**Supplementary Online Content**

eFigure 1. Hypothesis of the potential mechanism of microbial transfer from mother to infant.

eMethods. Detailed methodology and references

eTable 1. Comparisons of the demographic characteristics, maternal stress and infant weight gain between the selected and non-selected mother-infant pairs.

eFigure 2. Microbiome Analysis of Maternal Gut, Breast Milk, and Infant Gut samples

eFigure 3. Differences in microbiome community structures among maternal gut, breast milk, and infant gut

eTable 2. Numbers of observed species (α-diversity) in breast milk, maternal stool, and infant stool microbiota and comparison between intervention and control group

eFigure 4. Group differences in α-diversity based on Shannon index, ACE, and Chao1

eFigure 5. Relative abundance of the top 15 bacteria in maternal feces, breast milk, and infant feces at baseline

eFigure 6. Relative abundance of *Bifidobacterium* in maternal gut, breast milk, and infant gut by individuals

**eFigure 1. Hypothesis of the role played by the microbiome in the relationship between maternal stress and infant growth and behaviour.**

Notes: EMT= entero-mammary trafficking; HMO=human milk oligosaccharides.

This figure describes how the microbiome could be involved in mother-infant interaction (signalling). During lactation, maternal gut microbiome could be affected by maternal stress via gut-brain axis, and further influence the breast milk microbiome by entero-mammary trafficking, leading to the shifts in infant gut microbiome through breastfeeding.

A diagram of a plant

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**eMethods. Detailed methodology**

DNA Extraction, sequencing, and data processing

Frozen breast milk and stool sample from mother, and stool sample from child were determined by 16S ribosomal (rRNA) sequencing on an Illumina NovaSeq platform 6000 and 250 bp paired-end reads were generated. Total genome DNA from samples was extracted using CTAB method.

The sequencing targeted the V4 variable region, and amplified using univuniversal primers (515F-GTGCCAGCMGCCGCGGTAA, 806R-GGACTACHVGGGTWTCTAAT). PCR products was purified with Qiagen Gel Extraction Kit (Cat. # 28004, Qiagen, Germany). All samples reached band A “The total amount of PCR product meets the needs of one or more library constructions, which can be used for subsequent library constructions.”

Library preparation was performed using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index code were added. Library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system.

Bioinformatic workflow of 16S rRNA gene amplicon sequencing

We firstly removed primer from the raw sequence data (median 150,333 reads per specimen) and merged paired-end sequencing via FLASH 1 version 1.2.7 to obtain raw tags. These tags were then compared with the Silva database 7 via UCHIME (version 11) to remove chimeras. 2 Quantitative Insighs Into Microbial Ecology (QIIME2) version 2022.2.0 was used to forward process analysis. 3 We use q2-dada2 4 to process sequences into exact sequence features table. Features present less than a single sample, and features with a total frequency of less than 10 across all samples will be filtered from the feature table. To assign taxonomy to the sequences, q2-feature-classifier 5,6 was used with pre-trained Naive Bayes classifier via the SILVA rRNA database (version 138.1). 7 To filter out outliers from the sequencing results, features with a total abundance of less than 10 were removed, and features that appeared in only two samples were removed. Of the 228 samples sequenced, 5,729 of 22,610 features remained after these standard quality filtering methods for the following microbiota analysis. Moreover, we provide --p-sampling-depth 80000 to subsample the counts in each sample without replacement, so that each sample in the resulting table has a total count of 80000.

**Reference**

1. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics.* 2011;27(21):2957-2963.

2. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics.* 2011;27(16):2194-2200.

3. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature biotechnology.* 2019;37(8):852-857.

4. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods.* 2016;13(7):581-583.

5. Bokulich NA, Kaehler BD, Rideout JR, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin. *Microbiome.* 2018;6(1):1-17.

6. Robeson MS, O’Rourke DR, Kaehler BD, et al. RESCRIPt: Reproducible sequence taxonomy reference database management. *PLoS computational biology.* 2021;17(11):e1009581.

7. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research.* 2012;41(D1):D590-D596.

**eTable 1. Comparisons of the demographic characteristics, maternal stress, and infant weight gain between the selected and non-selected mother-infant pairs.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Involved in analysis | Mean  (SD) | | t-test for Equality of Means | | | | | | |
|  | t | Sig. (2-tailed) | | Mean Difference | 95% CI | |
| Lower | Upper |
| Gestational week | No (n=58) | 36.00  (1.01) | | Equal variances assumed | -1.372 | .173 | | -.263 | -.64 | .12 |
| Yes (n=38) | 36.26  (.76) | | Equal variances not assumed | -1.454 | .149 | | -.263 | -.62 | .10 |
| Birth weight | No (n=58) | 2.70  (.29) | | Equal variances assumed | .045 | .964 | | .003 | -.12 | .12 |
| Yes (n=38) | 2.70  (.30) | | Equal variances not assumed | .045 | .964 | | .003 | -.12 | .13 |
| Maternal age | No (n=58) | 29.36  (3.61) | | Equal variances assumed | -1.621 | .108 | | -1.138 | -2.53 | .26 |
| Yes (n=38) | 30.50  (2.95) | | Equal variances not assumed | -1.691 | .094 | | -1.138 | -2.48 | .20 |
| Full time education | No (n=58) | 15.35  (2.19) | | Equal variances assumed | -.309 | .758 | | -.155 | -1.15 | .84 |
| Yes (n=38) | 15.50  (2.70) | | Equal variances not assumed | -.296 | .768 | | -.155 | -1.20 | .89 |
| PSS at week 1 | No (n=58) | 20.02  (7.16) | | Equal variances assumed | -.191 | .849 | | -.299 | -3.40 | 2.80 |
| Yes (n=38) | 20.32  (7.95) | | Equal variances not assumed | -.187 | .852 | | -.299 | -3.48 | 2.88 |
| PSS at week 8 | No (n=58) | 18.93  (6.48) | | Equal variances assumed | .734 | .465 | | .984 | -1.68 | 3.64 |
| Yes (n=38) | 17.95  (6.32) | | Equal variances not assumed | .738 | .462 | | .984 | -1.67 | 3.63 |
| Change in PSS | No (n=58) | -1.09  (5.18) | | Equal variances assumed | 1.285 | .202 | | 1.282 | -.70 | 3.26 |
| Yes (n=38) | -2.37  (4.09) | | Equal variances not assumed | 1.350 | .180 | | 1.282 | -.61 | 3.17 |
| Infant weight gain | No (n=58) | 2.57  (.58) | | Equal variances assumed | .379 | .706 | | .044 | -.19 | .27 |
| Yes (n=38) | 2.52  (.51) | | Equal variances not assumed | .390 | .697 | | .044 | -.18 | .27 |
| Chi-Square Test | | | | | | | | | | |
| Infant sex | Involved male | | 16 (42%) | | | | Fisher’s Exact Test (2-sided) | | | |
| Non-involved male | | 36 (62%) | | | | .063 | | | |

Notes: PSS=perceived stress scale; CI=confidence interval; SD=standard deviation.

**eFigure 2.** **Microbiome** **Analysis of Maternal Gut, Breast Milk, and Infant Gut samples**

Notes:The distribution of read counts in maternal gut after standard sequencing quality control between control and intervention group at 1 week and 8week as shown in A; the distribution in breast milk as shown in B; the distribution in infant gut as shown in C; rarefaction curve as shown in D shown subsampled feature counts for each sample among maternal gut, breast milk, and infant gut, using a sampling depth of number 80000. CG=control group, IG=intervention group, BM=breast milk, IS=infant gut, MS=maternal gut.



**eFigure 3. Differences in microbiome community structures among maternal gut, breast milk, and infant gut.**

Notes: The microbiome community difference among maternal gut, breast milk and infant gut were measured using Bray-Curtis distance matrix, and presented using principal coordinates analysis plot (PCoA). Statistical difference was assessed by using analysis of similarities (ANOSIM).

A diagram of breastfeeding

Description automatically generated

eTable 2. α-diversity in breast milk, maternal stool, and infant stool microbiota and comparison between intervention and control group.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Observed Features | Shannon | ACE | Chao 1 |
| 1-week | Maternal gut | Median  (IQR) | CG | 374(124) | 4.24(1.1) | 379(125) | 381(123) |
| IG | 366(140) | 3.93(0.5) | 370(139) | 372(139) |
| Sig. (2-sided) | | 0.74 | 0.45 | 0.69 | 0.71 |
| Breast milk | Median  (IQR) | CG | 286(152) | 2.62(1.0) | 295(158) | 298(159) |
| IG | 237(89) | 2.77(1.0) | 247(247) | 250(84) |
| Sig. (2-sided) | | 0.56 | 0.75 | 0.56 | 0.51 |
| Infant gut | Median  (IQR) | CG | 68(101) | 1.90(0.8) | 69(105) | 69.5(105) |
| IG | 104(67) | 2.28(0.8) | 105(67) | 104(68) |
| Sig. (2-sided) | | 0.21 | 0.12 | 0.2 | 0.2 |
| 8-weeks | Maternal gut | Median  (IQR) | CG | 399(97) | 4.14(0.7) | 405(98) | 406(98) |
| IG | 368(86) | 4.08(0.6) | 371(86) | 370(86) |
| Sig. (2-sided) | | 0.67 | 0.3 | 0.71 | 0.71 |
| Breast milk | Median  (IQR) | CG | 296(110) | 3.03(0.4) | 304(111) | 302(113) |
| IG | 221(56) | 2.87(0.5) | 229(57) | 228(59) |
| Sig. (2-sided) | | **0.032** | 0.16 | **0.037** | **0.027** |
| Infant gut | Median  (IQR) | CG | 62(24) | 1.94(0.7) | 62(24) | 62(24) |
| IG | 102(69) | 2.27(0.3) | 106(73) | 106(75) |
| Sig. (2-sided) | | **<0.001** | **0.015** | **<0.001** | **<0.001** |

Notes: IG=intervention group; CG=control group. Maternal and infant gut microbiome were examined using their feces sample. Significance tested using Wilcoxon Rank-Sum Test.

**eFigure 4. Group differences in α-diversity based on Shannon index, ACE, and Chao1.**

Notes: Alpha diversity was measure using the Shannon index, ACE and chao1 in the maternal gut at 1 and 8 weeks, as shown in A; alpha diversity in breast milk is illustrated in B; alpha diversity in infant gut is depicted in C. Group differences in alpha diversity were assessed using Wilcoxon rank-sum test.

A group of colorful squares

Description automatically generated

**eFigure 5. Relative Abundance of The Top 15 Bacteria in Maternal Feces, Breast Milk, and Infant Feces at Baseline.**

Notes: The relative abundance of the top 15 most abundant genera in maternal feces, breast milk, and infant feces at baseline was examined. Statical difference between the intervention and control groups were compared using the Wilcoxon rank-sum test, and adjusted using the FDR method. CG=control group; IG=intervention group.A group of blue and orange lines

Description automatically generated

**eFigure 6. Relative** **Abundance of Bifidobacterium in Maternal Gut, Breast Milk, and Infant Gut by Individuals.**

Notes: The figure displays the relative abundance of *Bifidobacterium* for each individual (with unique study ID) in the maternal gut, breast milk and infant gut at 1 and 8 weeks, comparing the control and intervention groups. MS=maternal gut, BM=breast milk, IS=infant gut.

