Supplementary Information

Supplement to: Gaccioli F, Lager S, de Goffau MC, et al. Fetal inheritance of chromosomally integrated HHV-6 predisposes to preeclampsia in the mother.
Supplementary Results

Detection of viral DNAs in placental samples
We analyzed placentas collected during the POP study from the following pregnancy complications: FGR customized birth weight <5th percentile, n=100), preeclampsia (diagnosed according to the 2013 ACOG Guidelines, n=100), and pre-term deliveries (<37wkGA, n=100). Pregnancies with FGR and preeclampsia were matched to healthy controls (n=198; two controls were used twice) based on the following criteria: mode of delivery (absolute match), maternal BMI, maternal age, gestational age, sample collection time, maternal smoking, and fetal sex. All matched cases and controls were term deliveries (>37wkGA). In total, the study cohort included 498 unique pregnancies and the clinical characteristics of the patients are presented in Supplementary Table 2. No placental DNA sample was positive for Adenovirus, CMV, EBV, HPV-6, HPV-11, HPV-16, HPV-18, HSV-1, or HSV-2. Two placentas were positive for Parvovirus (one FGR and one preeclampsia), one placenta was positive for VZV (one pre-term), and HHV-6 was detected in 9 placental samples.

HHV-6 genomic integration: proof of principle
Contigs assembled from discordant read pairs were analyzed using BLAST and they typically resulted in contigs that were completely human. The reason for this is that most discordant read pairs have one read which is mapped erroneously to the HHV-6B genome due to the very high degree of similarity between some of the repetitive regions of the HHV-6B genome and the various repetitive parts of the human genome. However, in one parent and child pair, the ciHHV-6B integration site was identified at the telomeric side of 4p16.3 (https://www.ncbi.nlm.nih.gov/nucleotide/Z95704.1). In both samples, discordant read pairs that mapped to the 4p16.3 region either perfectly mapped to the 4p16.3 region, extending into the telomeric repetitive sequence of chromosome 4p16.3, or there was a gap close to the telomeric repetitive sequence. The sequence on either side of this gap was identical to the reference human genome. This integration site is identical to a previously described insertion site for ciHHV-6A (https://www.ncbi.nlm.nih.gov/nucleotide/KF366418.1): GATCCTTCCTTTCAGCC-GAGAATATTAGGGTGGGTTAGG.

Analysis of the association in women of non-European ethnicity
The POP study cohort was 93% white European (Table S1). Only 9 of the cases of preeclampsia occurred in women of non-European ancestry. Before exclusion, the odds ratio (95% CI) in the POP study (n=3,847) was 2.52 (1.05 to 6.19) whereas after the exclusion (n=3,847-216=3,631) it was 2.56 (1.06 to 6.32), i.e. the results were virtually identical with or without this exclusion. The ethnicity of the participants was not available for the case control study and the women recruited by the GOPEC consortium were all of white Western European ancestry. However, at this stage it is not possible to rule out population stratification due to the low number of women of non-European ethnicity. Further studies are needed to address this issue.

Analysis of the association with stillbirth and spontaneous preterm birth (sPTB)
The analysis of cord DNAs from the POP study cohort included 8 stillbirth cases. This group was not analysed separately as the numbers were too small to demonstrate the presence or absence of an association. Our analysis included 100 pregnancies with sPTB and there were no iciHHV-6 positive samples in this group (the expected number under the null was one), but the number of sPTB cases is too small to draw any meaningful conclusion. Further large studies are needed to evaluate the association between iciHHV-6 and stillbirth or sPTB.

Meta-analysis of large-scale population studies excluding Japanese populations
After exclusions of the two Japanese studies (Tanaka-Taya 2014 and Miura 2018) from the analysis reported in Figure 2C, the summary proportion of ciHHV-6 derived from the meta-analysis of large-scale population studies was 0.7% (392/58,211). Similarly to the main analysis, this sensitivity analysis demonstrated a two to three fold risk of preeclampsia associated with iciHHV-6 (OR=2.4, 95% CI: 1.4 to 4.3, P=0.0019). Heterogeneity between the remaining studies was also similar to what was observed in the main analysis (I²=89%, 2-sided P=4x10⁴).
### Supplementary Tables

#### Supplementary Table 1. Custom-made qPCR primers and probes.

| Custom-made Taqman assay | Primer/Probe | Sequence (5’ to 3’) | Final concentration |
|--------------------------|-------------|---------------------|---------------------|
| Adenovirus               | Forward     | GCC ACS GTG GGG TTT CTA AAC TT | 600 nM |
|                          | Probe       | [JUN]-TGC ACC AGA CCC GGR CTC AGG TAC TCC GA-[QSY] | 400 nM |
|                          | Reverse     | GCC CCA GTG GKC TTA CAT GCA CAT C | 600 nM |
| CMV                      | Forward     | GCA TGC GCG AGT GTC AGG AC | 600 nM |
|                          | Probe       | [JUN]-TGC GCC GTG TGC TGC TCG ACA-[QSY] | 400 nM |
|                          | Reverse     | GTT ACT TTG AGC GCC ATC TGT TCC T | 600 nM |
| EBV                      | Forward     | CCG GTG TGT TCG TAT ATG GAG | 600 nM |
|                          | Probe       | [JUN]-TGC CCT TGC TAT TCC ACA ATG TCG T-[QSY] | 400 nM |
|                          | Reverse     | GGG AGA CGA CTC AAT GGT GTA | 600 nM |
| HPV-6                    | Forward     | TGG GGT AAT CAA CTG TTT GTT ACT GTG GTA | 400 nM |
|                          | Probe       | [ABY]-GAC ATT ATG TGC ATC CTA AAT TCC AG-[QSY] | 200 nM |
|                          | Reverse     | GCA TGT ACT CTT TAT AAT CAG AAT TGG TGT ATG TG | 400 nM |
| HPV-11                   | Forward     | CTG GGG AAA CCA CTT GTT TGT TAC TGT G | 400 nM |
|                          | Probe       | [JUN]-GAC ACT TGG TAC ATC TGT TGC TAA-[QSY] | 800 nM |
|                          | Reverse     | CCG ATG TAT TCC TTA TAA TCT GAA TTA GTG TAT GTA | 400 nM |
| HPV-16                   | Forward     | TTG TTG GGG TAA CCA ATG TGT TAT TAT TGT T | 400 nM |
|                          | Probe       | [FAM]-GTC ATT ATG TGC CAT ATC TGT TCA ATC ACT[TCA]-[QSY] | 400 nM |
|                          | Reverse     | CCC CCC CAT GTC TGA GTT ACT CCT TAA AG | 400 nM |
| HPV-18                   | Forward     | GCA TAA TCA ATT ATT TGC TAT TGT AGA TAC CAC T | 400 nM |
|                          | Probe       | [VIC]-AAC AAT ATG TGC TCC TAC ACA ATG GCC TCC TGT-[QSY] | 100 nM |
|                          | Reverse     | GCT ATA CTG TTT AAA TTT GTT AGC ATC AT TTT G C | 400 nM |
| HSV-1                    | Forward     | TTC TGC AGC TGG CAC CAC | 600 nM |
|                          | Probe       | [FAM]-CGA TGG CAA CGC GCC CCA ACA TAT GTG TGA C-[QSY] | 300 nM |
|                          | Reverse     | GGA GCG CAT CAA GAC CAC C | 600 nM |
| HSV-2                    | Forward     | TGC GTG GCG TGG TAC TT | 800 nM |
|                          | Probe       | [ABY]-CAA ACA TGG GGT CGA TGG CCT C-[QSY] | 300 nM |
|                          | Reverse     | CCA TCT CGA CCA CCT TCA C | 800 nM |
| Parvovirus               | Forward     | TCC CTG GAA TTA ATG CAG ATG C | 1200 nM |
|                          | Probe       | [FAM]-ACC TCC AAA CCA CCC CAA TTT TGA TCA CA-[QSY] | 400 nM |
|                          | Reverse     | CAC TGC TGC TGA TAC TGG TGT CT | 200 nM |
| VZV                      | Forward     | CAC GTA TTT TCA GTC TCC TTG TTT AAG TG | 940 nM |
|                          | Probe       | [VIC]-TAC CGC TGG AGC GCC CCG-[QSY] | 400 nM |
|                          | Reverse     | TTA GAC GTG GAG TTG ACA TCG TTT | 940 nM |
| HHV-6 U67/68             | Forward     | TTC CGG TAT ATG ACC TCC TGA AGC | 300 nM |
|                          | Probe HHV-6A | [6-FAM]-ACA TTA TAT ATG TGC GAA CTT GAC ACT ACC TCC CG-[QSY] | 250 nM |
|                          | Probe HHV-6B | [VIC]-CAT TAT ATG TGC AAT CGC ATG CTG TCG CCT TCC G-[QSY] | 250 nM |
|                          | Reverse     | GAT GTC TCA CCT CCA AAT CTT TAG AAA T | 300 nM |
| HHV-6 U100               | Forward     | CGA CTT GCC TCA CAA ATA TTG TC | 18µM |
|                          | Probe       | [FAM]-CCA TCG TAA GCG CAT TGT GGC ACT C-[BHQ-1] | 5µM |
|                          | Reverse     | ATG GTG CAT AAT GCG GGA | 18µM |

The Taqman assay HHV-6 U67/68 was designed to discriminate between the HHV-6A and HHV-6B genes. The Taqman assay HHV-6 U100 was designed to detect transcripts coded by the U100 gene of both HHV-6A and HHV-6B, due to the high homology of the 2 sequences (gene ID: 1487972 for HHV-6A and gene ID: 1497092 for HHV-6B).
for HHV-6B). CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HPV-6, HPV-11, HPV-16, HPV-18: Human Papillomavirus types 6, 11, 16 and 18; HSV-1 and HSV-2: Herpes Simplex Viruses types 1 and 2; VZV: Varicella Zoster Virus; HHV-6A: human herpesvirus 6, variant A; HHV-6B: human herpesvirus 6, variant B; ciHHV-6 U67/68: human herpesvirus 6, U67/68 gene; HHV-6 U100: human herpesvirus 6, U100 gene; 6-FAM: 6-carboxyfluorescein reporter dye; QSY: QSY quencher; VIC: VIC reporter dye; BHQ-1: Black Hole Quencher-1 quencher.
Supplementary Table 2. Clinical characteristics of the patients analyzed by qPCR for detection of placental viral DNAs.

|                              | FGR     | Control (FGR) | PE      | Control (PE) | Pre-term |
|------------------------------|---------|---------------|---------|---------------|----------|
| **N**                        | 100     | 100           | 100     | 100           | 100      |
| Maternal BMI (kg/m²)         | 24.9    | (22.7 to 27.8)| 24.7    | (22.6 to 26.6)| 25.7     | (22.7 to 30.1) | 24.1 | (22.6 to 27.5) | 25.0 | (22.8 to 28.5) |
| Maternal age (years)         | 30.9    | (25.9 to 34.1)| 30.6    | (27.4 to 32.7)| 29.7     | (26.5 to 33.6) | 29.9 | (27.4 to 33.9) | 30.8 |
| Gestational age (weeks)      | 40.3    | (39.2 to 41.3)| 40.3    | (39.4 to 41.0)| 39.9     | (38.6 to 41.1) | 40.0 | (39.1 to 40.9) | 34.1 |
| Sample collection time (hours)| 2.2     | (0.3 to 7.7)  | 2.6     | (0.2 to 7.4)  | 4.1      | (0.4 to 9.2)   | 3.3  | (0.3 to 8.1)   | 0.8  |
| Fetal sex: female            | 57 (57%)| 54 (54%)      | 44 (44%)| 47 (47%)      | 42 (42%) |
| Smoking at booking (yes/no)  | 28 (28%)| 18 (18%)      | 10 (10%)| 7 (7%)        | 15 (15%) |
| Age stopped FTE (years)      | 20.0    | (18.0 to 23.0)| 21.0    | (18.0 to 23.0)| 21.0     | (18.0 to 23.0) | 21.0 | (18.0 to 23.0) | 21.0 |
| Maternal height (cm)         | 165     | (160 to 170)  | 165     | (161 to 169)  | 165      | (161 to 169)   | 164  | (160 to 170)   | 164  |
| Deprivation quartile         |         |               |         |               |          |
| 1 (lowest)                   | 28 (28%)| 23 (23%)      | 32 (32%)| 26 (26%)      | 25 (25%) |
| 2                            | 19 (19%)| 17 (17%)      | 21 (21%)| 20 (20%)      | 22 (22%) |
| 3                            | 25 (25%)| 32 (32%)      | 22 (22%)| 25 (25%)      | 31 (31%) |
| 4 (highest)                  | 21 (21%)| 23 (23%)      | 20 (20%)| 22 (22%)      | 19 (19%) |
| Missing                      | 7 (7%)  | 5 (5%)        | 5 (5%)  | 7 (7%)        | 3 (3%)   |
| Ethnicity                    |         |               |         |               |          |
| Non-white                    | 4 (4%)  | 9 (9%)        | 2 (2%)  | 4 (4%)        | 7 (7%)   |
| White                        | 94 (94%)| 91 (91%)      | 97 (97%)| 92 (92%)      | 91 (91%) |
| Missing                      | 2 (2%)  | 0 (0%)        | 1 (1%)  | 4 (4%)        | 2 (2%)   |
| Married                      | 59 (59%)| 73 (73%)      | 69 (69%)| 72 (72%)      | 66 (66%) |
| Any alcohol consumption      | 3 (3%)  | 6 (6%)        | 2 (2%)  | 6 (6%)        | 4 (4%)   |
| Type I or type II diabetes   | 1 (1%)  | 0 (0%)        | 1 (1%)  | 0 (0%)        | 2 (2%)   |
| UTA Doppler mean PI          | 25 (25%)| 7 (7%)        | 20 (20%)| 7 (7%)        | 22 (22%) |
| highest decile               | 5 (5%)  | 1 (1%)        | 2 (2%)  | 3 (3%)        | 7 (7%)   |
| Birth weight (g)             | 2660    | (2429 to 2810)| 3530    | (3325 to 3705)| 3493     | (3182 to 3790) | 3495 | (3263 to 3680) | 2110 |
| Induction of labor           | 37 (37%)| 20 (20%)      | 63 (63%)| 26 (26%)      | 5 (5%)   |
| Mode of delivery             |         |               |         |               |          |
| Vaginal                      | 80 (80%)| 80 (80%)      | 56 (56%)| 56 (56%)      | 56 (56%) |
| Intrapartum Caesarean        | 13 (13%)| 13 (13%)      | 29 (29%)| 29 (29%)      | 5 (5%)   |
| Prelabor Caesarean           | 7 (7%)  | 7 (7%)        | 15 (15%)| 15 (15%)      | 39 (39%) |

Median (interquartile range) or number (%) are given as appropriate. Smoking, maternal age and BMI were recorded at the booking appointment (~12 weeks of gestation) and other maternal characteristics were obtained from the 20 weeks questionnaire. Two women are controls for both an FGR case and a PE case. FGR: fetal
growth restriction, PE: preeclampsia, BMI: body mass index, FTE: full-time education, UtA: uterine artery, PI: pulsatility index.
Supplementary References

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