**SUPPLEMENTAL MATERIALS**

Cytokine levels at birth in children who developed acute lymphoblastic leukemia

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**RUNNING TITLE**

Neonatal cytokines and childhood ALL

**KEYWORDS**

Acute lymphoblastic leukemia; Cytokines; Prenatal exposure delayed effects; Biomarkers; Children’s health

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**CONFLICT OF INTEREST STATEMENT**

The authors declare no potential conflicts of interest.

**Supplemental Figure S1.** SCONE diagnostics. Figure S1 Diagnostics for SCONE normalization using IL-8 as an example.



**Supplemental Table S1.** Prenatal exposures that are putative risk factors for childhood acute lymphoblastic leukemia.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   |   |   |   |   |
|   | **Assessment** | **Group** | **Exposure** |   |
|   | Neonatal blood spots | Endogenous metabolites at birth (36) | Tetracosadienoic acid, hexadecadienoic acid, linolenic acid, linoleic acid, 3 phosphaditylcholines and 2 lysophosphaditylcholines, 2 lysophosphatidylethanolamines, 2 phosphatidylserines, a sphingomyelin, C64H102O6, C36H71NO3, C26H44O2, and 11 unknown compounds |   |
|   | Neonatal blood spots | Serum albumin adducts at birth (35) | Additions of acrolein, crotonaldehyde and cysteine |   |
|   | Settled home dust | Polychlorinated biphenyls (14) | PCBs 105, 118, 138, 153, 170, 180 |   |
|   | Settled home dust | Polycyclic aromatic hydrocarbons (13) | Benzo(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, indeno(*1,2,3-cd*)pyrene, dibenzo(*a,h*)anthracene, dibenzo(*a,e*)pyrene, Coronene |   |
|   | Settled home dust | Polybrominated diphenyl ethers (15) | BDEs 28, 47, 99, 100, 153, 154, 183, 196, 197, 203, 206, 207, 209 |   |
|   | Questionnaire | Maternal infections (44)  | Cold, pneumonia, flu, sexually-transmitted disease, or infections during pregnancy |   |
|   | Questionnaire | Maternal smoking (43)  | No. of cigarettes smoked before and during pregnancy |   |
|   | Questionnaire | Pesticide use at home (42) | Use of any pesticides or insecticides during pregnancy |   |
|   | Questionnaire | Paint use at home (45) | Any paint use in the home or paint use during the first 3 years of life |   |
|   |   |   |   |   |

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**Supplemental Figure S2.** Diagram of mediation analysis comparing the total effect between endogenous metabolites and cancer versus the direct effect between endogenous metabolites and cancer after adjusting for cytokine levels as a potential mediator.

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**Supplemental Table S2.** Childhood acute lymphoblastic leukemia (ALL) odds ratios associated with an interquartile range increment in cytokine levels at birth among participants of the California Childhood Leukemia Study (1995-2015); stratified by age at diagnosis date (or corresponding reference age for healthy control children).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |   |   |   |   |
|   |   |   | Under age 2; 96 cases and 118 controls |   | Ages 2 to 5; 538 cases and 569 controls |   | Ages 6 to 14; 386 cases and 316 controls |   |   |
|   |   |   |   |   |   |   |   |   |   |
|   | Single-cytokine models |   |   |   |   |   |   |
|   | IL1β |   | 1.27 (0.91, 1.92) |   | 1.16 (0.98, 1.38) |   | 1.20 (0.98, 1.49) |   |   |
|   | IL4 |   | 1.32 (0.96, 1.85) |   | 1.14 (0.97, 1.34) |   | 0.83 (0.68, 1.01) |   |   |
|   | IL6 |   | 1.02 (0.80, 1.30) |   | **0.91 (0.82, 1.00)** | \* | 1.10 (0.98, 1.23) |   |   |
|   | IL8 |   | 1.14 (0.75, 1.73) |   | 1.09 (0.91, 1.31) |   | **1.33 (1.07, 1.67)** | \* |   |
|   | GM-CSF |   | 1.01 (0.66, 1.55) |   | 1.15 (0.96, 1.37) |   | 0.77 (0.63, 0.95) |   |   |
|   | TNFα |   | 1.03 (0.73, 1.45) |   | **1.19 (1.00, 1.41)** | \* | 1.14 (0.95, 1.38) |   |   |
|   | VEGF |   | 0.88 (0.61, 1.24) |   | **1.21 (1.02, 1.44)** | \* | 1.22 (0.99, 1.51) |   |   |
|   |   |   |   |   |   |   |   |   |   |
|   | Two PCs in one model |   |   |   |   |   |   |
|   | PC1 |   | 1.19 (0.79, 1.79) |   | 1.19 (0.99, 1.44) |   | 1.12 (0.90, 1.40) |   |   |
|   | PC2 |   | 1.07 (0.77, 1.48) |   | 1.12 (0.96, 1.32) |   | 0.74 (0.62, 0.89) |   |   |
|   |   |   |   |   |   |   |   |   |   |

\* Association between cytokine levels and case/control outcome is significant in logistic regression, *p*-value < 0.05 (highlighted with **bold** font)

Model includes covariates: case/control outcome as a function of cytokine levels mutually adjusted for child’s sex, mother’s race/ethnicity, household annual income, mother’s age at birth, delivery method, gestational age at birth, birth weight, breastfeeding, and blood spot collection time

**Supplemental Table S3.** Childhood acute lymphoblastic leukemia (ALL) odds ratios associated with an interquartile range increment in cytokine levels at birth among participants of the California Childhood Leukemia Study (1995-2015); stratified by year of birth.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |   |   |   |   |
|   |   |   | 2000 or later; 407 cases and 232 controls |   | 1995 to 2000; 388 cases and 488 controls |   | Before 1995; 225 cases and 283 controls |   |   |
|   |   |   |   |   |   |   |   |   |   |
|   | Single-cytokine models |   |   |   |   |   |   |
|   | IL1β |   | 1.22 (0.96, 1.56) |   | **1.19 (1.00, 1.44)** | \* | 1.12 (0.88, 1.43) |   |   |
|   | IL4 |   | 1.03 (0.84, 1.27) |   | 1.00 (0.85, 1.18) |   | 1.03 (0.80, 1.33) |   |   |
|   | IL6 |   | 1.02 (0.89, 1.18) |   | 0.95 (0.85, 1.05) |   | 1.04 (0.91, 1.18) |   |   |
|   | IL8 |   | **1.36 (1.05, 1.79)** | \* | 1.06 (0.87, 1.28) |   | **1.30 (1.01, 1.69)** | \* |   |
|   | GM-CSF |   | 0.88 (0.67, 1.15) |   | 0.99 (0.83, 1.19) |   | 0.88 (0.69, 1.12) |   |   |
|   | TNFα |   | 1.22 (0.97, 1.55) |   | 1.12 (0.94, 1.33) |   | 1.15 (0.92, 1.43) |   |   |
|   | VEGF |   | **1.29 (1.03, 1.64)** | \* | 1.08 (0.90, 1.30) |   | 1.13 (0.88, 1.46) |   |   |
|   |   |   |   |   |   |   |   |   |   |
|   | Two PCs in one model |   |   |   |   |   |   |
|   | PC1 |   | 1.27 (0.97, 1.67) |   | 1.09 (0.90, 1.32) |   | 1.14 (0.88, 1.47) |   |   |
|   | PC2 |   | 0.89 (0.72, 1.11) |   | 0.98 (0.84, 1.15) |   | 0.86 (0.68, 1.08) |   |   |
|   |   |   |   |   |   |   |   |   |   |

\* Association between cytokine levels and case/control outcome is significant in logistic regression, *p*-value < 0.05 (highlighted with **bold** font)

Model includes covariates: case/control outcome as a function of cytokine levels mutually adjusted for child’s sex, mother’s race/ethnicity, household annual income, mother’s age at birth, delivery method, gestational age at birth, birth weight, breastfeeding, and blood spot collection time

**Supplemental Table S4.** Childhood acute lymphoblastic leukemia (ALL) odds ratios associated with an interquartile range increment in cytokine levels at birth among participants of the California Childhood Leukemia Study (1995-2015); stratified by child’s sex.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |   |   |
|   |   |   | Boys; 586 cases and 574 controls |   | Girls; 434 cases and 429 controls |   |   |
|   |   |   |   |   |   |   |   |
|   | Single-cytokine models |   |   |   |   |
|   | IL1β |   | **1.21 (1.03, 1.43)** | \* | 1.15 (0.97, 1.38) |   |   |
|   | IL4 |   | 1.10 (0.94, 1.27) |   | 0.98 (0.83, 1.16) |   |   |
|   | IL6 |   | 0.97 (0.89, 1.05) |   | 0.99 (0.88, 1.12) |   |   |
|   | IL8 |   | **1.19 (1.01, 1.40)** | \* | 1.16 (0.94, 1.44) |   |   |
|   | GM-CSF |   | 0.98 (0.84, 1.15) |   | 0.96 (0.79, 1.17) |   |   |
|   | TNFα |   | 1.15 (0.99, 1.34) |   | 1.10 (0.93, 1.31) |   |   |
|   | VEGF |   | 1.09 (0.93, 1.28) |   | 1.21 (1.00, 1.47) |   |   |
|   |   |   |   |   |   |   |   |
|   | Two PCs in one model |   |   |   |   |
|   | PC1 |   | 1.15 (0.98, 1.36) |   | 1.16 (0.94, 1.43) |   |   |
|   | PC2 |   | 0.98 (0.85, 1.14) |   | 0.94 (0.79, 1.11) |   |   |
|   |   |   |   |   |   |   |   |

\* Association between cytokine levels and case/control outcome is significant in logistic regression, *p*-value < 0.05 (highlighted with **bold** font)

Model includes covariates: case/control outcome as a function of cytokine levels mutually adjusted for mother’s race/ethnicity, household annual income, mother’s age at birth, delivery method, gestational age at birth, birth weight, breastfeeding, and blood spot collection time

**Supplemental Table S5.** Childhood acute lymphoblastic leukemia (ALL) odds ratios associated with an interquartile range increment in levels of endogenous metabolites among participants of the California Childhood Leukemia Study (1995-2015); with or without adjustment for levels of cytokines found in the dried blood spots and stratified by age at diagnosis.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |   |   |
|   | Accurate mass, m/z | Endogenous metabolite name† | Total Effect | % Direct | % Mediated |   |
|   |   |   |   |   |   |   |   |
|   | *Diagnosed at Ages 2 to 5* |   |   |   |   |   |
|   | 363.3263 | Tetracosadienoic acid | 0.84 (0.64, 1.10) |   | 100.0 | 0.0 |   |
|   | 431.3151 | Unknown | 1.35 (0.99, 1.84) |   | 100.0 | 0.0 |   |
|   | 570.3401 | Unknown | 1.29 (0.94, 1.78) |   | 100.0 | 0.0 |   |
|   | 604.3610 | Lysophosphatidylcholine (20:3) | 1.23 (0.93, 1.64) |   | 100.0 | 0.0 |   |
|   | 638.2760 | Unknown | 1.40 (1.02, 1.93) | \* | 100.0 | 0.0 |   |
|   | 696.3573 | Unknown | 1.24 (0.94, 1.66) |   | 100.0 | 0.0 |   |
|   | 766.5589 | Phosphatidylserine (16:0/16:0) | 1.36 (1.02, 1.82) | \* | 100.0 | 0.0 |   |
|   | 775.2985 | Unknown | 1.32 (0.97, 1.82) |   | 100.0 | 0.0 |   |
|   | 884.6004 | Phosphaditylcholine (18:0/20:4(OH)) | 1.29 (1.01, 1.67) | \* | 99.9 | 0.1 |   |
|   |   |   |   |   |   |   |   |
|   | *Diagnosed at Ages 6 to 14* |   |   |   |   |   |
|   | 251.2011 | Hexadecadienoic acid | 0.70 (0.45, 1.09) |   | 100.0 | 0.0 |   |
|   | 277.2170 | Linolenic acid | 1.46 (1.07, 2.01) | \* | 99.6 | 0.4 |   |
|   | 279.2329 | Linoleic acid | 1.37 (1.02, 1.87) | \* | 100.0 | 0.0 |   |
|   | 377.1419 | Unknown | 1.36 (0.96, 1.95) |   | 99.2 | 0.9 |   |
|   | 387.3264 | C26H44O2 | 1.94 (1.24, 3.09) | \* | 100.0 | 0.0 |   |
|   | 449.3629 | Unknown | 0.65 (0.44, 0.93) | \* | 100.0 | 0.0 |   |
|   | 476.2778 | Lysophosphatidylethanolamine (18:2) | 1.41 (0.98, 2.06) |   | 100.0 | 0.0 |   |
|   | 500.2782 | Lysophosphatidylethanolamine (20:4) | 1.57 (1.07, 2.35) | \* | 99.7 | 0.3 |   |
|   | 530.3234 | Unknown phosphatidylethanolamine | 1.47 (0.99, 2.20) |   | 100.0 | 0.0 |   |
|   | 552.2956 | Unknown | 0.56 (0.37, 0.85) | \* | 99.8 | 0.2 |   |
|   | 564.5344 | Putative ceramide, C36H71NO3 | 0.67 (0.43, 1.02) |   | 100.0 | 0.0 |   |
|   | 578.3458 | Lysophosphatidylcholine (18:2) | 1.46 (0.98, 2.20) |   | 99.6 | 0.4 |   |
|   | 592.3238 | Unknown | 0.50 (0.31, 0.79) | \* | 100.0 | 0.0 |   |
|   | 760.5126 | Phosphatidylserine (16:0/18:1) | 1.50 (1.03, 2.22) | \* | 99.6 | 0.4 |   |
|   | 789.6123 | Sphingomyelin (d16:1/20:4) | 0.64 (0.43, 0.96) | \* | 100.0 | 0.0 |   |
|   | 824.5793 | Phosphaditylcholine (P16:0/20:4) | 1.63 (1.14, 2.37) | \* | 100.0 | 0.0 |   |
|   | 844.6069 | Phosphaditylcholine (18:0/18:2) | 1.51 (1.04, 2.25) | \* | 100.0 | 0.0 |   |
|   | 897.6298 | Unknown | 0.65 (0.44, 0.95) | \* | 100.0 | 0.0 |   |
|   | 965.7627 | C64H102O6 | 1.41 (0.94, 2.15) |   | 100.0 | 0.0 |   |
|   |   |   |   |   |   |   |   |

† Names refer to putative annotations described by Petrick et al. (2017) in the format of the bCommon names used in the Human Metabolome Database (HMDB)

\* Association between endogenous metabolite levels and case/control outcome is significant in logistic regression, *p*-value < 0.05

Model includes covariates: case/control outcome as a function of endogenous metabolite evels mutually adjusted for child’s sex, mother’s race/ethnicity, household annual income, mother’s age at birth, delivery method, gestational age at birth, birth weight, breastfeeding, and blood spot collection time.

**Supplemental Table S6.** Comparison of three studies that have evaluated the relationship between cytokine levels at birth and childhood acute lymphoblastic leukemia (ALL) risk.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |
|   | **Study characteristics** | **Current Study** | **Chang *et al.* (11)** | **Soegaard *et al.* (12)** |   |
|   |   |   |   |   |   |
|   | No. of participants | 1,020 cases, 1,003 controls | 116 cases, 116 controls | 178 cases, 178 controls |   |
|   | Ethnicity of participants | ALL risk observed among Latinos only | Predominately Latino or white, analyzed together | Predominately white |   |
|   | Study location | California, USA | California, USA | Denmark |   |
|   | Birth years | 1984-2012 | 1995-2006 | 1995-2008 |   |
|   | Type of childhood leukemia to which the cytokine levels were linked | Any ALL,especially ALL with high hyperdiploidy | Any ALL | B-cell ALL |   |
|   | Cytokine assay | Luminex magnetic beads | Luminex polystyrene beads | Meso-scale multiplex sandwich |   |
|   | Antibody brand | R&D Systems | Bio-Rad | R&D Systems |   |
|   | Median DBS storage time | 21 years | 8 years | ~17 years |   |
|   | Typical DBS collection time | 1 to 2 days after birth | 1 to 2 days after birth | 7 days after birth |   |
|   | ALL associated with... | Higher levels of IL1β, IL8, TNFα, VEGF | Lower levels of IL4, IL6, IL10, IL13 | Higher levels of IL6, lower levels of IL8  |   |
|   |   |   |   |   |   |

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