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Maternal health status is associated with paired maternal and cord blood virome and mother-to-infant transmission



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The viromes of maternal peripheral blood (MPB) and umbilical cord blood (UCB) provide crucial insights into mother-to-infant transmission and the associations of maternal health with early-life viral colonization. Using viral metagenomic sequencing of 433 MPB and 426 UCB samples, we assembled 57 near-complete genomes from four core viral families (*Anelloviridae*, *Circoviridae*, *Parvoviridae*, *Flaviviridae*). MPB viromes were primarily composed of bacteriophages and *Anelloviridae*, while UCB exhibited relatively increased abundances of *Parvoviridae* and *Human Endogenous Retroviruses*. Maternal disease correlated with reduced α -diversity in MPB but elevated richness in UCB. β -Diversity varied significantly with both health status and sample type. Differential abundance analysis identified health-specific signatures, including enriched *Parvoviridae* in diseased UCB. Phylogenetic evidence indicated possible vertical transmission and high genetic diversity among identified viruses. This study systematically characterizes the maternal–fetal blood virome and reveals associations between maternal health status and viral community structure, providing a basis for understanding early-life viral exposure and informing future preventive strategies.

Umbilical cord blood (UCB) forms a critical bridge between the maternal and fetal circulatory systems during pregnancy. Beyond its well-established role in mediating nutrient and gas exchange, growing evidence suggests UCB may also serve as a conduit for early microbial and viral exposure. This distinctive biological compartment captures a snapshot of the perinatal virome, offering a unique window into mother-to-infant viral transmission, initial microbial colonization, and the developing neonatal immune system^{1,2}. Notably, emerging research on the cord blood virome is shedding light on its potential role in early-life viral exposure and the subsequent immunological consequences for the neonate, as well as its implications for maternal–fetal immune crosstalk³.

Physiologically, UCB constitutes a specialized environment enriched with hematopoietic cells, immunomodulatory factors, and signaling molecules⁴. In recent years, metagenomic analyses have progressively overturned the traditional “sterile womb” hypothesis,

demonstrating that blood carries complex viral communities comprising both eukaryotic viruses and bacteriophages, whose composition often reflects the host’s health status². Among the viral families consistently identified in UCB are *Herpesviridae*, *Anelloviridae*, and *Papillomaviridae*^{5,6}. It should be noted that not all of these families undergo true latency; while *Herpesviridae* can establish latent infections, *Anelloviridae* typically cause persistent infections. Viruses within these families can persist in various states and may participate in the developmental process of the fetal immune system. Particularly compelling is the detection of bacteriophage DNA in human cord blood at delivery, providing molecular evidence of in utero viral exposure and supporting the possibility of vertical viral transmission⁷. Maternal viromes may also indirectly modulate fetal immune development by shaping the intrauterine environment. Dynamic correlations between maternal and infant viral communities have been observed across body sites, including the

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gut, before and after birth. Moreover, the abundance of certain blood-borne viruses such as *Anelloviridae* has been correlated with immunological status and inflammatory markers, underscoring their potential utility as biomarkers for immune monitoring⁸. Although viral populations in maternal plasma, vaginal secretions, and other sites have been increasingly mapped⁹, systematic metagenomic comparisons between paired MPB and UCB samples remain limited¹⁰. Perhaps more importantly, the influence of maternal health status on the blood virome and its implications for vertical transmission have not been thoroughly elucidated. Our study seeks to address these unanswered questions by exploring mother–infant virome dynamics and their health relevance¹¹.

Strong epidemiological evidence links maternal viral infection and immune activation during pregnancy to adverse outcomes, including preterm birth, stillbirth, fetal growth restriction, and neurodevelopmental disorders in offspring^{12–14}. Even without direct fetal infection, viral presence can disrupt the intrauterine milieu by provoking placental or fetal inflammatory responses. The composition and dynamics of the cord blood virome may thus reflect maternal health status, including immune competence, co-infections, and microbial stability, highlighting its potential as a biomarker for assessing infant exposure and developmental risk⁶. Profiling the cord blood virome metagenomically remains technically demanding, primarily due to low viral nucleic acid abundance and high background host DNA. These challenges necessitate optimized purification protocols and deep-sequencing strategies.

Metagenomic sequencing powerfully captures the complete genetic landscape of all microorganisms, including viruses, within biological samples like UCB¹⁵. Unlike culture-dependent or PCR-based methods, metagenomics requires no prior knowledge of viral sequences, enabling unbiased detection of both known and novel viruses¹⁶. This advantage proves essential in UCB studies, where viral loads are typically low and many viruses remain uncultivable or poorly characterized¹⁷. The application of metagenomic approaches allows for broad profiling of eukaryotic viruses and bacteriophages in UCB. Recent bioinformatic advances—including refined machine learning algorithms—have substantially improved the prediction of virus–host interactions and enhanced the accuracy of viral genome assembly and taxonomic classification¹⁸. Together, these developments establish metagenomic sequencing as an indispensable tool for characterizing the UCB virome and illuminating its role in maternal–infant health.

In this study, we leveraged viral metagenomic strategies to systematically analyze the viromes of paired MPB and UCB samples. Combining deep sequencing with advanced bioinformatic pipelines, we compared viral community structures and diversity patterns between maternal peripheral blood and umbilical cord blood. Phylogenetic investigations further elucidated evolutionary relationships among key viral families and assessed their potential for cross-generational transmission. Our findings offer new insights into the architecture of the perinatal virome and uncover potential mechanisms governing vertical virus transmission.

Results

Viral metagenomic overview

To comprehensively profile the blood virome in maternal and umbilical cord blood samples, we collected 433 qualified maternal peripheral blood (MPB) and 426 paired umbilical cord blood (UCB) specimens following quality control assessment. These 7 MPB samples lacking UCB pairs were pooled into a single sequencing library. In total, 175 sequencing libraries were constructed and analyzed on the Illumina NovaSeq platform, generating 3,583,289,218 raw reads, ranging from 193,972 to 116,998,298 reads per library, with an average GC content of 44.7%. All sequences were demultiplexed, de novo assembled, and aligned against the GenBank non-redundant protein database using BLASTx to identify viral reads. Altogether, 5,208,976 viral genomic reads, ranging from 234 to 391,377 per library, were identified as belonging to known eukaryotic viruses. Based on maternal health status and sample type, the 175 libraries were classified into four groups: the Maternal Peripheral Blood Healthy group ($n = 37$, Blood01–Blood37), the Maternal Peripheral Blood Disease group ($n = 51$,

Blood38–Blood88), the Umbilical Cord Blood Healthy group ($n = 37$, Blood89–Blood125), and the Umbilical Cord Blood Disease group ($n = 50$, Blood126–Blood175). The Disease group included individuals diagnosed with a single, specific pregnancy complication, namely Gestational Diabetes Mellitus ($n = 13$), Hypothyroidism in Pregnancy ($n = 10$), Anemia in pregnancy ($n = 9$), or Hypertensive Disorders of Pregnancy ($n = 19$), where n indicates the number of maternal peripheral blood sequencing libraries. Maternal health status determined the classification of all groups. Detailed metadata for each library are provided in Supplementary Data 1.

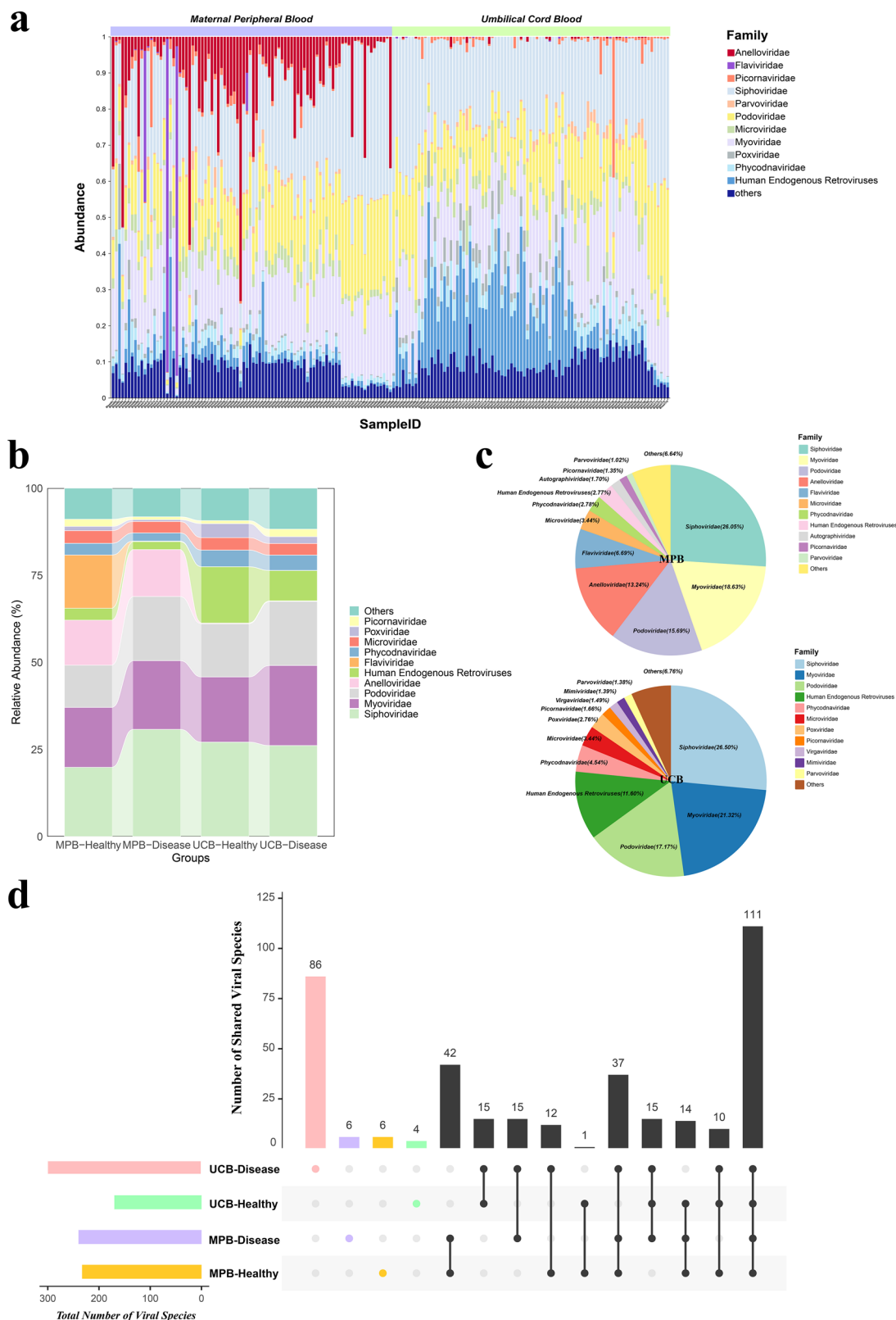
Through sequence assembly, a total of 57 distinct complete or near-complete viral genomes (CDS) were identified in this study. These genomes were classified into four defined viral families. Among them, 51 originated from maternal peripheral blood and 6 from umbilical cord blood. These included DNA viruses from *Anelloviridae* ($n = 41$, ssDNA), *Circoviridae* ($n = 6$, ssDNA), and *Parvoviridae* ($n = 7$, ssDNA), and RNA viruses from *Flaviviridae* ($n = 3$, ssRNA). Phylogenetic analyses were performed using amino acid or nucleotide sequences from the most conserved genomic regions, specifically ORF1 for *Anelloviridae*, the Rep protein for *Circoviridae*, the NS1 protein for *Parvoviridae*, and the RNA-dependent RNA polymerase (RdRp) domain for *Flaviviridae*.

Composition of the virome in maternal and umbilical cord blood

At the family level, viral reads were assigned to 37 families, including 22 DNA and 15 RNA virus families. The overall taxonomic profiles are shown in a circular heatmap (Supplementary Fig. 1). Relative abundance analyses revealed key structural and compositional patterns across libraries (Fig. 1a, b). *Myoviridae*, *Siphoviridae*, and *Podoviridae* were consistently dominant. *Anelloviridae* were markedly higher in MPB than in UCB. Pie charts (Fig. 1c) show that in maternal peripheral blood (MPB), *Siphoviridae* constituted the largest proportion (26.05%), followed by *Myoviridae* (18.63%), *Podoviridae* (15.69%), and *Anelloviridae* (13.24%); together these families dominated the MPB virome, with *Flaviviridae* also present at notable levels (6.69%). In UCB, *Siphoviridae* remained most abundant (26.5%), trailed by *Myoviridae* (21.32%) and *Podoviridae* (17.17%). *Human Endogenous Retroviruses* were substantially enriched in UCB (11.6%). Conversely, *Anelloviridae* were frequently detected in MPB but scarce in UCB, suggesting confinement mainly to maternal circulation. *Flaviviridae* were present in MPB (6.69%) but not significantly detected in UCB. Although *Parvoviridae* occurred at low abundance in MPB (1.02%), their relative abundance increased in UCB (1.38%), suggesting either neonatal-specific enrichment or distinct structural organization of the UCB virome. UpSet analysis (Fig. 1d) identified 374 viral species across all groups. The UCB-Disease group exhibited the highest species richness ($n = 86$), substantially surpassing the other three groups. A total of 111 viral species were shared among all groups, representing a core virome. Notably, only a single viral species was uniquely shared between the MPB-Healthy and UCB-Healthy groups. This exclusively shared virus was identified as Gokushovirinae Bog1183_53, a member of the *Microviridae* phage family. In contrast, a significantly greater number of species ($n = 15$) were uniquely shared between the MPB-Disease and UCB-Disease groups. These results suggest that maternal health status may be associated with specific viral distribution patterns and could potentially influence mother–infant viral transmission characteristics. Collectively, this study reveals potential relationships between maternal health status and the structure of the cord blood virome, as well as viral exchange features between mothers and their infants.

Virome diversity analysis of maternal and umbilical cord blood

To evaluate species richness and sampling completeness, we constructed rarefaction and accumulation curves. The rarefaction curves approached saturation with increasing sequencing depth, suggesting sufficient sequence coverage (Supplementary Fig. 2). The percentage contribution of viral sequence reads from each library is shown in a pie chart (Supplementary Fig. 3). Similarly, the species accumulation curves plateaued as sample size increased, confirming representative sampling (Supplementary Fig. 4). Overall, more than 300 viral species were identified across all libraries. We



compared α -diversity among the four groups using Shannon and Simpson indices (Fig. 2a). The MPB-Disease group exhibited significantly lower Shannon and Simpson indices compared to MPB-Healthy ($P < 0.05$). Between UCB-Healthy and UCB-Disease, Shannon diversity differed significantly ($P < 0.05$), though the Simpson index did not reach significance. Under healthy conditions, α -diversity did not differ significantly between

MPB-Healthy and UCB-Healthy. In contrast, under disease conditions, both Shannon and Simpson indices were significantly higher in UCB-Disease than in MPB-Disease ($P < 0.001$), indicating enhanced virome diversity in cord blood relative to maternal blood in the context of maternal disease. β -diversity was assessed using PCoA based on Bray–Curtis distances, which revealed clear separation among groups (Fig. 2b). MPB

Fig. 1 | Analysis of the composition of the maternal and infant umbilical cord blood virome. **a** Comparison of community composition at the viral family level in the maternal and umbilical cord blood samples. The stacked bar chart shows the relative abundance of the top 11 viral families in maternal peripheral blood and neonatal umbilical cord blood, with the remaining viral families grouped under “others”. Detailed information on the number of samples pooled per library is provided in Supplementary Data 1. **b** Based on maternal health status and sample type, the bar chart shows the top 10 viral families in relative abundance across four groups, with the remaining viral families grouped under “others”. **c** Pie charts

showing the relative abundance composition of viral families in maternal peripheral blood (MPB) and umbilical cord blood (UCB). Only the top 11 viral families by read count are listed, with the remaining low-abundance viral families grouped under “others”. **d** UpSet plot showing the number of shared viral species across the four groups. Filled dots with connected vertical lines represent intersections, and unfilled dots in light gray represent sets that do not belong to the intersections. The vertical bars represent the number of viral species within the intersections, and the horizontal bars represent the total number of viral species in each group.

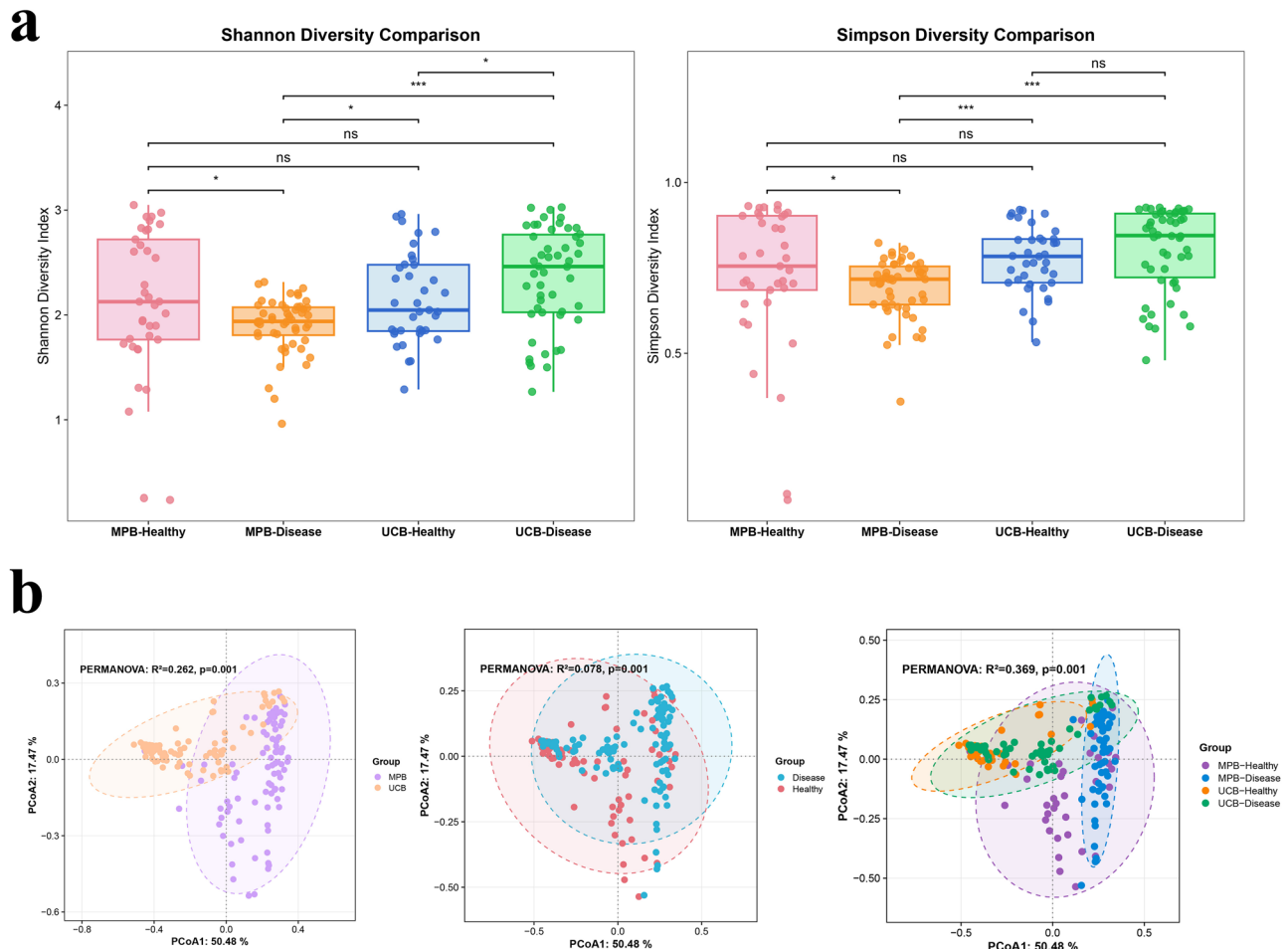


Fig. 2 | Analysis of the diversity of the maternal and umbilical cord blood virome. **a** Alpha diversity analysis. The alpha diversity of maternal and umbilical cord blood samples across four groups was assessed using the Shannon and Simpson indices, with the significance of intergroup differences tested using the Wilcoxon rank-sum test. In the box plots, the horizontal line within the box represents the median, the upper edge of the box corresponds to the 75th percentile (Q3, upper quartile), and

the lower edge corresponds to the 25th percentile (Q1, lower quartile). Statistical significance thresholds: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **b** Beta diversity analysis. Beta diversity analysis based on Bray-Curtis distances, visualized through principal coordinates analysis (PCoA). The percentages of variance explained by PCoA1 and PCoA2 are indicated on the respective axes. Ellipses represent the 95% confidence intervals for each group.

samples showed greater dispersion than UCB samples, and sample type (MPB vs. UCB) significantly influenced community structure ($P = 0.001$). Maternal health status also markedly affected viral community composition ($P = 0.001$). Multi-group comparisons confirmed highly significant β -diversity differences among all four groups. These results indicate that both sample source and maternal health status interact to shape viral β -diversity.

Differential virome profiles in maternal and umbilical cord blood under healthy and diseased conditions

STAMP analysis is widely used for detecting metagenomic differences and provides confidence intervals for biological interpretation¹⁹. STAMP

identified significant differences in viral abundance between healthy and diseased groups at both the family and species levels.

In MPB, 17 viral families exhibited significant differences (Fig. 3a). *Human Endogenous Retroviruses* and *Poxviridae* were enriched in MPB-Healthy, while *Siphoviridae* and *Podoviridae* were more abundant in MPB-Disease. At the species level (Fig. 3b), nearly 40 species exhibited significant differences, with the majority being enriched in MPB-Healthy. Notably, *Escherichia* phage Lambda and *Enterobacteria* phage O276 (*Siphoviridae*) showed elevated abundance in MPB-Disease. In UCB, 23 families demonstrated differential abundance (Fig. 3c). *Human Endogenous Retroviruses*, *Poxviridae*, *Herpesviridae*, among others, were enriched in UCB-Healthy, whereas *Myoviridae*, *Podoviridae*, *Virgaviridae*, and several other

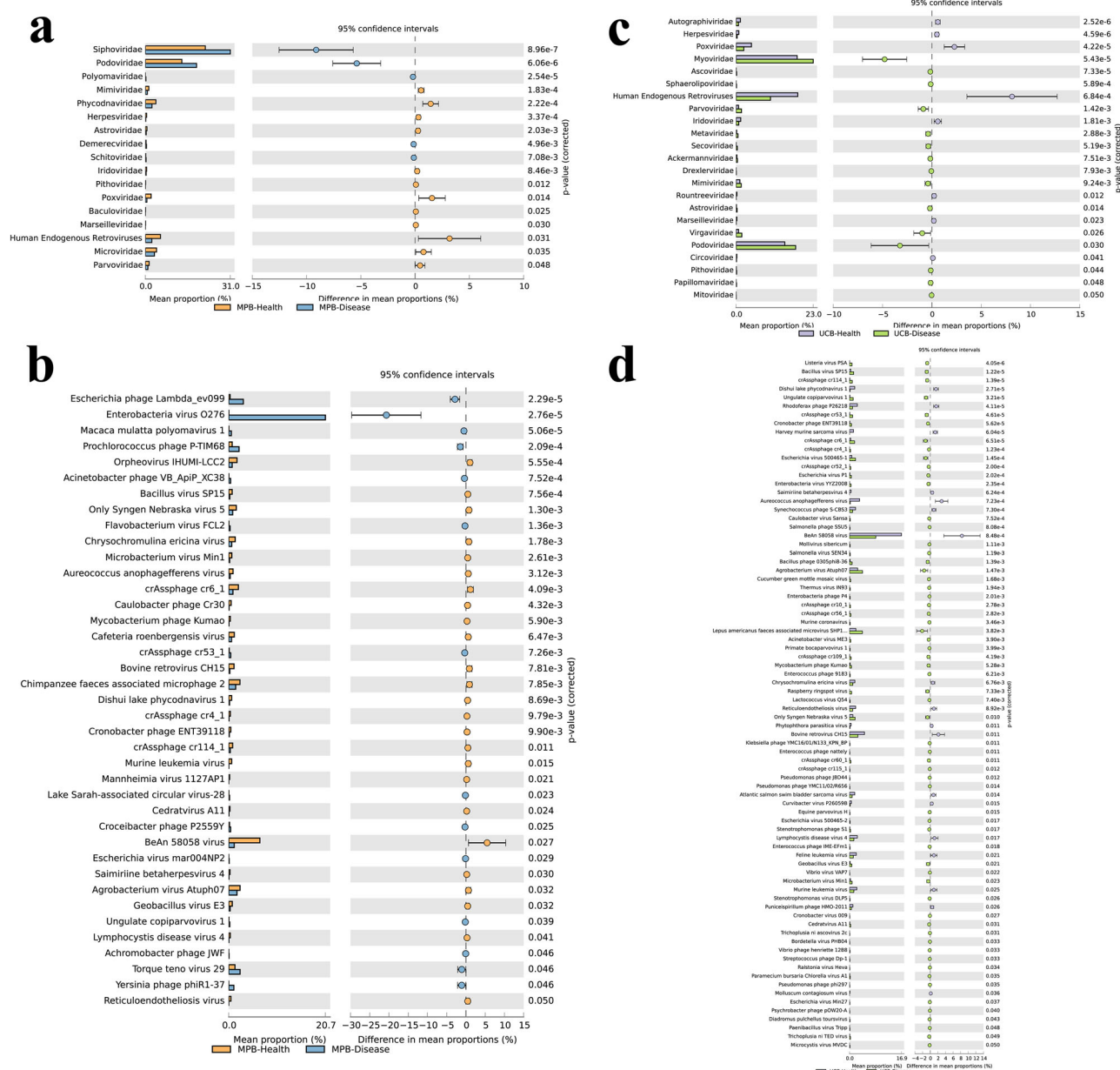


Fig. 3 | STAMP analysis of differences in the maternal and umbilical cord blood virome at the viral family and species levels. a, b STAMP analysis of differences in relative abundance of viral families and viral species between MPB-Healthy and MPB-Disease. **c, d** STAMP analysis of differences in relative abundance of viral

families and viral species between UCB-Healthy and UCB-Disease. For all panels, differences were tested using a two-sided Welch's *t*-test, and *p*-values were FDR-corrected for multiple comparisons (Benjamini-Hochberg method).

viral families were predominated in UCB-Disease. Interestingly, *Parvoviridae* were significantly more abundant in UCB-Disease—a pattern contrasting with MPB—suggesting possible vertical transmission and fetal replication²⁰. At the species level (Fig. 3d), 80 taxa differed significantly, most being enriched in the disease group. Notably, the BeAn 58058 virus, an unclassified virus within the family *Poxviridae* was relatively enriched in both healthy MPB and UCB samples. This unique pattern reveals a previously unrecognized ecological distribution of viruses at the maternal-fetal interface, the underlying mechanisms of which warrant further investigation.

To assess the influence of maternal health on viral community structure across taxonomic ranks, viromes were compared at class, order, and family levels. Among 21 detected viral classes, five differed significantly (fold change ≥ 2 ; Supplementary Data 3). *Flasuviricetes* were predominant in the healthy group (7.87%; 102.28-fold higher than diseased), with *Herviviricetes*

and *Pokkesviricetes* also enriched. In contrast, *Laserviricetes* and *Monjiviricetes* were more abundant in the diseased group. Co-occurrence analysis revealed widespread negative correlations among seven classes, indicating competitive interactions (Supplementary Fig. 5). At the order level, six orders differed significantly (Supplementary Data 3). Although *Caudovirales* dominated both groups (healthy: 58.78%; diseased: 67.29%), other orders varied considerably. *Chitovirales*, *Herpesvirales*, and *Amarillovirales* were enriched in healthy samples, whereas *Zurhausenvirales*, *Halopanvirales*, and *Mononegavirales* were more abundant in diseased samples. Network analysis showed predominantly negative correlations, except for a positive association between *Picornavirales* and *Martellivirales*, suggesting distinct interaction dynamics relative to class-level patterns (Supplementary Fig. 6). At the family level, 12 of 52 families exhibited significant differences (Supplementary Data 3). Thirteen families exceeded 1% relative abundance in the healthy group, with *Flaviviridae* (9.14%), *Poxviridae* (2.34%), and

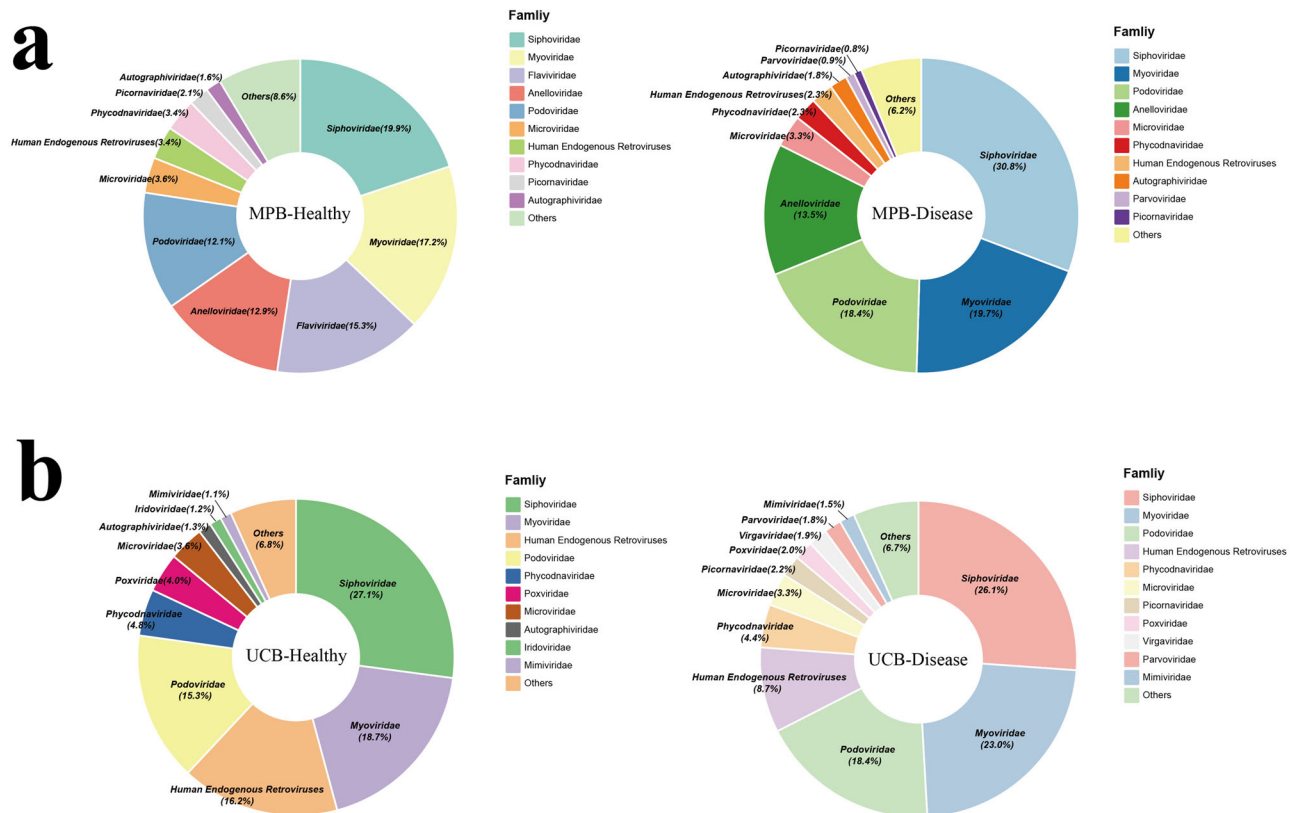


Fig. 4 | Pie charts showing the viral family composition of the four groups. a Viral family composition of MPB-Healthy and MPB-Disease. **b** Viral family composition of UCB-Healthy and UCB-Disease. Different colors are used to distinguish viral

family categories, and the legend indicates the names and colors of the corresponding viral families.

Herpesviridae (0.53%) being most prominent. In the diseased group, *Papillomaviridae* and *Sphaerolipoviridae* were markedly enriched (0.06% each), showing > 20-fold increases. In contrast to the competitive relationships observed at higher taxonomic levels, family-level networks revealed positive correlations among several families (e.g. *Picornaviridae* with *Virgaviridae* and *Iridoviridae*), suggesting potential cooperative ecological interactions (Supplementary Fig. 7).

Based on 175 metagenomic libraries, samples were categorized into four groups by maternal health status and sample type: MPB-Healthy, MPB-Disease, UCB-Healthy, and UCB-Disease. Viral family composition within each group is depicted in pie charts (Fig. 4a, b). *Human Endogenous Retroviruses* were substantially enriched in UCB compared to MPB, implying a possible role in neonatal virome establishment and maternal–fetal transmission. *Flaviviridae* abundance varied with maternal health status, being higher in MPB-Healthy but reduced in MPB-Disease and UCB samples. Conversely, *Parvoviridae* consistently increased in diseased groups across sample types, indicating a strong association with maternal–infant health and suggesting potential utility as a disease biomarker, though causal mechanisms remain unclear.

To further clarify maternal–infant differences and possible transmission routes, we analyzed four viral families with fully assembled genomes—*Anelloviridae*, *Circoviridae*, *Parvoviridae*, and *Flaviviridae*—evaluating their distribution in MPB and UCB and the influence of maternal health status. A paired analysis of 87 matched MPB–UCB samples (excluding Library 88, which lacked a UCB pair) was conducted. After normalizing raw read counts for library size and applying Wilcoxon signed-rank tests, *Anelloviridae* ($p < 0.0001$), *Circoviridae* ($p < 0.0001$), and *Flaviviridae* ($p < 0.05$) were significantly more abundant in MPB than UCB, whereas *Parvoviridae* showed the opposite trend, with higher UCB abundance ($p < 0.001$). Stratifying the pairs into healthy ($n = 37$) and diseased ($n = 50$) groups revealed consistent MPB enrichment of *Anelloviridae* in both groups ($p < 0.0001$).

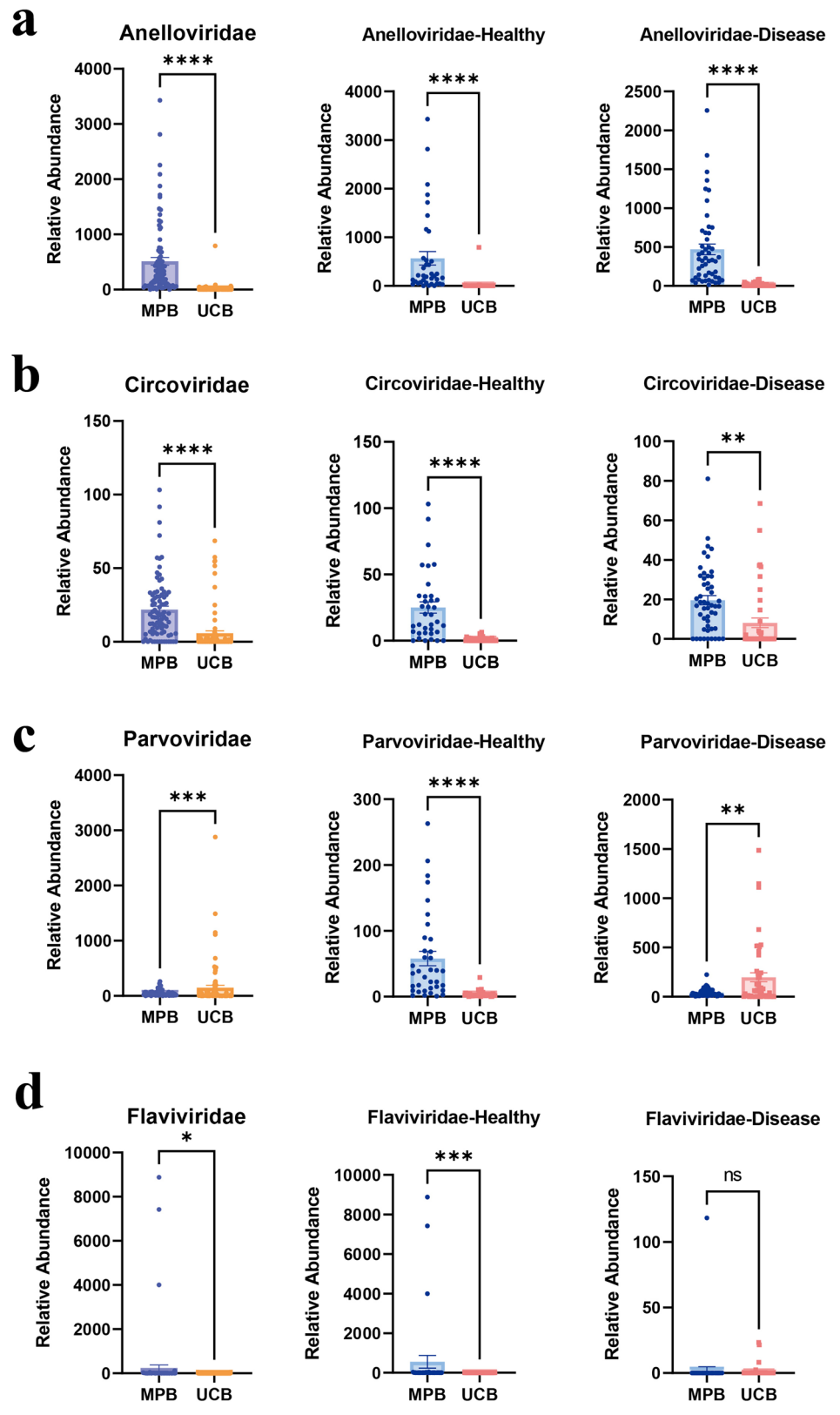
Circoviridae also displayed MPB enrichment in both subgroups, with a stronger effect in healthy ($p < 0.0001$) than diseased ($p < 0.01$) pairs. *Parvoviridae* exhibited a striking reversal: in healthy pairs, it was more abundant in MPB ($p < 0.0001$), but in diseased pairs, UCB abundance was significantly higher ($p < 0.01$). *Flaviviridae* showed MPB enrichment overall ($p < 0.05$) and in the healthy group ($p < 0.001$), but no significant difference was found in diseased pairs ($p > 0.05$). These results suggest that maternal health status may modulate placental barrier function, leading to increased UCB abundance of *Circoviridae* and *Parvoviridae* under disease conditions, in contrast to the consistently MPB-enriched *Anelloviridae* (Fig. 5a–d).

Anelloviruses found in the study

Anelloviridae are small, nonenveloped viruses with circular single-stranded negative-sense DNA genomes ranging from 1.6–3.9 kb. Viral particles exhibit icosahedral capsid symmetry, with diameters of 18–30 nm, and lack a lipid envelope²¹. To date, three genera within this family are known to infect humans: *Alphatorquevirus* (including torque teno virus, TTV), *Betatorquevirus* (including torque teno mini virus, TTMV), and *Gammatorquevirus* (including torque teno midi virus, TTMV). These viruses are ubiquitously detected in human plasma and represent a core component of the blood virome in healthy individuals⁶. A hallmark genomic feature of this family is the presence of a single ORF1, which encodes the capsid protein containing a conserved jelly-roll fold. Because ORF1 sequences exhibit both cross-genus conservation and genus-level specificity, and because their features are directly linked to viral taxonomy, ORF1 has become the principal genetic marker for phylogenetic analyses of *Anelloviridae*.

Anelloviridae are widely distributed in the human population, with an estimated adult infection rate of ~90%. They can establish persistent colonization in multiple organs such as the liver and kidney, as well as in body fluids including blood and urine. Within the blood virome of

Fig. 5 | Differential abundance analysis of four viral families in maternal and umbilical cord blood. a–d Abundance differences of *Anelloviridae*, *Circoviridae*, *Parvoviridae*, and *Flaviviridae* in MPB and UCB are shown. Based on 87 maternal–infant paired samples (excluding Library 88, which lacked a UCB pair, further divided into healthy subgroups $n = 37$ and disease subgroups $n = 50$), the raw read counts were normalized and analyzed using GraphPad Prism 9.5 software with the Wilcoxon signed-rank test to assess the differences between groups.



healthy individuals, *Anelloviridae* account for >70% of the total viral community, making them one of the most abundant viral groups in humans²². Large-scale viromic studies have revealed their extraordinary genetic diversity, frequent recombination events, and worldwide distribution, with hundreds of distinct lineages detectable even within healthy individuals²³. Importantly, both the viral load and diversity of

Anelloviridae are closely associated with host immune function. In immunocompromised populations, such as organ transplant recipients and HIV-1-infected patients, viral levels are typically elevated. This observation suggests that *Anelloviridae*, particularly TTV, may serve as surrogate markers of immune competence and could be useful for monitoring maternal immune status during pregnancy^{24,25}.

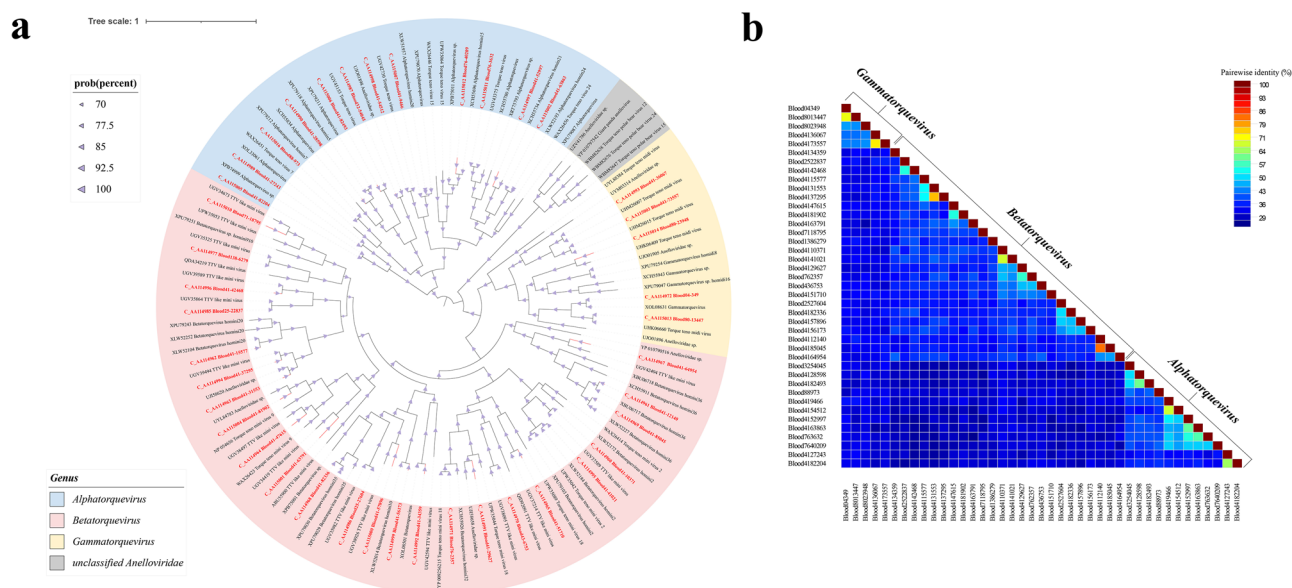


Fig. 6 | Phylogenetic relationship analysis of Anelloviridae. a Phylogenetic tree constructed based on the amino acid sequences of ORF1 and visualized with iTOL. Names in red indicate sequences obtained in this study. The scale bar represents the number of amino acid substitutions per site. **b** Whole-genome sequence similarity

analysis of Anelloviruses assembled from individual libraries. The analysis was performed on near-complete genomes derived from each specific library, illustrating the genetic relationships and clear demarcations among the genera *Alphatorquevirus*, *Betatorquevirus*, and *Gammatorquevirus*.

Based on metagenomic data from MPB and UCB, we analyzed the distribution and vertical transmission potential of *Anelloviridae*. Assembly recovered 41 complete ORF1 sequences, and their taxonomic assignments revealed marked differences across genera and sample sources. At the genus level, 12 sequences clustered within *Alphatorquevirus*, while 29 belonged to *Betatorquevirus*/*Gammatorquevirus*. With respect to sample origin, only one ORF1 sequence was derived from UCB, whereas the remaining 40 sequences originated from MPB. This result confirms the strong enrichment of *Anelloviridae* within maternal circulation and reveals a characteristic composition, with *Betatorquevirus*/*Gammatorquevirus* accounting for 70.7% and *Alphatorquevirus* representing 29.3%. This pattern is consistent with Timmerman et al., who observed that *Betatorquevirus*/*Gammatorquevirus* became dominant in maternal serum and breast milk 6 months postpartum, suggesting that MPB at delivery may already be shifting toward dominance by *Betatorquevirus*/*Gammatorquevirus*²⁶.

From 175 MPB and UCB libraries, a total of 41 Anelloviruses were assembled, including 11 complete circular genomes. Genome sizes ranged from 1920 to 2939 bp, with representative circular structures illustrated in Supplementary Fig. 8. These genomes exhibited a conserved organization of ORF1, ORF2, and ORF3. BLASTx analysis showed that the amino acid sequences of the 41 viruses shared 67.36%–99.11% identity with previously deposited sequences in GenBank. Considerable genetic heterogeneity was observed, as multiple Anelloviruses detected within the same library were not completely identical. Subsequently, a phylogenetic tree was constructed based on ORF1 amino acid sequences (Fig. 6a). The phylogenetic analysis included 86 publicly available reference sequences to provide taxonomic context (see Supplementary Data 2 for accession numbers). The 41 Anelloviruses clustered into three distinct clades, containing 12, 24, and 5 viruses, respectively. To further investigate genetic relationships and evolutionary divergence among the three genera of *Anelloviridae*, whole-genome similarity analyses were conducted (Fig. 6b). Pairwise alignment of all genomes revealed clear boundaries among *Alphatorquevirus*, *Betatorquevirus*, and *Gammatorquevirus*, with no evidence of cross-genus clustering or overlap. Notably, multiple Anelloviruses from different genera were identified in library Blood41, and their sequence similarities strictly adhered to genus-specific boundaries. This finding highlights the enrichment and considerable genetic diversity of *Anelloviridae* in maternal and umbilical cord blood.

Circoviruses found in the study

Circoviridae, a member of the CRESS (circular Rep-encoding single-stranded) DNA virus family, has attracted considerable attention in virological research. Viruses within this family possess circular, covalently closed single-stranded DNA (ssDNA) genomes, typically ranging from 1.7 to 2.4 kilobases (kb) in length²⁷. The genomes exhibit an ambisense organization, containing two major open reading frames (ORFs): the replication-associated protein (rep) gene and the capsid protein (cap) gene²⁸. This streamlined genomic architecture underlies their ability to efficiently replicate and adapt to diverse hosts. According to the classification criteria established by the International Committee on Taxonomy of Viruses (ICTV), *Circoviridae* is divided into two genera: *Circovirus* and *Cyclovirus*²⁹. Species demarcation within these genera is primarily based on whole-genome nucleotide sequence identity, with sequences sharing <80% identity typically classified as distinct species²⁷.

In this study, six *Circoviridae* sequences were recovered from maternal and umbilical cord blood samples, including five sequences assigned to *Circovirus* and one to *Cyclovirus*. Genome lengths ranged from 657 to 1,969 base pairs (bp), with an average GC content of 43.81%. The observed genus-level distribution aligns with prior knowledge of *Circoviridae* host range and epidemiological characteristics: *Circovirus* members are more commonly reported in mammalian hosts, including humans, whereas *Cyclovirus* demonstrates rapidly expanding genetic diversity and potential for cross-host transmission. However, consistent detection of *Cyclovirus* in defined human populations or under specific exposure conditions remains limited, emphasizing the need for further validation and integration of host exposure history in future analyses^{30,31}.

Phylogenetic analysis based on the Rep protein (Fig. 7a) revealed that Blood41-1808 clustered with a feline-derived cyclovirus (Feline cyclovirus, GenBank accession: YP_009052458), exhibiting 84.21% amino acid identity and supporting its classification within the genus *Cyclovirus*. In contrast, *Circovirus* sequences formed four distinct clades. Blood41-54603 clustered with a parrot-derived circovirus (GenBank accession: WGL41063) with 96.22% amino acid identity, while Blood133-235 was most closely related to the integrated Rep gene of *Cynopterus sphinx* circular DNA virus 6 (GenBank accession: WOE49735), sharing 95.1% sequence identity. Blood50-6147 showed highest similarity to a turtle-derived circovirus (GenBank accession: WRQ56666), with 96.01% amino acid identity.

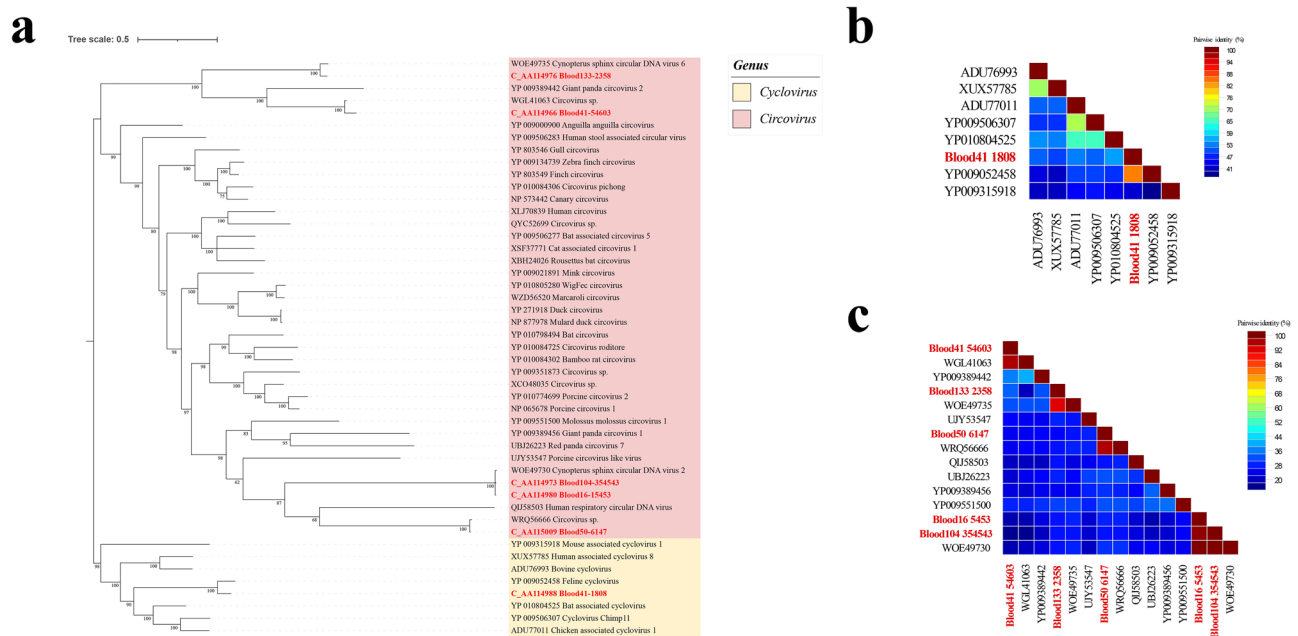


Fig. 7 | Phylogenetic relationship analysis of *Circoviridae*. **a** Phylogenetic tree constructed based on the amino acid sequences of the Rep protein, visualized using iTOL software. Viruses identified in this study are highlighted in red within the tree. The scale bar represents the number of amino acid substitutions per site. **b** Pairwise

comparison analysis of whole-genome sequences of *Cyclovirus* within the phylogenetic tree. One *Cyclovirus* identified in this study is highlighted in red. **c** Pairwise comparison analysis of whole-genome sequences of *Circovirus*. *Circoviruses* identified in this study are highlighted in red.

Notably, Blood16-15453 and Blood104-354543, derived from maternal peripheral blood and umbilical cord blood, respectively, clustered within *Cynopterus sphinx* circular DNA virus 2 (GenBank accession: WOE49730) with 99% amino acid sequence identity, exhibiting highly consistent phylogenetic patterns. This result supports the possibility of mother-to-child transmission for this viral family and suggests potential phylogenetic conservation between paired samples. The phylogenetic analysis was based on an alignment of 41 publicly available reference sequences (accession numbers and details are provided in Supplementary Data 2).

Pairwise whole-genome comparison of the Blood41-1808 strain revealed 40%–82.40% sequence identity with other *Cyclovirus* members (Fig. 7b). Within *Circovirus*, aside from the nearly identical Blood16-15453 and Blood104-354543, pairwise comparisons of Rep proteins from all circoviruses detected in maternal and umbilical cord blood revealed sequence identities as low as 30%, indicating a high level of genetic diversity among *Circovirus* genomes from these samples (Fig. 7c).

Parvoviruses found in the study

Parvoviridae comprises non-enveloped, single-stranded DNA viruses with genomes ranging from 4 to 6 kb, typically encoding two major open reading frames (ORFs), NS1 and VP1, which correspond to non-structural protein 1 and structural protein 1, respectively³². Based on molecular evolution and host specificity, *Parvoviridae* is currently divided into three core subfamilies: *Parvovirinae*, which specifically infects vertebrates; *Densovirinae*, primarily targeting arthropods; and *Hamaparvovirinae*, which infects both invertebrate and vertebrate hosts. This classification framework reflects the remarkable diversity of *Parvoviridae* at both the genomic and host-spectrum levels and provides a critical reference for phylogenetic, epidemiological, and functional studies of the family³³. According to the latest standards, taxonomic classification within *Parvoviridae* relies primarily on NS1 protein sequence homology. Typically, members within the same genus share ≥35%–40% amino acid identity in NS1, and if two strains exhibit >85% NS1 sequence identity with >80% coverage, they can be considered the same species³⁴.

In maternal–neonatal contexts, *Parvoviridae* has attracted considerable attention due to its potential involvement in vertical transmission and interactions with the placental barrier. Recent studies indicate that

parvoviruses, particularly *human parvovirus B19* (B19V), are capable of crossing the placenta to achieve mother-to-child vertical transmission. During maternal infection, B19V can traverse the placental barrier, enter the fetal circulation, and efficiently replicate in cord blood as well as fetal hematopoietic cells³⁵. This process is primarily mediated by the recognition of P antigen (globoside) on placental and fetal cell surfaces, with higher expression levels of P antigen during early pregnancy increasing the risk of transplacental viral passage. Although the placenta generally provides a biological barrier against most pathogens, B19 infection can induce apoptosis and functional impairment of trophoblast cells, thereby weakening the barrier and facilitating maternal–fetal transmission. Furthermore, maternal B19 infection has been associated with a significantly elevated risk of adverse fetal outcomes, including anemia, hydrops fetalis, and miscarriage, underscoring the critical importance of viral monitoring and preventive measures during pregnancy³⁶.

Based on NS1 amino acid sequences, a phylogenetic tree was constructed (Fig. 8a), which included 29 publicly available reference sequences to establish the phylogenetic framework (see Supplementary Data 2 for accession numbers). The tree shows that parvovirus sequences derived from maternal and umbilical cord blood (highlighted in red) clustered with multiple known mammalian parvoviruses. Blood145-18746 grouped with bovine parvovirus 2 (GenBank accession: QYW06847), indicating close evolutionary relatedness to cattle-derived parvoviruses. Blood18-11667, Blood133-165528, Blood19-7574, and Blood140-361998 clustered with ovine copiparvoviruses (GenBank accessions: QYW06845 and QYW06831), suggesting a potential evolutionary link between parvoviruses detected in cord blood and viruses originating from ruminants. Additionally, two sequences, Blood48-3924 and Blood84-19672, were assigned to the subfamily *Densovirinae* and clustered closely with two bat-derived viruses (GenBank accessions: XBS25702 and XBS26080), implying that bats may serve as hosts or reservoirs and potentially contribute to transmission to humans, including via maternal and umbilical cord blood.

Flaviviruses found in the study

Flaviviridae is an important family of zoonotic RNA viruses, with a single-stranded positive-sense RNA genome of ~9–13 kb, typically containing a single open reading frame (ORF). This ORF encodes a polyprotein that is

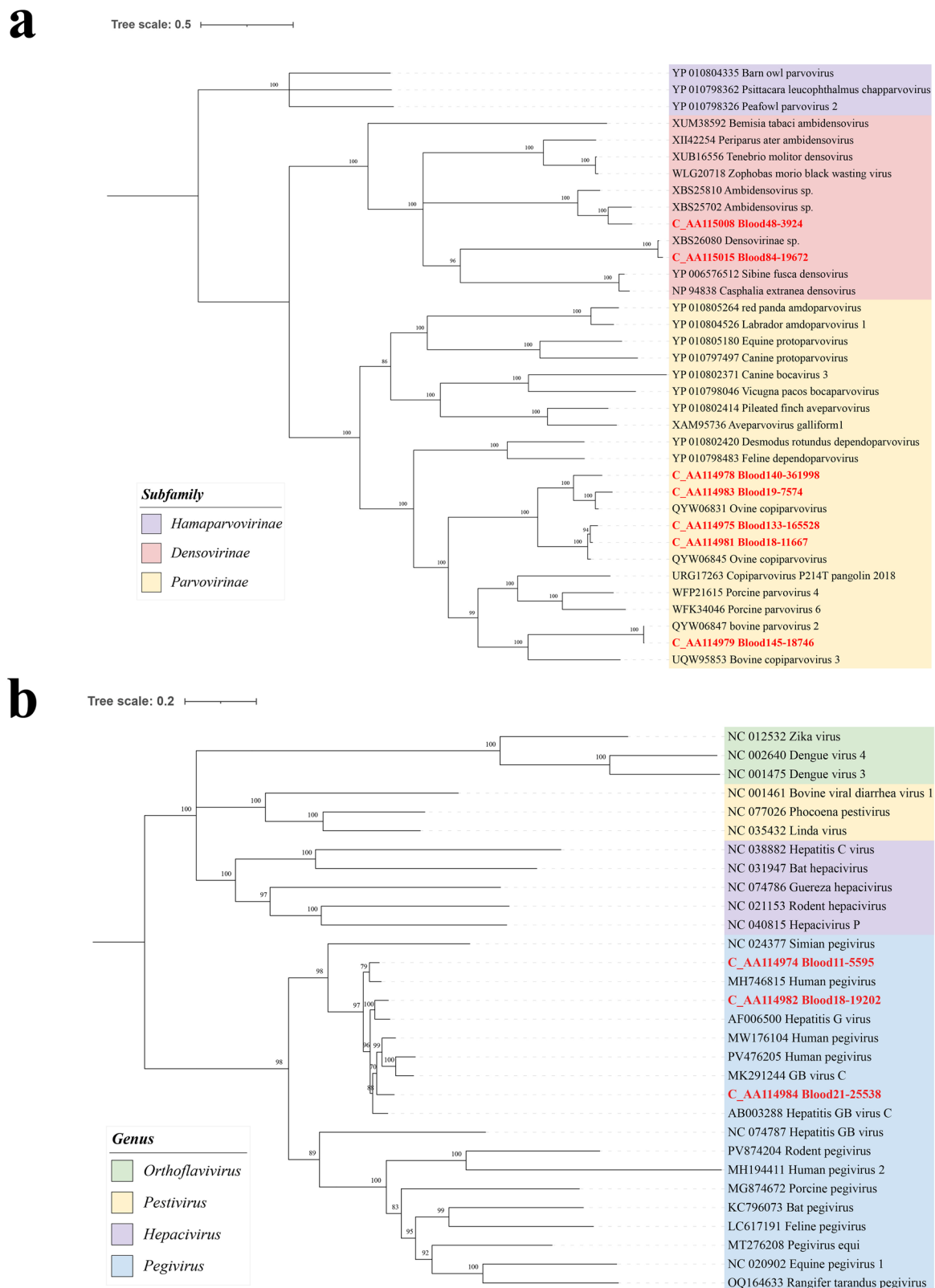


Fig. 8 | Phylogenetic relationship analysis of Parvoviridae and Flaviviridae.

a Phylogenetic tree constructed based on the amino acid sequences of the NS1 protein of parvoviruses, with sequences obtained in this study highlighted in red.

b Phylogenetic tree constructed based on the nucleotide sequences of the RNA-dependent RNA polymerase (RdRp) region of flaviviruses, with viruses identified in this study highlighted in red.

cleaved by viral and host proteases to generate structural proteins (C, prM/M, E) and nonstructural proteins (NS1–NS5). Among these, NS5 serves as the core component of the viral replication complex, exhibiting both methyltransferase and RNA-dependent RNA polymerase activities. It is

highly conserved and plays a crucial role in viral replication and host adaptation^{37,38}.

According to the latest classification by the International Committee on Taxonomy of Viruses (ICTV), the family *Flaviviridae* is divided into four

genera: *Orthoflavivirus*, *Hepacivirus*, *Pegivirus*, and *Pestivirus*. *Orthoflavivirus* primarily comprises arthropod-borne viruses, including dengue virus, yellow fever virus, West Nile virus, Japanese encephalitis virus, and Zika virus, which are mainly transmitted by mosquitoes and ticks, infect humans as well as a wide range of wild animals, and constitute a major global public health threat³⁹. *Hepacivirus* is dominated by hepatitis C virus, which primarily infects the human liver and can lead to chronic hepatitis and hepatocellular carcinoma. *Pegivirus* infects humans and other mammals, typically causing persistent infections with relatively low pathogenicity. *Pestivirus* mainly infects ruminants, causing various animal diseases with significant implications for the livestock industry⁴⁰.

Recent studies investigating mother-to-child transmission mechanisms of *Flaviviridae* have increased, particularly regarding Zika virus (ZIKV), which can cross the placental barrier, leading to fetal infection and severe congenital defects⁴¹. This highlights the ability of flaviviruses to traverse host barriers and underscores the significance of vertical transmission in viral epidemiology. In the present study, three *Pegivirus* sequences were assembled from maternal peripheral blood samples, while no complete flavivirus sequences were recovered from umbilical cord blood. Although *Pegivirus* typically establishes persistent infections, its pathogenicity remains largely unclear. Regarding vertical transmission, a retrospective study in Brazil reported that 25% of HIV-infected pregnant women were positive for human *Pegivirus* (HPgV), with a vertical transmission rate of 31% to neonates⁴². However, other reports indicate that even in cases of high maternal viremia, vertical transmission may not occur; for example, one mother displayed high HPgV levels during two pregnancies, and while placental tissues tested positive, both chorionic villi and umbilical cord blood were negative⁴³. These observations suggest that the vertical transmission mechanisms of *Pegivirus* require further investigation.

Phylogenetic analysis based on the nucleotide sequences of the RNA-dependent RNA polymerase (RdRp) region of *Flaviviridae* (Fig. 8b) was conducted using 27 publicly available reference sequences to establish the phylogenetic context (see Supplementary Data 2 for accession numbers). The analysis revealed that the three *Pegivirus* sequences assembled exclusively from maternal peripheral blood (highlighted in red) formed a distinct cluster within the genus *Pegivirus*, grouping with known human *pegivirus* reference sequences (MH746815 Human *pegivirus*, AF006500 Hepatitis G virus, etc.) in an independent branch. Notably, no complete *Flaviviridae* genomes were assembled from umbilical cord blood samples. BLASTn analysis indicated nucleotide sequence identities exceeding 90%, suggesting a close evolutionary relationship with human *pegiviruses* and providing a robust phylogenetic framework for further studies on the vertical transmission potential and evolutionary adaptation of *Pegivirus* in the maternal–fetal context.

Discussion

Utilizing high-throughput metagenomic sequencing, this study systematically profiled the viromes of 433 maternal peripheral blood and 426 umbilical cord blood samples, offering the first large-scale and comprehensive characterization of viral communities across the maternal–neonatal axis. We delineated virome diversity patterns and their associations with maternal health status, confirming the presence of complex viral assemblages in cord blood and demonstrating that their composition is influenced by both sample origin and maternal health status. Critically, the correlated viral distribution patterns observed in some mother–neonate pairs offer new perspectives for understanding the existence and dissemination characteristics of viruses across the placental interface.

Analysis of the virome composition revealed that bacteriophages, including *Siphoviridae*, *Myoviridae*, and *Podoviridae*, dominated both maternal and neonatal viral communities, consistent with previous human blood virome studies^{44,45}. These phages likely originate from gut or other microbial reservoirs and may enter systemic circulation via mucosal barriers, reflecting immune activity rather than active infection⁴⁶. Notably, our data also showed a relative increase in bacteriophage abundance under maternal disease conditions, in both maternal and cord blood samples.

While this trend may potentially reflect underlying microbial dysbiosis or altered host immunity, recent reports have speculated that phage dynamics could serve as a surrogate marker for disease risk or severity. However, the mechanisms driving this increase remain to be elucidated, and further studies are warranted to assess the reliability and specificity of phage-based biomarkers in perinatal contexts⁴⁷. A notable observation was the relative enrichment of sequences derived from *Human Endogenous Retroviruses* (HERVs) in umbilical cord blood. This trend may hold potential biological relevance⁴⁸. It should be noted, however, that due to inherent technical limitations of the analytical approach, the current data cannot determine whether these detected sequences are transcriptionally active. Furthermore, the majority of *HERV* elements are thought to have accumulated mutations that impair their coding capacity; thus, the detection of these sequences alone does not support the inference that intact viral particles are being produced. As remnants of ancient retroviral integration events, *HERV* sequences constitute ~8% of the human genome⁴⁹. Some studies have suggested that they may participate in specific epigenetically regulated processes during embryonic development. Evidence also indicates that certain *HERV* families, such as *HERV-W*, may contribute—possibly through their expressed products—to processes including trophoblast fusion and syncytiotrophoblast formation during placental development^{50,51}. In this context, the observed enrichment of *HERV*-derived sequences in cord blood in our study provides a basis for further investigating the potential role of these endogenous elements in the dynamic regulation of the fetal–placental interface⁵². Determining whether these *HERV* loci are transcriptionally active and elucidating their potential biological roles remain to be investigated in future studies, which should constitute important next steps. Specifically, transcriptomic approaches such as RNA-seq will be critical for this purpose.

A central observation of this work is the pronounced effect of maternal health status on the maternal–neonatal virome. Diseased mothers exhibited reduced α -diversity (Shannon and Simpson indices) in peripheral blood, likely indicative of immune dysregulation that permits dominance of certain viruses and suppresses others, thereby homogenizing the viral community. Notably, cord blood from neonates of diseased mothers displayed higher viral diversity than their maternal counterparts and sometimes even exceeded that of healthy neonate–mother pairs. This suggests a more permissive intrauterine environment under maternal disease, potentially facilitating crossing of diverse viruses across the placenta. β -diversity analysis (PCoA) clearly distinguished viral communities between health and disease groups, reinforcing maternal condition as a key determinant of virome structure. The analysis of Upset plots indicated a greater number of viral species unique to diseased mother–neonate pairs (15 species) compared to those unique to healthy pairs (1 species). STAMP analysis identified several viral families and species enriched under disease conditions, suggesting that maternal disease could potentially affect placental selectivity and might contribute to vertical transmission, thereby possibly influencing the assembly of the neonatal virome⁵³. Notably, the BeAn 58058 virus (family *Poxviridae*) has been recurrently identified across distinct human anatomical compartments—demonstrating remarkable predominance in the pulmonary virome of COPD patients (relative abundance: 90–94%)⁵⁴ and exhibiting comparable prevalence in lacrimal sac specimens from PANDO cases⁵⁵. These consistent observations provide compelling evidence for its establishment within the human microbiome. Within this established context, the viral enrichment we observed specifically in healthy mother–neonate dyads delineates a previously unrecognized ecological configuration at the maternal–fetal interface. We hypothesize that while the BeAn 58058 virus may colonize multiple human ecological niches, its abundance dynamics and phenotypic expression appear to be tightly regulated by both local microenvironmental conditions and host systemic immune status. In localized pathological milieus—such as the COPD-affected lung or obstructed lacrimal sac—specific microenvironmental characteristics may facilitate its aberrant proliferation, whereas its relative enrichment in the systemic circulation might reflect a state of balanced coexistence under conditions of immune homeostasis. The precise

mechanisms underlying these observations—potentially involving virus–host interactions and specific immunomodulatory pathways—warrant further investigation.

Substantial disparities in viral abundance were noted between maternal and umbilical cord blood, especially among *Anelloviridae*, *Circoviridae*, *Parvoviridae*, and *Flaviviridae*. *Anelloviridae* were far more abundant in maternal blood, consistent with limited vertical transmission and suggesting that mothers serve more as reservoirs than direct transmitters^{23,56}. This underscores the importance of maternal immunity and the perinatal environment in early virome establishment⁵⁷. Notably, the disease group in our study showed a modest reduction in the abundance of *Anelloviridae*. Although viral loads of this family typically increase in states of systemic immunosuppression, its replication dynamics may be influenced by the local immune milieu and viral competition⁵⁸. The specific inflammatory or immune-activated state associated with maternal disease in our cohort may have partially compromised the persistence of *Anelloviridae*, thereby contributing to the observed decrease in abundance. Alternatively, the expansion of other viral families, such as *Parvoviridae*, under disease conditions could also contribute to the relative reduction in *Anelloviridae* abundance at the community level. Importantly, despite these fluctuations in viral abundance within the maternal circulation, the consistently observed disparity between MPB and UCB underscores that the placental barrier may effectively restrict the mother-to-infant transmission of *Anelloviridae* under both physiological and pathological conditions⁵⁹. The minimal presence of Anelloviruses in umbilical cord blood aligns with longitudinal human and nonhuman primate studies, supporting that neonatal virome assembly occurs largely after birth via feeding, colonization, and environmental exposure^{60–62}. Circovirus sequences displaying high homology (>99% identity) were detected in paired maternal–umbilical samples, offering compelling evidence supporting transplacental passage. Similar phenomena have been reported in animal models, with porcine circoviruses crossing the placenta and maintaining high sequence conservation^{63,64}. Although human and porcine placentas exhibit anatomical differences⁶⁵, the high sequence identity identified here suggests that human *Circoviridae* may cross the placental barrier, highlighting the need to explore viral tropism and immune evasion within the human placenta^{66,67}. In addition to detecting highly homologous circovirus sequences suggestive of potential transplacental transmission, this study identified a sequence (Blood41-54603) exhibiting 96.22% amino acid identity with the Rep protein of a parrot-derived circovirus strain. This finding offers new perspectives for understanding viral transmission mechanisms: it may reflect cross-species transmission events or indicate functional conservation of the Rep protein during evolution. With continued advances in research on human-associated circoviruses and the ongoing discovery of novel sequences, it is anticipated that human circovirus variants showing even higher similarity to the sequences identified in this study may be discovered in the future. Collectively, these results suggest the possible existence of a previously underrecognized community of circoviruses within the human blood virome, whose diversity appears to extend beyond current understanding, warranting further investigation into their transmission routes and adaptation mechanisms. *Parvoviridae* demonstrated a more complex distribution pattern, with significantly higher abundance in the umbilical cord blood of diseased mothers. This observation supports the possibility that maternal disease status may alter placental permeability and facilitate fetal replication—a pattern consistent with the well-documented behavior of human parvovirus B19. On the other hand, their detection in maternal peripheral blood from healthy individuals may reflect only low-level persistent or latent infection or asymptomatic carriage, as widely documented for human parvovirus B19 in healthy populations. This presence does not necessarily indicate active infection, but rather may represent a baseline viral component that contributes to overall viral community diversity^{68,69}. In contrast, although *Flaviviridae* (*Pegivirus*) sequences were detected in maternal peripheral blood, no complete genomes were assembled in umbilical cord blood, implying constrained transmission due to placental regulation or complex viral dynamics. Notably, all pegivirus sequences identified in our cohort were derived from

healthy pregnant women without HIV co-infection or apparent immunosuppression. This finding provides a unique perspective for understanding the ecological behavior of HPgV-1 in immunocompetent hosts and lends support to the emerging view of its potential role as a commensal member of the blood virome. This observation does not contradict its established function as a biomarker in immunocompromised individuals, but rather collectively reveals the context-dependent nature of virus–host interactions: the ecological behavior of the same virus may vary considerably depending on the host's immune status.

These findings suggest that maternal–fetal viral transmission varies substantially across viral families and appears to be influenced by a combination of environmental factors, placental selectivity, and maternal health status^{70–73}. The marked enrichment of *Anelloviridae*, *Circoviridae*, and *Flaviviridae* in MPB implies that the placenta serves as an effective filter limiting transmission—a phenomenon consistent with clinical observations that many orthoflaviviruses (e.g. *Zika virus*) are transmitted vertically only at low rates, likely due to placental immune defense⁷⁴. Phylogenetic analysis further substantiates this restricted transmission pattern. For *Anelloviridae*, the only UCB-derived sequence had no complete sequence assembled from its matched MPB sample and did not cluster with any MPB-derived sequences in the ORF1-based phylogeny. In parallel, no complete *Flaviviridae* genomes were recovered from any UCB samples. Together, these results underscore the efficiency of the placental barrier in limiting transmission of these viral families. Notably, an increased abundance of *Parvoviridae* was specifically observed in UCB from diseased mothers, raising important questions about the underlying transmission mechanism. Our phylogenetic analysis, however, revealed a more complex picture: although seven sequences were assembled (four from MPB and three from UCB), none exhibited direct mother–neonate pairing. One plausible interpretation is that maternal disease status may alter placental permeability, potentially facilitating the entry of environmental or reservoir-derived parvoviruses into the fetal circulation, rather than enabling direct vertical transmission of homologous maternal strains. This could suggest a distinct mode of viral exposure in utero, where the diseased maternal environment permits the passage of parvoviruses that are phylogenetically unrelated to those circulating in maternal blood. In contrast, *Circoviridae* provided more suggestive evidence for potential vertical transmission. Sequences from one matched MPB–UCB pair co-clustered in Rep-based phylogeny with high (99%) amino acid sequence identity, providing phylogenetic support for transplacental passage. These findings indicate that *Parvoviridae* and *Circoviridae* may occupy distinct ecological niches in vertical transmission, warranting further mechanistic investigation. Therefore, risk assessment of vertical viral transmission should account for taxonomic differences and avoid uniform interpretation across viral families.

However, this study has several limitations worthy of consideration. Although we employed strategies such as random primers and optimized PCR cycling conditions, PCR-based metagenomic approaches remain susceptible to amplification bias (including PCR jackpotting effects), which may affect the detection of low-abundance viruses²¹. To mitigate these potential biases, we implemented multiple safeguards in our experimental design: the use of random primers during reverse transcription and library preparation helped reduce sequence-specific bias, while our sample pooling strategy combining 4 to 7 individual samples served to average out amplification variations. Additionally, we employed a moderate 18 PCR cycles during library construction to balance material yield and amplification artifacts. Furthermore, our primary focus on comparative analyses between groups rather than absolute quantification enhances the robustness of our findings against systematic technical variations. While these measures strengthen the validity of our comparative results, we acknowledge that some degree of amplification bias may persist. Beyond methodological considerations, the study design itself presents inherent constraints. As a cross-sectional analysis, this work reveals associations between maternal health status and the maternal–neonatal virome but cannot establish causal relationships or fully delineate the dynamics of viral transmission³. On the other hand, the generally low viral load in blood samples, combined with the

inherent limitations of short-read sequencing technology in genome assembly, may somewhat restrict the detection capability for low-abundance viruses and impede fine-resolution analysis of viral transmission routes²⁷. Although phylogenetic analysis provides preliminary evidence supporting the possibility of vertical viral transmission, the precise transplacental pathways and underlying mechanisms still require further validation through approaches such as ex vivo placental models or longitudinal sampling throughout pregnancy⁷⁵.

In summary, this large-scale metagenomic study reveals that both maternal peripheral blood and umbilical cord blood host complex viral communities, whose composition is influenced by the selective permeability of the placental barrier and maternal health status. Notably, we demonstrate that maternal disease alters viral profiles in both compartments and may enhance viral sharing and cord blood virome diversity^{3,76}. Together, these findings establish a foundational framework for elucidating the ecological principles governing maternal–neonatal virome interactions and their impact on perinatal health. Our work thus provides a basis for potential strategies aimed at monitoring or modulating the maternal virome to improve infant outcomes, while also guiding future research into the evolutionary dynamics and functional mechanisms of viral assembly in early life.

Methods

Subjects and clinical data

To investigate the virome composition of maternal peripheral blood (MPB) and umbilical cord blood (UCB) and its potential association with maternal health status, we recruited parturients from the Affiliated Wujin Hospital of Jiangsu University. Initially, 434 maternal peripheral blood (MPB) samples and 426 paired umbilical cord blood (UCB) samples were collected. Following quality control assessment, one MPB sample was excluded due to quality issues. Ultimately, a total of 433 eligible MPB samples (including 7 without matched UCB specimens) and 426 eligible paired UCB samples were obtained for subsequent analysis. Based on two primary factors—sample type (Maternal Peripheral Blood, MPB, or Umbilical Cord Blood, UCB) and maternal health status (Healthy or Disease)—the samples were categorized into 175 subgroups. This classification defined four main cohorts: MPB-Healthy (37 subgroups), MPB-Disease (51 subgroups), UCB-Healthy (37 subgroups), and UCB-Disease (50 subgroups). Each subgroup corresponds to a single sequencing library and was constructed by pooling 4–7 individual samples that were identical in both sample type and maternal health status. Among these, subgroups 1–87 and 89–175 consisted of maternal peripheral blood and their paired umbilical cord blood samples, respectively, whereas subgroup 88 contained only maternal peripheral blood owing to the absence of a paired umbilical cord blood sample. Detailed metadata for each sequencing library are provided in Supplementary Data 1. All specimens were obtained through routine clinical procedures, anonymized prior to analysis, and exempted from individual informed consent. The study protocol was reviewed and approved by the Ethics Committee of the Affiliated Wujin Hospital of Jiangsu University (Approval Number: 2023-SR-103).

Sample collection, processing, and sequencing

Umbilical cord blood was collected immediately after delivery in accordance with international NetCord-FACT guidelines. Following neonatal cord clamping, trained obstetric staff performed aseptic collection using two clinical methods depending on the delivery process⁷⁷: (i) In utero collection: before placental expulsion, a 10–15 cm segment near the placental end of the cord was disinfected. A sterile collection bag preloaded with CPDA-1 anticoagulant was then used to puncture the umbilical vein, and blood was allowed to drain by gravity. (ii) Ex utero collection: after placental delivery, the placenta was transferred to a sterile workbench, suspended with the fetal surface facing downward, and the umbilical vein was punctured for gravity drainage using the same method. Throughout both procedures, strict aseptic technique was maintained, and thorough mixing of blood with anticoagulant was ensured. The interval between cord clamping and sample

collection was maintained within 5 min to prevent coagulation. Collected samples were labeled, stored in cold-chain boxes at 4°C, and delivered to the laboratory within 6 h for downstream processing⁷⁸. Whole blood was centrifuged (10 min, 15,000 × g) to collect the supernatant, which was aliquoted into 1.5 mL sterile tubes. For each subgroup, an equal volume of ~100 µL of supernatant from each constituent individual sample was pooled to create a single sequencing library. This pooling strategy was essential to increase viral nucleic acid yield for robust metagenomic detection from low-biomass, low-viral-load blood samples. The pooled supernatant was then filtered through a 0.45 µm membrane (Millipore, Darmstadt, Germany) to remove eukaryotic and bacterial cell-sized particles⁷⁹. The filtrates enriched in viral particles were incubated at 37 °C with DNase (Ambion) and RNase (Fermentas) for 90 min to digest unprotected host and microbial nucleic acids⁸⁰. Following enzymatic digestion, total nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN), which recovers both viral RNA and DNA, according to the manufacturer's instructions. No DNase treatment was applied post-extraction. Reverse transcription was carried out with SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) and random hexamer primers to generate first-strand cDNA, followed by second-strand synthesis with the Klenow fragment polymerase (New England BioLabs), yielding double-stranded DNA (dsDNA) templates. Finally, 175 sequencing libraries were constructed using the Nextera XT DNA Library Preparation Kit (Illumina) with 18 cycles of PCR amplification and subjected to 150 bp paired-end sequencing on the Illumina NovaSeq 6000 platform^{81,82}.

Bioinformatics analysis

Raw 150 bp paired-end reads were first processed using Illumina-provided software. The data were processed on an internal analysis pipeline running on a 32-node Linux cluster. Adapter sequences and low-quality bases were trimmed with Trim Galore v0.6.7 (<https://github.com/FelixKrueger/TrimGalore>) using the parameters: trim_galore -q 25 -j 40 --phred33 --clip_R1 10 --clip_R2 10 --length 35 --stringency 3 --paired. PCR duplicates were removed based on the internal analysis pipeline. Specifically, reads with identical bases from positions 5 to 55 were identified as duplicates, with only one copy randomly retained and clonal reads discarded. Subsequently, Host-derived nucleic acids were removed by aligning reads against the human reference genome (Homo sapiens, GCF_000001405.40) using Bowtie2 v2.4.5^{83,84}. All host-mapped reads were discarded. De novo assembly of host-filtered reads was conducted independently for each library with MEGAHIT v1.2.9^{85,86} using default parameters. To minimize false negatives, unmapped reads and contigs shorter than 500 bp were further assembled with the de novo assembler in Geneious Prime (<https://www.geneious.com>). After reassembly, only contigs ≥1500 bp were retained, and sequences containing frameshift mutations were manually inspected and removed⁸⁷. Following sample pooling at the subgroup level for library construction, all bioinformatic processes, including de novo assembly, were performed on a per-library basis. The high-quality contigs (≥1500 bp) obtained thus preserved their library-specific identity, ensuring traceability throughout the downstream analyses.

Viral genome identification and annotation

Viral sequences derived from MPB and UCB libraries were characterized through a multi-step pipeline. First, all assembled contigs were queried against the viral subset of the non-redundant (nr) protein database (downloaded on March 30, 2025) using BLASTx in DIAMOND v2.1.12^{88,89} with an *E*-value threshold of <10⁻⁵. To increase annotation accuracy and exclude false positives, candidate sequences were subsequently compared against the complete nr database. The resulting contigs were then imported into Geneious Prime® 2024.0.5 for refinement and inspection⁹⁰. Potential vector-derived contaminants were removed using VecScreen (<https://www.ncbi.nlm.nih.gov/tools/vecscreen>), an NCBI BLASTn-based tool. Open reading frames (ORFs) were predicted in Geneious Prime with parameters set to a minimum length of 100 bp and ATG as the start codon. Conserved protein domains were annotated using the Conserved Domain Database

(CDD) v3.21 (E-value < 10^{-5})⁹¹, which integrates curated NCBI models and entries from Pfam, SMART, COG, PRK, and TIGRFAM⁹². Contigs containing viral marker genes representative of major taxonomic groups were retained for downstream phylogenetic inference.

Phylogenetic analysis

Phylogenetic relationships among viral groups were inferred using predicted viral amino acid or nucleotide sequences, the closest homologs identified by BLASTx/ BLASTn searches against GenBank, and representative reference sequences retrieved from the GenBank non-redundant protein database using targeted queries: “ORF1 AND *Anelloviridae*” for *Anelloviridae*, “Rep AND *Circoviridae*” for *Circoviridae*, “NS1 AND *Parvoviridae*” for *Parvoviridae*, and “RdRp AND *Flaviviridae*” for *Flaviviridae*. During the selection process, we prioritized complete and representative sequences that encompassed the diversity of major subfamilies or genera within each viral family. A total of 86, 41, 29, and 27 reference sequences were included for each family, respectively, with detailed accession numbers provided in Supplementary Data 2. Multiple sequence alignments were performed in MEGA v11.0.13⁹³ using MUSCLE with default settings. Sites with >50% missing data were excluded. Bayesian inference (BI) phylogenetic trees were generated in MrBayes v3.2.7⁹⁴, with the substitution model set as prset aamodelpremixed. Two independent Markov chain Monte Carlo (MCMC) runs were conducted until the average standard deviation of split frequencies fell below 0.01. The first 25% of sampled trees were discarded as burn-in. Maximum likelihood (ML) trees were reconstructed in MEGA v11.0.13⁹³ to validate BI topologies. Final tree visualization was performed using iTOL⁹⁵ and graphically edited in Adobe Illustrator 2025 (<https://www.adobe.com/products/illustrator/free-trial-download.html>)⁹⁶.

Statistical analysis

Viral composition across 175 libraries was analyzed with MEGAN v6.22.2⁹⁷, which applies the lowest common ancestor (LCA) algorithm to assign reads to taxonomic groups and generate hierarchical summaries⁹⁸. Furthermore, the “compare” function in MEGAN was utilized to systematically compare viral community structures across different libraries. To evaluate the coverage of viral diversity by sequencing depth, rarefaction curves were generated using the built-in rarefaction analysis tool in MEGAN. The hierarchical summaries and comparative community data obtained from these analyses were subsequently exported and served as the foundation for further statistical analysis. Statistical analyses and visualizations were performed in R v4.4.2 and ChiPlot (<https://www.chiplot.online/>). Viral α -diversity (Shannon and Simpson indices) was calculated using the R packages vegan v2.6.6 and picante v1.8.2. Intergroup differences were tested with the Mann–Whitney *U* test. β -diversity was estimated using Bray–Curtis distance matrices and visualized with principal coordinate analysis (PCoA). Intergroup variation in community structure was assessed with PERMANOVA (Adonis) based on permutation tests. Statistical significance was defined as $P < 0.05$. Differential abundance analysis at the family and species levels was performed using STAMP v2.1.3¹⁹. Specifically, two-sided Welch’s *t*-test was used to compare viral abundance between healthy and diseased groups, with confidence intervals calculated by the DP: Welch’s inverted method. The Benjamini–Hochberg false discovery rate (FDR) correction was applied, and an FDR-corrected *p*-value < 0.05 was considered statistically significant. Community composition and abundance were visualized with heatmaps, stacked bar charts, pie charts, and UpSet plots generated in R and ChiPlot. Family-level abundance differences were further assessed using GraphPad Prism v9.5, with $P < 0.05$ considered significant.

Quality control

All procedures were performed in a BSL-2 cabinet with aerosol-resistant filter tips to avoid nucleic acid contamination. All tubes and consumables were certified nuclease-free. DEPC-treated water (Sangon Biotech) was used throughout nucleic acid handling.

Data availability

The datasets generated in this study are available at the National Genomics Data Center (NGDC, <https://ngdc.cncb.ac.cn>) under BioProject accession number PRJCA045326. Details are provided in Supplementary Data 1. All sequences have also been deposited in NGDC without restrictions, with accession numbers listed in Supplementary Data 2.

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Author contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; W.Z., L.J., Y.L., H.Y., J.T. and C.Z. contributed to the conception and design; X.S., Y.F., H.X., H.W., J.C., S.H., Y.C., J.X., W.L., J.Z., P.W., Q.S., S.Y., X.W. and Y.L. contributed to the collection and assembly of data; X.S., Y.F., H.X. and H.W. contributed to the data analysis and interpretation.

Competing interests

The authors declare no competing interests.

Additional information

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