Transmission of gut bacteria and viruses from mothers to infants

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Transmission of gut bacteria and viruses from mothers to infants

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Abstract

Seeding and development of the gut ecosystem are crucial for health, both in childhood and later in life. While the composition of infant gut bacterial communities has been described, the composition and origin of the infant gut virome remains under-studied. Here, we explore mother-to-infant transmission of bacteria and viruses in 30 mother–infant pairs in a longitudinal collection of faecal samples taken during pregnancy and the first 3 months after birth. We demonstrate that infant bacterial strains resemble maternal strains more than those of unrelated mothers. We quantify viromes using a complementary approach examining both total metagenomes and viral metagenomes. The virome composition is highly consistent between viral and total metagenomes. The infant gut viromes are dominated by active temperate bacteriophages, which are more abundant in infants than mothers (p -value=7.2e-06). We observe that the proportion of shared viruses between maternal and infant gut is only 11.3% when considering the active virome fraction alone, but increases to 37.6% when taking into account temperate phages in the form of prophages. These findings indicate that viruses are vertically transmitted from mothers to infants early in life and that pioneering phages can reach the infant gut via vertical transmission of their bacterial hosts.

Introduction

The human early life gut ecosystem has garnered much interest in recent years because of its links to health and disease later in life¹, but the core aspects of its origin and development remain less understood. Studies have characterised the development of the infant gut microbiome through the first 2–3 years of life, after which the gut microbiome reaches a state of high microbial richness equivalent to that of an adult^{2,3}. Thus far, the focus of research has been on the development of gut bacteria. However, the gut ecosystem also comprises viruses,

archaea and eukaryotes such as fungi and protists, whose role in early gut ecosystem development has received less attention.

Both vertical transmission (during pregnancy and birth) and horizontal transmission (from environmental sources like infant diet) have been described as sources of infant gut microbiota⁴. Recent studies provide increasing support for the maternal gut bacterial reservoir as a key source of microbial transmission from mothers to infants^{5,6}. Although the transmission of infectious viruses such as cytomegalovirus and herpes simplex virus in the context of maternal and infant morbidity has been established⁷, little is known about the transmission of bacteriophages (bacterial viruses) from maternal to infant gut. This is partially due to difficulties in isolating and annotating metaviromes⁸. As environmental studies have clearly demonstrated that bacteriophages are key players in the modulation of bacterial communities^{9,10}, it is crucial to study them in the context of the developing human gut ecosystem as the bacterial community is established in the months following birth.

A recent study in 20 healthy infants using viral-like particle (VLP) data provided substantial functional evidence that the bacteriophages colonising the infant gut arise from excisions from pioneering infant gut bacteria¹¹. However, the origin of these pioneering bacteriophages, as well as their hosts and their possible roots in the maternal gut ecosystem, remain underexplored. We therefore sequenced 183 total metagenomes (TMs) obtained by isolating all microbial DNA from stool and 66 viral metagenomes (VMs) using a VLP enrichment isolation protocol from 30 mother–infant pairs for whom we have longitudinal samples collected during pregnancy and the first 3 months of life. We first described the bacterial and viral composition in both mothers and infants and elucidated the degree of inter-individual and intra-individual variability. We then compared bacterial strains and viruses across and within mother–infant pairs and found that infant bacterial strains resemble

their mother's strains more than those of unrelated mothers. We found that the proportion of shared active viruses within mother–infant pairs was lower than the proportion of shared bacteria. However, we also found significantly more viruses that were shared between mother–infant pairs when taking into account bacteriophages incorporated in the bacterial genomes of maternal TMs, which indicates that maternal prophages are a source of pioneering infant gut bacteriophages.

Results

Study sample and gut bacterial composition in mothers and infants

We first characterised the gut bacteriome of mothers and infants and described the degree of sharedness of bacterial species in 183 TMs from 30 mothers and 32 infants, including 2 twin pairs (Figure 1a). Mothers collected their faeces at months 3 and 7 during pregnancy, at birth and at 3 months after birth (Figure 1a). Faecal samples from infants were collected at months 1, 2 and 3 after birth. Although meconium samples collected at birth were available, only 3 of the 32 meconium samples had a sufficient number of microbial reads, reflecting a low bacterial abundance in these samples, and these 3 meconium samples were therefore excluded from the analysis. In this study, mothers had a median age of 32.0 ± 4.4 years, a median pre-pregnancy BMI of 23.0 ± 2.6 kg/m² (approximately 5 years prior to the pregnancy) and a median gestational age of 39.6 ± 1 week. The infants were 56% female and had a median birth weight of $3,705 \pm 380.4$ grams. 87.5% of the infants were vaginally delivered, with one twin pair having different delivery modes, and 28% were born at home. In terms of feeding, 53.6% were exclusively breastfed at month 1, 56.3% were exclusively breastfed at month 2 and 53.3% were exclusively breastfed at month 3 (Supplementary Table S1).

The infant gut bacteriome showed a remarkably lower species-level alpha-diversity compared to the maternal gut bacteriome (mean mother=2.62, mean infant=1.26, p-value=1.12e-34, (Supplementary Fig. 1a)), consistent with previous findings⁵. Over the entire study period, maternal alpha-diversity did not show any significant association patterns to maternal phenotypes or timepoint (Supplementary Fig. 1c), although this analysis may have been hampered by low sample size. The alpha-diversity of bacterial species in infants also did not change significantly during the first 3 months (Supplementary Fig. 1b). At the phylum level, mothers and infants showed remarkably different compositions (Figure 1b, c), with infants having more Actinobacteria and Proteobacteria and mothers having more Bacteroidetes and Firmicutes (FDR<0.05) (Supplementary Table S2).

The species-level bacteriome composition was significantly different in mothers and infants (permutational multivariate analysis of variance (PERMANOVA) p-value=0.001, 999 permutations, Figure 1d). The maternal bacteriome showed greater inter-individual variation than intra-individual variation across timepoints (Supplementary Fig. 2b). Infants also demonstrated more inter-individual variation than intra-individual variation (Supplementary Fig. 2a). We next explored the relation between host phenotypes and the abundance of microbial species and for example found that infants who were exclusively breastfed had a significantly higher abundance of *Cutibacterium acnes*, a bacterium typically known to be a member of skin flora (FDR=0.001) (Supplementary Table S3).

Figure 1: Study description and differences and sharedness in the bacterial composition between mothers and infants.

a, Distribution of successfully sequenced samples across mothers and infants at month 3 (P3) and month 7 of pregnancy (P7), birth (B) and the first, second and third month after birth (M1, M2 and M3, respectively). **b-c**, Phylum-level relative abundance profiles for infant (**b**) and maternal (**c**) samples. **d**, PCoA analysis showing the differences in overall bacterial composition between mothers and infants calculated using Bray-Curtis dissimilarity matrix. Ellipses show 95% confidence intervals. **e**, Number of the bacterial species in infants shared with own mother. Number of dots represents the number of available timepoints for each infant.

Maternal to infant vertical transmission of bacteria

Although microbiome composition differed between mothers and infants, $43.6 \pm 10.0\%$ of bacterial species were shared across related mother – infant pairs (considering all timepoints in mothers and infants, Figure 1e, Supplementary Table S4). We next aimed to explore whether bacterial species shared between related mother–infant pairs are represented by the same strains. For 12 of the 17 species for which strain-level data could be constructed for both mothers and infants, we found at least one incidence of a mother–infant pair sharing an identical dominant strain. These species were *Akkermansia muciniphila*, *Alistipes finegoldii*, *Bacteroides dorei*, *Bacteroides vulgatus*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bilophila wadsworthia*, *Collinsella aerofaciens*, *Parabacteroides distasonis* and *Bifidobacterium uniformis*. In 10 of these species (*B. dorei*, *B. longum*, *A. finegoldii*, *B. uniformis*, *B. vulgatus*, *B. adolescentis*, *B. bifidum*, *B. wadsworthia*, *C. aerofaciens* and *P. distasonis*), the phylogenetic distances between related mothers and infants were significantly lower than the distances between unrelated individuals (999 permutations, $FDR < 0.05$, Figure 2c), suggesting either horizontal or vertical strain transmission of these species from mothers to infants.

We hypothesised that birth mode and delivery location might affect dominant strain-sharing between mothers and infants. The phylogenetic distances of strains of *B. bifidum*

104 showed a different pattern of transmission from mothers to infants depending on the place
 105 of delivery (PERMANOVA, 999 permutations, $p=0.001$). For home deliveries (28% of
 106 deliveries), the *B. bifidum* phylogenetic distances between infants and their mothers were
 107 small, whereas the *B. bifidum* phylogenetic distances between infants and mothers tended to
 108 be larger for hospital deliveries (Figure 2a). Interestingly, in family 20, twin pairs shared
 109 identical dominant strains of *B. uniformis* and *B. breve* despite having opposing modes of
 110 delivery. Furthermore, we observed an interesting pattern in one family where the strain of
 111 *A. muciniphila* showed a strain replacement during pregnancy, with infant strains being
 112 similar to those of mother after birth in contrast to maternal strains just before birth (Figure
 113 2b).

Figure 2: Patterns of within- species phylogenetic distances between mothers and infants.

a, pairwise distances between *B. bifidum* strains between mother–infant pairs delivered at the hospital (HS) and mother–infant pairs delivered at home (HM). **b**, PCoA based on phylogenetic distances in *A. muciniphila* strains in mother-infant pairs. Labels represent timepoints. Only strains present in at least one mother-infant pair are depicted **c**, Phylogenetic distances within all combinations of samples for related mothers and infants and distances between unrelated individuals.

Gut viral composition in mothers and infants

We performed VLP DNA extraction for 93 faecal samples from mothers (pregnancy and birth timepoints) and infants (birth, month 1, month 2 and month 3) that were selected for total metagenome analysis based on sufficiency of faecal material (Figure 1a). In all, 66 VMs were successfully sequenced (Figure 1a) and 51,449 viral-representative contigs were reconstructed from all successfully sequenced VMs, constituting a curated viral database (Figure 3a). Of these, 616 contigs, ranging in size from 3 to 253kbp, had identical ends, which suggests that they represent complete genomes of viruses with circular or terminally redundant linear genomes.

The total number of contigs with an assigned taxonomy at family level was 14,405 (28% of all detected viral contigs). Viral-representative contigs were assigned to 11 families of eukaryotic and prokaryotic viruses. Consistent with previous observations 99.7% of the taxonomically assigned contigs represented bacteriophages^{12,13}. Among the seven bacteriophage families, *Siphoviridae* represented the largest number of contigs. Based on similarity between viral contigs and CRISPR spacer sequences of microbial genomes, we predicted potential hosts for 630 viral contigs (1.2%; Supplementary Table S5; see Methods). Most infant bacteriophages were linked to bacteria of the phyla Actinobacteria, Firmicutes and Proteobacteria whereas maternal bacteriophages were linked to Firmicutes,

131 Actinobacteria and Bacteroidetes and a very low fraction were linked to Proteobacteria
 132 (Figure 3c).

Figure 3: Characteristics of the viral representative contigs.

a, Distribution of contigs from the curated database by length, taxonomic family and bacterial host. Blue and pink lines represent the spread of contig length in mothers and infants, respectively. The colour of the dots at the median length values indicates if a family includes eukaryotic (yellow), prokaryotic (chartreuse) or unassigned (black) viruses. **b**, Classification of viral contigs by taxonomy, host and viral family. **c**, Number of representative viral contigs linked to their potential bacterial phyla by CRISPR.

133 To obtain information about the prevalence and presence of viral-representative contigs in
 134 VMs, we aligned their reads to the curated viral database (see Methods). Here, we observed
 135 that virome composition at viral family level was mostly consistent across maternal samples,
 136 with high prevalence of viral contigs assigned to the bacteriophage families *Siphoviridae*,

Podoviridae, *Myoviridae* and crAss-like phages that were present in all maternal samples (Figure 4a). In maternal samples, the abundances of these most prevalent viral families did not change significantly over time (FDR>0.05, Figure 4a).

Similar to the maternal samples, the viral families *Siphoviridae*, *Podoviridae* and *Myoviridae* were present in all infant samples, and the crAss-like phages were present in 31 out of 32 infant samples. In infants, the abundances of these viral families did not change significantly with time. While abundances of the *Podoviridae* and *Myoviridae* viral families were similar between maternal and infant samples, the abundance of crAss-like phages was significantly higher in maternal samples compared to infant samples (FDR<0.05) and the abundance of the *Siphoviridae* family was higher in infants compared to mothers (FDR<0.05, Figure 4a). *Siphoviridae* dominated both maternal and infant viromes, showing a greater abundance variation and a nominally significant decrease with time in infants (p-value=0.04, FDR=0.17).

At the level of viral-representative contigs, the composition and diversity of maternal and infant samples showed remarkable differences. The alpha-diversity of the maternal virome was significantly higher than that of the infant virome (p-value=2.2e-05, Figure 4b) and did not change over time in mothers or infants. Similar to the bacterial community, we observed divergent faecal viral communities between mothers and infants, with maternal and infant samples forming two distinct clusters (PERMANOVA, p-value=0.001, 999 permutations, Figure 4c), although this clustering was not as distinct as for the bacteriome composition (beta-diversity R² 0.62 for bacteriome versus 0.37 for virome). Even though infant samples tended to cluster together in the first principal component (Figure 4c), infant viromes were more individual-specific than maternal viromes (PERMANOVA, p-value<0.001, 999 permutations, Figure 4d). Infant viromes also showed greater changes with time than

maternal viromes (PERMANOVA, p -value <0.001 , 999 permutations, BC distances: 0.79 ± 0.14 for infants and 0.56 ± 0.14 for mothers) (Figure 4d), indicating more dynamic viromes in the developing gut ecosystems.

Figure 4: Differences in viral composition between mothers and infants.

a, Dynamics of viral families in mothers and infants. Reads were associated with viral lineages based on the annotation of viral contigs. Only viral families present in at least five samples per timepoint

are shown. **b**, Difference in alpha-diversity (calculated using the Shannon diversity index) of viral-representative contigs in mothers and infants. **c**, PCoA based on the beta-diversity of the viruses in viral metagenomes (VMs) calculated using Bray-Curtis dissimilarity matrix. Ellipses show 95% confidence intervals. **d**, Between-individual and within-individual Bray-Curtis distances for infants and mothers.

In mothers, 105 viral contigs were shared across at least 50% of the samples, which is in contrast to only two viral contigs shared across at least 50% of the infant samples, demonstrating, yet again, the high individual-specificity of the infant gut virome.

In mothers, the number of shared viruses across two timepoints was, on average, 1,060, which comprised $26.0 \pm 18.6\%$ of all viruses ever detected in a mother (Supplementary Fig.3). This percentage is comparable with another longitudinal study in adults, where $19.4 \pm 13.5\%$ of the total viral pool was shared between two timepoints¹³. In infants, on average, 41 viruses, or $20.9 \pm 14.9\%$ of all viruses ever detected in an infant, were shared across two timepoints. These results indicate that the percentage of shared viruses across timepoints in an individual is the same for infants and mothers, even though the viral richness in infants was substantially lower.

Mother to infant transmission of viruses

A moderate proportion of all the viruses found in infants was shared with their mothers ($11.3 \pm 20.0\%$), which is in contrast to the proportion of bacterial species shared between mothers and infants ($43.6 \pm 10.0\%$). The proportion of shared viruses did not significantly correlate to any available infant phenotypes.

Previous studies have shown that the adult gut ecosystem observes “Piggyback-the-Winner” dynamics, a state where bacteriophage lysogeny is increasingly favoured in a state with a higher microbial density^{11,14,15}. In contrast, the developing infant gut ecosystem is thought to be characterised by a high frequency of bacterial lysis events induced by

bacteriophages¹⁶. We therefore sought to compare the cumulative relative abundance of temperate phages between mothers and infants using the presence of integrase and recombinase genes within each viral contig as a measure of temperate phages. The relative abundance of active temperate phages in infants was significantly higher than in mothers (p-value=7.2e-06, Figure 5a) and decreased with timepoint ($R_{\text{Pearson}}=-0.38$, p-value=0.03, Figure 5b). The difference in the abundances of temperate phages in infant and maternal samples may explain the low proportion of shared viruses in mother–infant pairs, as our VLP extraction method targets actively reproducing viruses and not prophages. We therefore investigated whether the proportion of shared viruses increased when accounting for prophages integrated in bacterial genomes in maternal TMs. To do so, we mapped reads from the TMs to the curated viral database and obtained the relative abundances of bacteriophages found in TMs. The viral richness of both mothers and infants was higher in TMs than in VMs, with 462 ± 2767.4 and 1508.5 ± 2968.3 viruses per sample for VMs and TMs, respectively (p-value=3.0e-4). This was presumably due to the presence of prophages in TMs that are not captured as part of the active fraction in the VMs. As in VMs, in TMs, the viral alpha-diversity of maternal samples was higher than those of infants (p-value<2e-16), and the samples from mothers and infants formed two distinct clusters based on Bray-Curtis dissimilarity (PERMANOVA, p-value<0.001, 999 permutations, Supplementary figure 4a). The composition of the virome was highly consistent between concurrent VMs and TMs (Supplementary figure 4b). Interestingly, the proportion of viruses shared between mothers and their infants in TMs was $37.6 \pm 22.5\%$ (Figure 5c), which is much higher than in VMs (on average 27.8% higher, p-value=7.6e-06). The proportion of shared viruses between mothers and infants is thus similar to the proportion of the shared bacterial species when the prophages are taken into account. There was no significant correlation between the proportion of shared bacterial species in

207 mother–infant pairs and the proportion of shared viruses in VMs (Figure 5d). The proportion
208 of shared bacterial species correlates moderately with the proportion of shared viruses in
209 TMs ($R_{\text{Pearson}}=0.47$, $p=0.006$, Figure 5e), further suggesting that vertically transmitted bacteria
210 could be a source for vertically transmitted bacteriophages. These findings together support
211 the argument that the phages colonising the infant gut are vertically transmitted from the
212 mother by means of vertically transmitted bacteria.

Figure 5: Maternal to infant transmission of viruses

a, Relative abundance of temperate phages in mothers (P7, B) and infants (M1, M2, M3). **b**, The relative abundance of temperate phages in infants decreasing with time. **c**, The number of the shared viral-representative contigs between related and unrelated infants and mothers in total metagenome (TM) samples. Number of dots represents the number of timepoints available for each infant and the red dots represent the availability of VM's for that sample **d**, Correlation of the proportion of shared bacterial and the proportion of shared viruses in VMs of mother–infant pairs. **e**,

Correlation of the proportion of shared bacterial species and the proportion of shared viruses in TMs of mother–infant pairs.

Discussion

This study describes the extent and nature of microbial transmission from mothers to infants, with a focus on viral transmission. A limitation of our study design is that we did not analyse RNA viruses, given their perceived low abundance in the healthy human gut, although future studies should also investigate this dark aspect of the virome. However, while we acknowledge that our sample size only allows us to describe a limited number of associations with phenotypes and only indicates trends in the co-development of bacteriome and virome, our study also has notable strengths. Our novel approach of complementary investigation of both TMs and VMs in both mothers and infants led to novel findings. In the VLP-specific analysis that selectively explores the active virome fraction in stool, we observe high individual-specificity and lower diversity in infants compared to mothers. The average shared fraction of active virome between infants and their mothers was 11.3%, which agrees with the results of one of the few studies to investigate the sharedness of viruses amongst mothers and their infants, where a low sharing of viruses in comparison with bacterial genera was reported in 28 mother–infant twin pairs¹⁶. However, in our study, when we took prophages into account, the average proportion of vertically transmitted viruses increased to 37.6% and became comparable to the sharedness of gut bacterial species. The majority of the temperate bacteriophages dominating the infant gut were not detected in maternal metaviromes, but were prevalent in maternal TMs, suggesting that infants partly obtain their virome from their mothers via vertically transmitted bacteria.

Our study also addressed previously unstudied factors for maternal to infant microbial transmission such as the place of deliveries. As home deliveries constitute 12.7% of the total

234 deliveries (2018) in the Netherlands, and 28% in our samples, we possess the unique
235 opportunity to also reveal trends concerning possible novel associations regarding the effect
236 of place of birth on infant bacterial strains like *Bifidobacterium bifidum* which need to be
237 further investigated in a larger cohort¹⁷. An additional strength of our study was that we did
238 not use amplification techniques during the generation of VMs, which allowed accurate
239 quantification of viruses and led to minimal bias in our estimation and characterisation of
240 double stranded DNA viral families.

241 In conclusion, we provide evidence for mother-to-child viral and bacterial
242 transmission events at high-resolution and novel insights into the early colonisation of the
243 infant gut ecosystem.

Methods

Study design

The samples for this (pilot) study were obtained from the Lifelines NEXT cohort, a birth cohort designed to study the effects of intrinsic and extrinsic determinants on health and disease in a four-generation design. Lifelines NEXT is embedded within the Lifelines cohort study, a prospective three-generation population-based cohort study recording the health and health-related aspects of 167,729 individuals living in Northern Netherlands¹⁸ In Lifelines NEXT, we aim to include 1,500 pregnant Lifelines participants and intensively follow them, their partners and their children up to at least 1 year after birth. During the Lifelines NEXT study, biomaterials including maternal and neonatal (cord) blood, placental tissue, faeces, breast milk, nasal swabs and urine are collected from the mother and child at 10 timepoints. The long-term health outcomes of these infants and their parents will be investigated. Furthermore, data on medical, social, lifestyle and environmental factors are collected via questionnaires at 14 different timepoints and via connected devices¹⁹.

Informed consent

The Lifelines NEXT study was approved by the Ethics Committee of the University Medical Center Groningen, document number METC UMCG METc2015/600. Written informed consent forms were signed by the participants or their parents/legal guardians.

Sample collection

Mothers collected their faeces during pregnancy at months 3 and 7, at birth and 3 months after birth (Figure 1a). Faeces from infants were collected from diapers by their parents at months 1, 2 and 3 of infant age. Parents were asked to freeze the stool samples at home at -20°C within 10 min of stool production. Frozen samples were then collected and transported to the UMCG and stored in a -80°C freezer until extraction of microbial and viral DNA. We collected a total of 217 samples for total microbiome analysis.

Total microbial DNA extraction

Microbial DNA was isolated using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) using the QIAcube (Qiagen) from 0.2–0.5 g faecal material, with a final elution volume of 100 µl. DNA eluates were stored at -20°C.

VLP extraction and nucleic acid extraction

Initially, a subset of 93 of 217 faecal samples was selected for viral -like particle extraction based on the availability of faecal material. This subset of 93 samples included maternal samples from pregnancy and birth and infant samples at birth, month 1, month 2 and month 3. The gut virome was studied using extraction of DNA from VLPs as described in Shkoporov et al. 2018²⁰. Briefly, 0.5 g of faecal material was resuspended in 10 ml of SM buffer. Samples were centrifuged at 4,800 rcf for 10 min at 4°C. Supernatant was collected and centrifuged again using the same settings. The supernatant was filtered twice through a 0.45-µm pore polyethersulfone membrane filter. VLPs were concentrated from the filtrate with Polyethylene glycol 8000 (Sigma-Aldrich, Cat#P2139) precipitation overnight and purified with chloroform treatment. The resulting fraction was treated with 8 U of TURBO DNase (Ambion/ThermoFisher Scientific) and 20 U of RNase I (ThermoFisher Scientific) at 37°C for 1 h before inactivating enzymes at 70°C for 10 min. Subsequently, proteinase K (40 µg, Sigma-Aldrich, Cat#2308) and 20 µl of 10% SDS were added to the samples and incubated for 20 min at 56°C. Finally, VLPs were lysed by addition of 100 µl of Phage Lysis Buffer (4.5 M guanidinium isothiocyanate, 44 mM sodium citrate pH 7.0, 0.88% sarkosyl, 0.72% 2-mercaptoethanol) and incubated at 65°C for 10 min. Nucleic acids were extracted twice from lysates using Phenol/Chloroform/Isoamyl Alcohol 25:24:1 (ThermoFisher Scientific) treatment followed by centrifugation at 8,000 g for 5 min at room temperature. The resulting aqueous phase was subjected to the final round of purification using the DNeasy Blood & Tissue Kit (Qiagen) with a final elution volume of 50 µl. Viral DNA was stored at -20°C.

Genomic library preparation and sequencing

Faecal microbial DNA and viral DNA samples were sent to Novogene, China for genomic library preparation and shotgun metagenomics sequencing. Sequencing libraries were prepared using the NEBNext® Ultra™ DNA Library Prep Kit or the NEBNext® Ultra™ II DNA Library Prep Kit, depending on the sample DNA concentration, and sequenced using HiSeq 2000 sequencing with 2 × 150 bp paired-end chemistry (Illumina). On average, 30.76±4.2 million paired-end total metagenome reads and 29.8±4.8 million paired-end viral metagenome reads were generated per sample. Of 217 collected samples for total microbiome analysis, 187 were successfully sequenced. Following this, three meconium samples and one mislabelled sample were excluded. For virome analysis, 66 of the 93 samples

were successfully sequenced. As with bacteriome, meconium samples failed sequencing due to their perceived low viral abundance.

Profiling of total gut microbiome composition

Total metagenome sequencing reads were trimmed and Illumina sequence adaptor sequences removed using KneadData tools (v0.7.4) and an average PHRED quality score of 33²¹. Following trimming, the KneadData-integrated Bowtie2 tool (v2.4.2)²² was used to remove reads that aligned to the human genome (GRCh37/hg19), and the quality of the processed data was examined using the FastQC toolkit (v0.11.9)²³. Samples with a clean read-depth <5 million were not considered in further analysis. Taxonomic composition of total metagenomes was profiled using the MetaPhlAn3 tool with the MetaPhlAn database of marker genes mpa_v30 and the ChocoPhlAn pan-genome database (201901).

Profiling of gut virome composition

VM sequencing reads were subject to quality trimming. Read mapping to the human (GRCh38.p12) reference genome was performed with KneadData (v0.5.1)²¹. On average, 22.1±4.5 million paired-end VLP reads passed quality control. The quality of the raw and clean reads was visualised with FastQC (v0.11.7)²³. Bacterial contamination of VMs was assessed by aligning reads to the single copy chaperonin gene cpn60 database²⁰. On average, VLP samples contained 3.7±7.9% of bacterial genomic DNA per sample.

We annotated the gut virome composition using the *de novo* assembly-based method. Whole metagenome *de novo* assembly was performed per VMs using SPAdes (v3.11.1) in metagenomic mode (*-meta*) with default settings²⁴. On average, 280,363 contigs were assembled for maternal samples and 46,830 contigs were assembled for infant samples. When pooled, 724,612 contigs from the whole dataset were subjected to a redundancy-removal procedure in which contigs with 90% nucleotide identity over 90% of the length of a shorter contig were considered redundant, and the shorter contig was removed. Overall, 274,870 representative pooled contigs larger than 1 kbp were subject to validation as viral.

For the 274,870-representative pooled contigs, we predicted the Open Reading Frames (ORFs) using Prodigal v2.6.3 in metagenomic mode²⁵. A Hidden Markov Model (HMM) algorithm (hmmsearch from HMMER v3.2.1 package)²⁶ was used to compare amino acid sequences of predicted protein products against the HMM database Prokaryotic Virus

Orthologous Groups (pVOGs) ²⁷. Hits were considered significant at an e-value threshold of 10^{-5} . Ribosomal proteins were identified using a BLASTp ²⁸ search (e-value threshold of 10^{-10}) against a subset of ribosomal protein sequences from the COG database (release 2014). We used VirSorter v1.0.3 ²⁹ with its expanded built-in database of viral sequences ('-db 2' parameter) in the decontamination mode as one of the steps for prediction of viral sequences. Representative contigs larger than 1 kbp were considered viral if they fulfilled at least one of six criteria (similar to those described in ^{12,13,30}): (1) they produced BLASTn alignments to a viral section of NCBI RefSeq (release 98) with e-value $\leq 10^{-10}$, covering >90% of contig length at >50% identity, (2) they had at least three ORFs, producing HMM-hits to the pVOG database with an e-value $\leq 10^{-5}$, with at least two ORFs per 10 kb of contig length, (3) they were VirSorter-positive (all six categories, including suggestive), (4) they were circular³¹, (5) they produced BLASTn alignments to 427 crAss-like reference genomes ³² & unpublished data) with an e-value $\leq 10^{-10}$ covering >90% of contig length at >50% identity, or (6) they were longer than 3 kbp with no hits to the nt database (release 235) (alignments >100 nucleotides with 90% identity and an e-value of 10^{-10}). 54,267 contigs fulfilled at least one of these six criteria, and these contigs were subjected to clustering with 427 crAss-like phage contigs from ³² and an unpublished custom database and genomes of the reference database "ProkaryoticViralRefSeq97-Merged", using vConTACT2 0.9.15 with default parameters³³. Contigs assigned the status 'Overlap', 'Singleton' and 'Outlier' by vConTACT2 were treated as viral clusters consisting of a single contig in all subsequent analyses. Contigs were further subject to a decontamination procedure based on the following criteria. Contigs were kept if they 1) were circular and contained at least 1 pVOGs hit, 2) were circular and VirSorter-positive, or 3) were VirSorter-positive and did not have ribosomal protein genes. Contigs were excluded if they 1) had more than three ribosomal protein genes (similar to the decontamination process described in^{12,13}), or 2) were longer than 200 kbp and co-clustered with contigs possessing more than three ribosomal protein genes.

The final curated database of reconstructed viral sequences generated based on our dataset included 51,455 representative contigs ranging in size from 1 kbp to >626 kbp, with low-to-high k-mer coverage (1.1–52,101.3X), which recruited 89% reads per sample on average.

Quality-filtered VM reads were aligned to the custom viral database consisting of 51,455 viral-representative contigs on a per-sample basis using Bowtie2 v2.3.4.1 in 'end-to-end' mode²². A count table was subsequently generated using SAMTools v1.9³⁴. Sequence coverage was calculated per contig per sample using the BEDtools v2.25.0 'coverage' command³⁵. To remove spurious Bowtie2 alignments, readcounts that featured a breadth of contig coverage less than $1 \times 75\%$ of a contig length were set to zero³⁶ resulting in 51,449 viral sequences being used for the construction of the final count table. RPKM value transformation was applied to the final count table, and the resulting RPKM count table was used for downstream analysis.

Family-level taxonomic annotations were assigned to viral contigs using the Demovir script (<https://github.com/feargalr/Demovir>) with default parameters and database. Manual curation of Demovir taxonomy assignment and expansion on unassigned contigs using vConTACT was performed as described previously¹³. Despite using sequencing adaptors binding only dsDNA, a few genomes of ssDNA viruses assigned to the families *Circoviridae*, *Inoviridae*, *Microviridae* and *Parvoviridae* were reconstructed. These could be either a result of taxonomy misassignment or of catching these viruses during their replication in the duplex form. Given the low bacterial contamination level, we did not exclude the putatively ssDNA virus contigs from the further analysis, assuming that even in case of taxonomy misassignment, these contigs are still of viral origin.

Virome annotation in TMs

Quality-filtered reads from 183 TMs were aligned to the custom viral database consisting of 51,455 viral-representative contigs on a per-sample basis using Bowtie2 v2.3.4.1 in 'end-to-end' mode²². A count table was generated and transformed as with VMs. The resulting RPKM count table was used to assess the number of shared viruses in related mother-infant pairs. The increase in the number of shared viruses in mother-infant pairs in TMs compared to VMs did not depend on the initial size of the virus pool or any of the infant or maternal phenotypes studied (Spearman correlation, $p\text{-value} > 0.05$). All comparisons regarding the VMs and TMs viromes were performed on the 66 samples that had both VM and TM samples.

Prediction of virus hosts based on CRISPR spacer sequences

CRISPR spacer sequences from the CRISPRCas++ database (18.06.2019)³⁷ were compared to the 51,455 viral contigs using BLASTN 2.7.1+²⁸ with the "-task 'blastn-short' -evalue 1e-5"

parameters. Hosts were predicted based on hits characterised by bitscore ≥ 45 and < 2 mismatches, gaps and unaligned spacer nucleotides. Host taxonomy was retrieved from GenBank using BioPerl 1.6.924^{38,39}.

Ecological measurements and taxonomic comparisons

To assess bacterial alpha-diversity for each sample, we used species reported in the MetaPhlAn3 output at $> 0.1\%$ mean relative abundance and present in at least two samples. For the virome alpha-diversity measurements, no filters were applied to the table of RPKM (reads per kilobase per million) counts. The alpha diversity for both bacteriome and virome was calculated using the Shannon diversity index using the *diversity()* function in R package 'vegan' v.2.5-7⁴⁰. To test the difference in Shannon diversity index between mother and infant and the effect of timepoint on infant Shannon diversity index, we used a linear model or a linear mixed model, by comparing the suitability of the random effects using the *exactLRT()* function from the 'RLRsim' package⁴¹.

To compare the bacterial phylum abundances of the five most abundant phyla of mothers and infants, we used a linear model or a linear mixed model, by comparing the suitability of the random effects using the *exactLRT()* function from the 'RLRsim' package⁴¹. Prior to analysis, a centred log-ratio (CLR) transformation was performed. FDR correction was applied to correct for multiple testing, with changes considered statistically significant at $FDR < 0.05$ using the Benjamini-Hochberg method.

The Bray-Curtis distances between samples were calculated based on the level of bacterial species and on viral representative contigs. For the Bray-Curtis distances calculation, we used the function *vegdist()* from the R package 'vegan' v.2.5-7⁴⁰. To compare the bacterial and viral composition between mothers and infants, PERMANOVA was used with 999 permutations. For these comparisons the aforementioned filters for abundance and presence of bacterial species were used. For the viral-representative contigs-level no filters were applied.

The dynamics of viral family abundances was only assessed families present in at least five individuals per every timepoint ($n=4$; crAss-like phages, *Myoviridae*, *Podoviridae* and *Siphoviridae*). To normalise the RPKM counts per sample for these viral families, we used a clr transformation. We further excluded outliers if they were not in the range $[Q1 - 1.5 \cdot IQR; Q3 + 1.15 \cdot IQR]$, where $Q1$ and $Q3$ are the 25th and 75th percentiles and IQR is the interquartile

range. Next, to determine if the abundance of the viral families changed over time, we applied a linear (mixed) model that took into account a timepoint (and the repeated measurements). Differentially abundant viral taxa between adults and infants were defined using a linear (mixed) model that took into account the origin of the sample (and the repeated measurements). The choice of model for every viral family was performed by comparing the suitability of the random effects, as described earlier. FDR correction was applied to correct for multiple testing as described previously.

Bacterial species-specific strain analysis

Strain SNP haplotypes were generated using StrainPhlAn3^{21,42}. This method is based on reconstructing consensus sequence variants within species-specific marker genes and using them to estimate strain-level phylogenies⁴². This method only takes into account the dominant strain of species and hence our method misses overlaps in secondary strains. We then performed multiple sequence alignment and used the Kimura 2-parameter method from the 'EMBOSS' package⁴³ to calculate phylogenetic distance matrices that contain the pairwise nucleotide substitution rate between strains. Using presence in more than 50 samples as a cut-off, we were able to construct SNP haplotype differences in 17 bacterial species. To identify distinct strain clusters within species, we performed hierarchical clustering using the R function *hcluster()*. To define identical strains between mother and infant, a definition of 0.0 of Kimura strain distance value was used. The significance of the difference between phylogenetic distances between related mother-infant pairs and unrelated mother-infant pairs was tested using PERMANOVA (999 permutations) and a FDR<0.05 was considered significant. Similarly, the effect of place of birth was tested using PERMANOVA (999 permutations) on the phylogenetic distances of *Bifidobacterium bifidum* correcting for samples origin (mother or infant) and repeated measurements.

Association with phenotypes

We used R package 'Multivariate Association with Linear Models (MaAsLin) 2'⁴⁴ to associate phenotype data with bacterial species. MaAsLin performs boosted, additive general linear models of associations between phenotypes and the relative abundance of species. We performed this analysis only in infant samples where we used a cut-off of 0.01% species abundance and selected only species occurring in at least 5% of the samples. Prior to input into MaAsLin, we performed a clr transformation. In MaAsLin, we set individual IDs as random

444 effects and added read-depth, sex, delivery mode, infant feeding type, timepoint, birth
445 weight, place of birth, maternal age, maternal pre-pregnancy BMI and gestational age as fixed
446 effects. We defined statistical significance at $FDR < 0.05$. Given the low sample size to study
447 association of viral contig abundances with phenotypes we did not perform this analysis.

Code availability

448 All codes used in this study can be found at: [https://github.com/GRONINGEN-MICROBIOME-](https://github.com/GRONINGEN-MICROBIOME-CENTRE/Lifelines_NEXT)
449 [CENTRE/Lifelines_NEXT](https://github.com/GRONINGEN-MICROBIOME-CENTRE/Lifelines_NEXT)

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Supplementary figures:

Supplementary Figure 1: Violin plots representing the alpha diversity in mothers and infants

a, Difference in alpha-diversity of bacterial species in mothers and infants (***) depicts a p value of $<2.2 \times 10^{-16}$. Alpha-diversity of the bacteria at species level was calculated using Shannon diversity index. **b**, Alpha-diversity of bacterial species in infants across timepoints. **c**, Alpha-diversity of bacterial species in mothers across timepoints.

Supplementary figure 2: Barplots representing beta-diversity in mothers and infants

a, Bray-Curtis within-individual distances between timepoints in infants: Month 1 (M1) to Month 2 (M2), M1 to Month 3 (M3) and M2 to M3. The Bray-Curtis dissimilarity matrix was calculated based on the species level of bacteria. **b**, Bray-Curtis within-individual distances in mothers between

timepoints: P3-P7, P3-B, P3-M3, P7-B, P7-M3, B-M3. Bray-Curtis dissimilarity matrix was calculated based on bacterial species.

Supplementary figure 3: Number of viral-representative contigs per subject present in a given number of timepoints. Where available (2 infants), three timepoints were subsampled to two timepoints, and the mean was taken into consideration. Y-axis is log-scaled. “1 timepoint” is viral richness for all the infants or mothers at every timepoint.

Supplementary figure 4: Overview of viral contigs in total metagenomes (TMs) and viral metagenomes (VMs).

a, PCoA based on the beta-diversity of the viruses in total metagenomes (TMs) calculated using Bray-Curtis dissimilarity matrix. Ellipses show 95% confidence intervals. **b**, heatmap showing the similarity between infant individual viromes in VMs and TMs.

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[§]Lifelines NEXT cohort study

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