010). Thereafter, hsa-miR-3622b has been found to have anti-tumor properties in several types of cancer (Bhagirath et al., 2019; Lu et al., 2017; Vernon et al., 2020) and has been implicated as a biomarker for Alzheimer's disease (Denk et al., 2015). Binding of this miRNA to the bacterial polymerase transcripts of *Odoribacter splanchnicus* could potentially directly impact DNA synthesis and thereby growth.

Previously, Liu et al. have shown a positive effect of hsa-miR-515-5p and hsa-miR-1226-5p on the growth of *Fusobacterium nucleatum* and *Escherichia coli* respectively (Liu et al., 2016). Using a similar approach, we cultured *O. splanchnicus* and *S. variabile* in the presence of their associated miRNA. These miRNAs and bacteria were selected from the univariate analysis because of the negative correlation, meaning the miRNA would impair the growth of the bacterium. We chose this approach since a growth-stimulatory effect of a miRNA on a bacterium was, in our opinion, biologically unlikely. In a highly competitive ecological environment as the intestine, being dependent on specific miRNAs from the host for an optimal growth is detrimental for survival and would be selected against.

Unfortunately, we did not observe any effect of the miRNAs on the growth. The absence of effect could be explained by the fact that there was no overlap between the miRNA and the genome of the tested strain (in the case of *S. variabile*). Since bacterial genes lack introns, alignment of a bacterial gene with a miRNA sequence is predictive for binding capacity of the miRNA to bacterial mRNA (Horne et al., 2019). Absence of a target bacterial mRNA which can be inhibited or degraded by the miRNA could explain the absence of growth inhibition. In addition, the miRNAs were not encapsulated in extracellular vesicles, nor bound to high-density lipoproteins or Argonaute proteins, which probably impaired the uptake by the bacteria and reduced the stability of the oligonucleotides (Creemers et al., 2012). However, there have been reports of uptake of free miRNA in extracellular vesicles *in vivo*, possibly mediated by electrostatic and hydrophobic interactions between the nucleotides and fatty acids (Chen and Walde, 2010; Liu et al., 2019). More likely, the interaction could be the other way around, whereby *Subdoligranulum* and *O. splanchnicus* are responsible for the decreased expression of the associated miRNA in the gut. Whether these microbes decrease these fecal miRNAs should be further elucidated in future studies.

Limitations of our study include the small sample size and the fact that solely Caucasian males were included, precluding generalization to females and people of other ethnicities. In current study, univariate correlations were based on the group that received allogenic FMT and we made no comparison between interventions or looked into a specific donor effect. In addition to the small sample size, the unbalanced groups and the fact that FMT effects depend both on the donor and the recipient made it impossible to perform any stratified analyses (*e.g.*, donor stratified correlations). In future studies, larger, more balanced groups should be compared, in which FMT is compared with a real placebo, since an autologous FMT will influence the microbiome as well. In addition, mapping of miRNA sequences to the miRbase database

resulted in reads with relatively high E-values, meaning many hits were assigned with low confidence. Unfortunately, the current setup of our study made it impossible to study the effect of donor miRNAs present in the administered FMT. Future studies should further investigate the role of miRNAs transplanted during the FMT and whether these contribute to the effect of the FMT.

Nevertheless, the use of a prospective cohort in which MetS subjects received an allogenic or autologous FMT does show that changes in the microbiome coincide with changes in miRNA expression, and thus provides further evidence for an involvement of the gut microbiota in regulating intestinal miRNA expression.

5. Conclusions

We found a correlation between the fecal miRNA profile and microbiome composition in human MetS subjects. Although FMT did not induce a significant global shift in the miRNA profiles, compositional changes in microbiome could be correlated to changes in miRNA profiles. Furthermore, changes in specific microbe abundance could be correlated to changes in miRNA abundance. Finally, we could not to show a direct effect of miRNAs on the growth of specific bacterial strains.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local Institutional Review Board of the Academic Medical Center (AMC) in Amsterdam, the Netherlands. The study was registered at the Dutch Trial Register (number 2705).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

Processed data and analysis are available at: https://github.com/AMCMC/FL_miRNA.

Author statement

Data curation: Koen Wortelboer, Guido Bakker and Maaike Winkelmeijer; Formal analysis: Koen Wortelboer, Guido Bakker, Evgeni Levin and Mark Davids; Supervision: Max Nieuwdorp and Hilde Herrema; Writing – original draft: Koen Wortelboer; Writing – review & editing: Guido Bakker, Maaike Winkelmeijer, Natal van Riel, Evgeni Levin, Max Nieuwdorp, Hilde Herrema and Mark Davids.

Declaration of Competing Interest

MN is co-founder and member of the Scientific Advisory Board of Caelus Health, the Netherlands. MN is on the Scientific Advisory Board of Kaleido Biosciences, USA. EL is founder and CEO of Horaizon BV.

None of these are directly relevant to the current paper.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.micres.2022.126972.

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