Figure S1: Study Population Derivation

Maternal enrollments in P1025 by June 21, 2013 (time of P1025 closing to accrual): **N = 3088**

Had a preterm \*\* (≤ 35 weeks gestation) or a term (≥ 37 weeks gestation) delivery: **N = 2623**

Final population in analysis: **N = 308**

Case = 103; Control = 205

Delivered a live-born singleton baby: **N = 2922 \***

* Infants had congenital anomalies associated with polyhydramnios corticosteroids: n = 42
* Women took immunosuppressive medication ≤ 2 weeks before plasma sample collection: n = 18
* Had blood transfusion ≤ 2 weeks before plasma sample collection: n = 5

**N = 1716**

Had at least 2 ml plasma sample collected ≥ 1 week before delivery: **N = 1781**

Sample for secondary objective: N = 1338

**N = 1574**

Women with multiple pregnancies: **N = 137** (kept the most recent pregnancy)

Eligible population for selecting matched cases and controls: **N = 1478 (**Preterm: n = 104; Term: n = 1374)

Women had unknown race (n=89) or reported multiple race including Black (n=7): **N = 96**

Matched population \*\*\*: **N = 311** (Case=104; Control=207)

1 case did not consent to future non-protocol specimen testing

*\* Selected case (≤35 weeks gestational age at delivery)-control (≥37 weeks gestational age at delivery) sample was weighted back to this study population in the analysis.*

*\*\* Preterm labor or membrane ruptures regardless of mode of delivery.*

*\*\*\* Cases and controls are matched on gestational age at the time of plasma specimen collection (14-20 vs. 21-27 vs. 28-34 weeks gestation), and race (black vs. non-black)*

**Table S1: Distributions of Covariates by Preterm Delivery**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** |  | **Preterm  (N=103)** | **Term  (N=205)** |
| Age (years) at delivery: Mean (SD) |  | 29.0 (6.0) | 28.7 (6.1) |
| Age (years) at delivery ≥ 35 |  | 20 (19%) | 41 (20%) |
| Black Race |  | 60 (58%) | 120 (59%) |
| Hispanic |  | 34 (33%) | 67 (33%) |
| Gestational age (weeks) at plasma sample collection | 14 - 20 | 3 (3%) | 5 (2%) |
|  | 21 - 27 | 30 (29%) | 60 (29%) |
|  | 28 - 34 | 70 (68%) | 140 (68%) |
| Last maternal CDC classification during pregnancy: category C |  | 26 (25%) | 25 (12%) |
| Last maternal CD4 at/prior to plasma sample collection: < 350 (cells/mm³) |  | 44 (43%) | 71 (35%) |
| Last maternal RNA at/prior to plasma sample collection > 400 (copies/mL) |  | 36 (35%) | 37 (18%) |
| Used alcohol during pregnancy |  | 31 (30%) | 57 (28%) |
| Used cigarettes during pregnancy |  | 29 (28%) | 43 (21%) |
| Used illicit drugs during pregnancy |  | 17 (17%) | 30 (15%) |
| First maternal BMI after delivery > 30 |  | 33 (32%) | 92 (45%) |
| Maternal STDs and other genital infections during pregnancy |  | 67 (65%) | 118 (58%) |
| Maternal Hepatitis B or C infection in pregnancy |  | 9 (9%) | 15 (7%) |
| Maternal infections other than STDs or Hepatitis B/C during pregnancy |  | 55 (53%) | 81 (40%) |
| Maternal vaccination received within 14 days before plasma collection |  | 2 (2%) | 7 (3%) |
| Maternal acute or chronic infections at the time of plasma sample collection |  | 22 (21%) | 30 (15%) |
| Fetal (congenital) infection |  | 2 (2%) | 1 (0%) |
| Maternal Vitamin D use in pregnancy |  | 2 (2%) | 2 (1%) |
| Last maternal CDC classification during pregnancy: category C |  | 26 (25%) | 25 (12%) |
| Last maternal CD4 at/prior to plasma sample collection: < 350 (cells/mm³) |  | 44 (43%) | 71 (35%) |
| Last maternal RNA at/prior to plasma sample collection ≤ 400 (copies/mL) |  | 67 (65%) | 168 (82%) |
| Time of initiating PI-containing ART | 1st Trimester or earlier | 38 (37%) | 64 (31%) |
| before plasma collection | 2nd/3rd Trimester | 49 (48%) | 96 (47%) |
|  | No PI-containing ART exposure | 15 (15%) | 45 (22%) |

Illicit drugs include marijuana, cocaine, heroin, amphetamines, methamphetamines, barbiturates, ecstasy, methadone, prescription drugs without prescription.

Covariates with missing data: Hispanic (preterm: 2%); alcohol use during pregnancy (preterm: 9%; term: 10%); cigarettes use in pregnancy (preterm: 22%; term: 20%); maternal BMI (preterm: 10%; term: 14%); timing of initiating PI-containing ART (preterm: 1%).

**Table S2: Distributions of Bio-marker Concentrations by Cases and Controls**

| **Type of Biomarker** | **Biomarker** |  | **Case (n=103)** | **Control (n=205)** |
| --- | --- | --- | --- | --- |
| Soluble markers of inflammation | TNF alpha (pg/ml) | Mean (s.d.) | 13.64 (9.01) | 14.09 (23.07) |
|  |  | Median (Q1, Q3) | 11.50 (8.22, 16.69) | 10.16 (7.49, 14.52) |
|  |  | Min, Max | 0.70, 57.93 | 0.70, 308.69 |
|  | IFN gamma (pg/ml) | < LLOD (0.800 pg/ml) | 38 (37%) | 78 (38%) |
|  |  | ≥ LLOD - < Q1 (7.275 pg/ml) | 19 (18%) | 29 (14%) |
|  |  | ≥ Q1 - < Q3 (30.275 pg/ml) | 26 (25%) | 70 (34%) |
|  |  | ≥ Q3 | 20 (19%) | 28 (14%) |
|  | IL-6 (pg/ml) | Mean (s.d.) | 3.96 (24.00) | 5.15 (52.47) |
|  |  | Median (Q1, Q3) | 1.25 (0.60, 2.12) | 1.15 (0.65, 1.74) |
|  |  | Min, Max | 0.11, 244.72 | 0.11, 752.00 |
|  | IL-8 (pg/ml) | Mean (s.d.) | 4.56 (4.89) | 12.70 (89.99) |
|  |  | Median (Q1, Q3) | 2.56 (1.84, 5.16) | 2.48 (1.52, 3.66) |
|  |  | Min, Max | 0.42, 31.44 | 0.29, 1,248.00 |
|  | IL-1 beta (pg/ml) | Mean (s.d.) | 2.82 (2.81) | 3.49 (8.64) |
|  |  | Median (Q1, Q3) | 2.03 (1.01, 3.57) | 1.98 (1.02, 3.31) |
|  |  | Min, Max | 0.14, 16.95 | 0.14, 114.46 |
|  | IL-18 (pg/ml) | Mean (s.d.) | 584.54 (918.66) | 538.19 (953.49) |
|  |  | Median (Q1, Q3) | 291.85 (187.12, 617.90) | 247.02 (141.29, 432.38) |
|  |  | Min, Max | 18.40, 7,183.59 | 18.40, 9,433.24 |
|  | IL-17 (pg/ml) | < LLOD (0.700 pg/ml) | 25 (24%) | 48 (23%) |
|  |  | ≥ LLOD - < LLOQ (3.300 pg/ml) | 32 (31%) | 74 (36%) |
|  |  | ≥ LLOQ - < Q2 (11.99 pg/ml) | 21 (20%) | 43 (21%) |
|  |  | ≥ Q2 | 25 (24%) | 40 (20%) |
|  | G-CSF (pg/ml) | Mean (s.d.) | 95.96 (148.86) | 75.93 (46.44) |
|  |  | Median (Q1, Q3) | 71.82 (47.75, 103.13) | 67.82 (46.65, 97.70) |
|  |  | Min, Max | 1.80, 1,507.23 | 1.80, 233.54 |
|  | MCP-1 (pg/ml) | Mean (s.d.) | 234.81 (120.67) | 224.45 (141.89) |
|  |  | Median (Q1, Q3) | 228.09 (152.45, 317.95) | 198.28 (139.76, 275.14) |
|  |  | Min, Max | 17.61, 560.34 | 1.90, 1,334.56 |
|  | IP-10 (pg/ml) | Mean (s.d.) | 309.25 (366.31) | 332.13 (612.09) |
|  |  | Median (Q1, Q3) | 188.69 (117.81, 338.81) | 145.09 (86.82, 312.01) |
|  |  | Min, Max | 18.40, 2,906.79 | 24.18, 6,428.60 |
|  | sIL-2R alpha (pg/ml) | Mean (s.d.) | 686.69 (432.41) | 507.90 (315.63) |
|  |  | Median (Q1, Q3) | 564.40 (365.61, 854.87) | 446.06 (313.57, 641.22) |
|  |  | Min, Max | 30.00, 2,033.87 | 30.00, 2,381.61 |
|  | sCD14 (pg/ml) | Mean (s.d.) | 1,919.50 (636.35) | 1,767.67 (593.25) |
|  |  | Median (Q1, Q3) | 1,852.87 (1,482.90, 2,276.77) | 1,733.16 (1,416.14, 2,092.71) |
|  |  | Min, Max | 359.28, 4,039.72 | 25.00, 3,428.06 |
|  | MMP9 (pg/ml) | Mean (s.d.) | 145,758.36 (127,616.11) | 131,205.84 (120,075.14) |
|  |  | Median (Q1, Q3) | 100,644.00 (49,413.50, 220,308.34) | 92,037.50 (44,053.89, 168,407.66) |
|  |  | Min, Max | 3,004.21, 500,000.00 | 100, 500,000 |
|  | VEGF-A (pg/ml) | < LLOD (26.300 pg/ml) | 29 (28%) | 60 (29%) |
|  |  | ≥ LLOD - < Q1 (143.31 pg/ml) | 18 (17%) | 36 (18%) |
|  |  | ≥ Q1 - < Q3 (364.09 pg/ml) | 38 (37%) | 72 (35%) |
|  |  | ≥ Q3 | 18 (17%) | 37 (18%) |
|  | GMC-SF (pg/ml) | Mean (s.d.) | 18.07 (27.42) | 15.68 (24.53) |
|  |  | Median (Q1, Q3) | 9.76 (4.82, 20.30) | 8.80 (3.85, 17.28) |
|  |  | Min, Max | 2.00, 207.85 | 2.00, 206.32 |
|  | GRO alpha (pg/ml) | Mean (s.d.) | 1,097.41 (1,385.42) | 1,173.79 (1,758.72) |
|  |  | Median (Q1, Q3) | 537.49 (171.08, 1,563.13) | 537.75 (179.30, 1,360.05) |
|  |  | Min, Max | 9.90, 8,227.60 | 9.90, 9,742.00 |
| Pro-inflammatory markers | PG E2 (pg/ml) | Mean (s.d.) | 261.65 (423.18) | 271.93 (414.64) |
|  |  | Median (Q1, Q3) | 150.60 (66.70, 267.30) | 126.75 (58.00, 302.70) |
|  |  | Min, Max | 6.00, 3,515.70 | 6.00, 3,657.70 |
|  | PG D2 (pg/ml) | Mean (s.d.) | 853.47 (747.34) | 785.52 (838.10) |
|  |  | Median (Q1, Q3) | 652.60 (412.90, 1,107.00) | 592.55 (365.10, 942.50) |
|  |  | Min, Max | 6.00, 4,645.90 | 6.00, 8,309.90 |
|  | PG Delta-12 (pg/ml) | Mean (s.d.) | 133.07 (173.62) | 134.76 (299.48) |
|  |  | Median (Q1, Q3) | 87.80 (49.50, 166.00) | 71.30 (37.90, 128.00) |
|  |  | Min, Max | 6, 1,321 | 6, 3,672 |
|  | PG J2 (pg/ml) | < LLOD (6.000 pg/ml) | 36 (36%) | 91 (45%) |
|  |  | ≥ LLOD - < Q1 (25.25 pg/ml) | 14 (14%) | 30 (15%) |
|  |  | ≥ Q1 - < Q3 (90.15 pg/ml) | 31 (31%) | 57 (28%) |
|  |  | ≥ Q3 | 20 (20%) | 24 (12%) |
|  | PG 15-deoxy D2 (pg/ml) | Mean (s.d.) | 157.74 (118.71) | 157.55 (128.10) |
|  |  | Median (Q1, Q3) | 133.80 (87.60, 198.30) | 124.90 (62.30, 217.80) |
|  |  | Min, Max | 6, 672 | 6.00, 842.50 |
|  | PG F2 alpha (pg/ml) | Mean (s.d.) | 167.27 (251.59) | 160.13 (367.98) |
|  |  | Median (Q1, Q3) | 96.50 (51.80, 177.40) | 72.30 (41.00, 138.10) |
|  |  | Min, Max | 6.00, 1,755.60 | 6.00, 4,218.70 |
|  | PG 8-Isoprostane (pg/ml) | Mean (s.d.) | 38.13 (44.70) | 36.07 (53.46) |
|  |  | Median (Q1, Q3) | 27.70 (11.30, 43.50) | 22.75 (9.30, 41.50) |
|  |  | Min, Max | 6.00, 326.10 | 6.00, 492.70 |
|  | 9-HODE (ng/ml) | Mean (s.d.) | 6.77 (14.03) | 5.47 (7.53) |
|  |  | Median (Q1, Q3) | 4.29 (2.44, 8.19) | 3.75 (2.01, 6.33) |
|  |  | Min, Max | 0.01, 137.93 | 0.01, 80.30 |
|  | 13-HODE (ng/ml) | Mean (s.d.) | 14.61 (24.32) | 11.43 (10.93) |
|  |  | Median (Q1, Q3) | 9.79 (6.03, 17.54) | 9.34 (5.52, 14.55) |
|  |  | Min, Max | 0.01, 233.36 | 0.01, 92.49 |
|  | 5-HEPE (ng/ml) | Mean (s.d.) | 0.15 (0.25) | 0.24 (1.97) |
|  |  | Median (Q1, Q3) | 0.09 (0.06, 0.18) | 0.08 (0.04, 0.13) |
|  |  | Min, Max | 0.01, 2.31 | 0.01, 28.10 |
|  | 8-HEPE (ng/ml) | Mean (s.d.) | 0.04 (0.07) | 0.04 (0.14) |
|  |  | Median (Q1, Q3) | 0.02 (0.01, 0.05) | 0.02 (0.01, 0.04) |
|  |  | Min, Max | 0.01, 0.50 | 0.01, 1.91 |
|  | 9-HETE (ng/ml) | Mean (s.d.) | 1.59 (2.75) | 1.85 (7.23) |
|  |  | Median (Q1, Q3) | 0.98 (0.59, 1.66) | 0.72 (0.36, 1.47) |
|  |  | Min, Max | 0.01, 23.76 | 0.01, 99.33 |
|  | 11-HETE (ng/ml) | Mean (s.d.) | 0.80 (1.34) | 0.78 (2.04) |
|  |  | Median (Q1, Q3) | 0.45 (0.26, 0.86) | 0.38 (0.17, 0.73) |
|  |  | Min, Max | 0.01, 10.41 | 0.01, 25.33 |
|  | 12-HETE (ng/ml) | Mean (s.d.) | 1.85 (3.28) | 1.58 (3.24) |
|  |  | Median (Q1, Q3) | 0.68 (0.25, 1.35) | 0.60 (0.27, 1.30) |
|  |  | Min, Max | 0.01, 20.67 | 0.01, 21.88 |
|  | 15-HETE (ng/ml) | Mean (s.d.) | 0.37 (0.61) | 0.38 (0.87) |
|  |  | Median (Q1, Q3) | 0.25 (0.15, 0.41) | 0.21 (0.12, 0.38) |
|  |  | Min, Max | 0.01, 5.40 | 0.01, 11.06 |
| Anti-inflammatory markers | IL-10 (pg/ml) | Mean (s.d.) | 13.60 (14.76) | 12.96 (12.42) |
|  |  | Median (Q1, Q3) | 11.11 (4.62, 17.96) | 8.76 (4.77, 17.40) |
|  |  | Min, Max | 0.56, 118.72 | 0.56, 65.08 |
|  | TGF beta (pg/ml) | Mean (s.d.) | 8,036.32 (5,372.31) | 7,289.10 (5,019.78) |
|  |  | Median (Q1, Q3) | 6,268.20 (3,771.04, 11,569.61) | 5,449.87 (3,793.82, 9,319.74) |
|  |  | Min, Max | 1,234.19, 20,000.00 | 1,401.24, 20,000.00 |
| Vitamin D | Vitamin D, 25-Hydroxy (ng/ml) | Mean (s.d.) | 16.77 (7.45) | 16.96 (8.55) |
|  |  | Median (Q1, Q3) | 15 (10, 21) | 14 (10, 22) |
|  |  | Min, Max | 10, 38 | 10, 62 |
|  |  | ≥ 20 ng/ml | 31 (30%) | 63 (31%) |

**Table S3: Associations of Time of Initiating PI-containing ART and 25OH-Vitamin D with Lipid Biomarkers**

|  | **Time initiating PI-containing ART (Ref: 1st trimester or earlier)** | | |  | **Vitamin D plasma concentration (continuous): per one unit increase** | |  | **Vitamin D plasma concentration ≥ 20 ng/ml (Ref: <20 ng/ml)** | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Biomarker (Outcome Measures)** |  | **Estimated Difference1 (95% CI)** | **P- value** |  | **Estimated Difference (95% CI)** | **P- value** |  | | **Estimated Difference1 (95% CI)** | **P- value** |
| PG E2 | 2nd/3rd Trimester | -0.107 (-0.252, 0.037) | 0.15 |  | **-0.271 (-0.488, -0.054)** | **0.01** |  | Yes | -0.133 (-0.271, 0.005) | 0.06 |
|  | No PI-containing ART exposure before plasma collection | -0.106 (-0.281, 0.068) | 0.23 |  |  |  |  |  |  |  |
| PG D2 | 2nd/3rd Trimester | -0.053 (-0.177, 0.072) | 0.41 |  | **-0.312 (-0.499, -0.124)** | **0.001** |  | **Yes** | **-0.256 (-0.373, -0.139)** | **< 0.001** |
|  | No PI-containing ART exposure before plasma collection | -0.109 (-0.260, 0.041) | 0.16 |  |  |  |  |  |  |  |
| PG Delta-12 | 2nd/3rd Trimester | -0.046 (-0.175, 0.083) | 0.48 |  | **-0.212 (-0.407, -0.016)** | **0.03** |  | **Yes** | **-0.180 (-0.302, -0.057)** | **0.004** |
|  | No PI-containing ART exposure before plasma collection | -0.128 (-0.283, 0.028) | 0.11 |  |  |  |  |  |  |  |
| PG 15-deoxy D2 | 2nd/3rd Trimester | -0.027 (-0.145, 0.091) | 0.65 |  | 0.016 (-0.167, 0.199) | 0.87 |  | Yes | 0.022 (-0.094, 0.137) | 0.71 |
|  | No PI-containing ART exposure before plasma collection | -0.081 (-0.222, 0.061) | 0.27 |  |  |  |  |  |  |  |
| PG F2 alpha | 2nd/3rd Trimester | -0.105 (-0.249, 0.039) | 0.15 |  | **-0.369 (-0.587, -0.150)** | **< 0.001** |  | **Yes** | **-0.163 (-0.302, -0.023)** | **0.02** |
|  | No PI-containing ART exposure before plasma collection | -0.053 (-0.227, 0.121) | 0.55 |  |  |  |  |  |  |  |
| PG 8-isoprostane | 2nd/3rd Trimester | -0.064 (-0.199, 0.071) | 0.35 |  | **-0.287 (-0.491, -0.082)** | **0.01** |  | **Yes** | **-0.191 (-0.320, -0.061)** | **0.004** |
|  | No PI-containing ART exposure before plasma collection | 0.015 (-0.148, 0.177) | 0.86 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
| 9-HODE | **2nd/3rd Trimester** | **-0.318 (-0.610, -0.027)** | **0.03** |  | 0.206 (-0.245, 0.657) | 0.37 |  | Yes | 0.233 (-0.051, 0.518) | 0.11 |
|  | No PI-containing ART exposure before plasma collection | -0.217 (-0.569, 0.134) | 0.23 |  |  |  |  |  |  |  |
| 13-HODE | **2nd/3rd Trimester** | **-0.345 (-0.665, -0.025)** | **0.03** |  | 0.226 (-0.269, 0.720) | 0.37 |  | Yes | 0.268 (-0.044, 0.579) | 0.09 |
|  | No PI-containing ART exposure before plasma collection | -0.204 (-0.590, 0.182) | 0.30 |  |  |  |  |  |  |  |
| 5-HEPE | **2nd/3rd Trimester** | **-0.233 (-0.390, -0.076)** | **0.004** |  | 0.224 (-0.020, 0.468) | 0.07 |  | **Yes** | **0.245 (0.092, 0.397)** | **0.002** |
|  | **No PI-containing ART exposure before plasma collection** | **-0.255 (-0.444, -0.066)** | **0.01** |  |  |  |  |  |  |  |
| 8-HEPE | **2nd/3rd Trimester** | **-0.141 (-0.268, -0.013)** | **0.03** |  | 0.186 (-0.010, 0.382) | 0.06 |  | **Yes** | **0.157 (0.033, 0.280)** | **0.01** |
|  | No PI-containing ART exposure before plasma collection | -0.149 (-0.303, 0.004) | 0.06 |  |  |  |  |  |  |  |
| 9-HETE | **2nd/3rd Trimester** | **-0.262 (-0.509, -0.015)** | **0.04** |  | 0.162 (-0.220, 0.544) | 0.41 |  | Yes | 0.165 (-0.076, 0.406) | 0.18 |
|  | No PI-containing ART exposure before plasma collection | -0.172 (-0.470, 0.126) | 0.26 |  |  |  |  |  |  |  |
| 11-HETE | **2nd/3rd Trimester** | **-0.238 (-0.459, -0.017)** | **0.03** |  | 0.089 (-0.254, 0.431) | 0.61 |  | Yes | 0.091 (-0.126, 0.307) | 0.41 |
|  | No PI-containing ART exposure before plasma collection | -0.144 (-0.410, 0.123) | 0.29 |  |  |  |  |  |  |  |
| 12-HETE | **2nd/3rd Trimester** | **-0.324 (-0.571, -0.078)** | **0.01** |  | 0.086 (-0.296, 0.468) | 0.66 |  | Yes | 0.191 (-0.050, 0.432) | 0.12 |
|  | No PI-containing ART exposure before plasma collection | -0.187 (-0.484, 0.111) | 0.22 |  |  |  |  |  |  |  |
| 15-HETE | **2nd/3rd Trimester** | **-0.223 (-0.416, -0.029)** | **0.02** |  | 0.130 (-0.170, 0.431) | 0.39 |  | Yes | 0.147 (-0.042, 0.337) | 0.13 |
|  | No PI-containing ART exposure before plasma collection | -0.167 (-0.401, 0.067) | 0.16 |  |  |  |  |  |  |  |

1 Estimated difference in adjusted mean biomarker concentration level per one unit increase in continuous exposure measurements; or estimated difference in adjusted mean biomarker concentration level between participants with vs. without a specific characteristic for categorical exposure measurements.

Separate models were built for each pair of exposure (i.e. Time initiating PI-containing ART, Vitamin D concentrations) and outcome (i.e. biomarkers) measure combinations, adjusting pre-select covariates as below:

Models for time of initiating PI-containing ART included race, age at delivery, alcohol/illicit drug use during pregnancy, maternal BMI, maternal acute or chronic infections at the time of plasma collection, maternal vaccination received within 14 days before plasma collection, last maternal CD4 count at/prior to plasma sample collection, last maternal CDC classification during pregnancy

Models for vitamin D concentration included race, age at delivery, alcohol/illicit drug use during pregnancy, maternal BMI, maternal acute or chronic infections at the time of plasma collection, maternal vaccination received within 14 days before plasma collection, maternal STDs and other genital infections during pregnancy, last maternal RNA at/prior to plasma sample collection, last maternal CDC classification during pregnancy.