Title

Increased neonatal level of arginase 2 in cases of childhood acute lymphoblastic leukemia implicates immunosuppression in the etiology.

Journal

Haematologica, 104(11)

ISSN

0390-6078

Authors

Nielsen, Amalie B
Zhou, Mi
de Smith, Adam J
et al.

Publication Date

2019-11-01

DOI

10.3324/haematol.2019.216465

Peer reviewed

haematologica
Journal of The Ferrata Storti Foundation

Increased neonatal level of arginase 2 in cases of childhood acute lymphoblastic leukemia implicates immunosuppression in etiology

by Amalie B. Nielsen, Mi Zhou, Adam J. de Smith, Rong Wang, Lucie McCoy, Helen Hansen, Libby Morimoto, Kirsten Grønbæch, Christoffer Johansen, Scott C. Kogan, Catherine Metayer, Paige M. Bracci, Xiaomei Ma, and Joseph L. Wiemels

Haematologica 2019 [Epub ahead of print]

Citation: Amalie B. Nielsen, Mi Zhou, Adam J. de Smith, Rong Wang, Lucie McCoy, Helen Hansen, Libby Morimoto, Kirsten Grønbæch, Christoffer Johansen, Scott C. Kogan, Catherine Metayer, Paige M. Bracci, Xiaomei Ma, and Joseph L. Wiemels. Increased neonatal level of arginase 2 in cases of childhood acute lymphoblastic leukemia implicates immunosuppression in etiology. Haematologica. 2019; 104:xxx
doi:10.3324/haematol.2019.216465

Increased neonatal level of arginase 2 in cases of childhood acute lymphoblastic leukemia implicates immunosuppression in etiology

Amalie B. Nielsen[1,2], Mi Zhou[1], Adam J. de Smith[1,3], Rong Wang[4], Lucie McCoy[5,6], Helen Hansen[5], Libby Morimoto[6], Kirsten Grønbæk[2], Christoffer Johansen[2], Scott C. Kogan[7], Catherine Metayer[6], Paige M. Bracci[1], Xiaomei Ma[4*] and Joseph L. Wiemels[1,3*]

[1]Department of Epidemiology and Biostatistics, University of California San Francisco

[2]Department of Hematology, Rigshospitalet, Biotech Research and Innovation Centre, BRIC, Faculty of Health and Medical Sciences, University of Copenhagen

[3]Centre for Genetic Epidemiology, Keck School of Medicine, University of Southern California

[4]Department of Chronic Disease Epidemiology, School of Public Health, Yale University

[5]Department of Neurosurgery, University of California San Francisco

[6]Division of Epidemiology, School of Public Health, University of California Berkeley

[7]Department of Laboratory Medicine, University of California San Francisco

*Co-senior authors

Running Title: Arginase-2 and pediatric acute lymphoblastic leukemia

Corresponding Author:

Joseph Wiemels, PhD
Centre for Genetic Epidemiology, Norris Comprehensive Cancer Center
University of Southern California
1450 Biggy Street, NRT 1506A
Los Angeles, CA 90033
wiemels@usc.edu

Word count: main text 1493 words.

Acknowledgements

The authors would like to thank the Lundbeck Foundation, Clinical Research Fellowship granted to A.B.N. Research reported in this publication was supported by funding from the National Institutes of Health (grants R01CA175737, R01ES009137 and P50ES018172) and the Environmental Protection Agency (grant RD83615901) of the United States. The content is solely the responsibility of the authors and does not represent the official views of any funding agencies.

The biospecimens and/or data used in this study were obtained from the California Biobank Program at the California Department of Public Health, SIS request number 600, in accordance with Section 6555(b), 17 CCR. The authors acknowledge Robin Cooley and Marty Kharrazi of the California Department of Public Health for their assistance providing banked specimens and record linkage services for this portion of the study. This study used birth data obtained from the Center for Health Statistics and Informatics, California Department of Public Health. The California Department of Public Health is not responsible for the analyses, results, interpretations, or conclusions drawn by the authors regarding the birth data used in this publication.

The collection of cancer incidence data used in this study was supported by the California Department of Public Health pursuant to California Health and Safety Code Section 103885; Centers for Disease Control and Prevention's National Program of Cancer Registries, under cooperative agreement 5NU58DP003862-04/DP003862; the National Cancer Institute's Surveillance, Epidemiology and End Results Program under contract HHSN261201000140C awarded to the Cancer Prevention Institute of California, contract HHSN261201000035C awarded to the University of Southern California, and contract HHSN261201000034C awarded to the Public Health Institute. The ideas and opinions expressed herein are those of the author(s) and do not reflect the opinions of the State of California, Department of Public Health, the National Cancer Institute, and the Centers for Disease Control and Prevention or their Contractors and Subcontractors.

Letter to the Editor

Acute lymphoblastic leukemia (ALL) afflicts 2,250 children diagnosed annually in the United States (0-14 yrs).[1] Modern treatment regimens cure approximately 90% of those afflicted, but survivors suffer from long-term sequelae.[2] Epidemiologic evidence for ALL development points to a role for infection and immune development.[3] Clinically diagnosed infections in the first year of life have been associated with a higher risk of childhood ALL,[4,5] whereas increased exposure to common infections based on proxy measures of childhood social contacts (e.g., daycare attendance) may reduce risk.[6] The role of neonatal immune development in ALL is further supported by the increased ALL risk in children delivered by elective cesarean section.[7] Emerging evidence suggests that neonatal infection susceptibility is related to active immune suppression within the neonatal environment. A key regulator within perinatal immunity is arginase 2 (ARG2),[8,9] which suppresses T-cells through an anti-inflammatory cascade resulting from arginine depletion.[10] Given the function of ARG2 in neonatal immune function and response to early-life infections, we

investigated whether variation in ARG2 levels at birth may be associated with risk of developing ALL in childhood. For the current study, we selected a total of 137 children who were born in seven counties of California and diagnosed with ALL at the ages of 0-14 years during 2000-2009, as well as 500 cancer-free control children who were matched to the cases by birth year and county of birth (3 or 4 controls per case). We obtained data on cancer diagnosis from the California Cancer Registry, data on birth characteristics from the California Center for Health Statistics and Informatics, and archived neonatal blood spots from the California Biobank Program which is part of the Genetic Disease Screening Program. The blood spots, which were used in this study to measure newborn ARG2 levels, were leftover material from statewide disease screening in newborns. All available cases were obtained within the catchment time and space, along with appropriate matched controls chosen via a population-based registry. Our study protocol has been approved by the State of California Committee for the Protection of Human Subjects, and the institutional review boards at all agencies from which we obtained data or blood spots as well as the academic institutions involved (University of California, San Francisco and Berkeley, Yale University, University of Southern California). We obtained a 14-mm diameter blood spot on filter paper (also known as Guthrie card) collected via a heel-prick of the newborns, usually within two days after birth (median = 28 hours). These blood spots are stored at a central State archive under frozen (-20°C) conditions. For each subject, one third of a blood spot was excised and placed in 300 μL of extraction buffer [PBS, pH 7.4, 0.5% Tween-20 and 2x complete protease inhibitor cocktail (Roche)], shaken at 600 rpm under room temperature for 1 hour and spun 30 sec at 20,000 x *g*. Extracts were assayed in duplicate and block randomized on 96-well plates, with each plate containing a 7-point standard ARG2 dilution in duplicate, and the same proportion of cases and controls and racial/ethnic groups. ARG2 was measured using enzyme-linked immunosorbent assay (ELISA) (MyBioSource). Serum-protein levels were determined using Pierce BCA protein assay.

Four parameter logistic regression was used to calculate the standard curves for each batch. ARG2 levels were estimated from standard curves. ARG2 levels were averaged across duplicates and normalized to the sample-specific total serum-protein concentration. Categorical variables are shown using frequencies and percentages, and continuous variables (eg., age at neonatal blood collection) were summarized by median and interquartile range. Baseline characteristics between cases and controls were compared using chi-square test for categorical variables and Wilcoxon rank sum test for age at neonatal blood collection. ARG2 levels were categorized into quartiles according to the distribution among controls. An unconditional multivariable mixed-effect logistic regression model with batch as a random variable was used to estimate the association between ARG2 level and risk of ALL, adjusting for birth characteristics (listed in Figure 1 legend). All analyses were conducted using SAS Version 9.4 (SAS Institute Inc., Cary, NC) with two-sided tests and a type I error of 5% as the threshold for statistical significance. A p-value for trend with increasing ARG2 was calculated by treating arginase quartile as an ordinal variable in the logistic regression model. Eight control samples were excluded due to a coefficient of variance greater than 30% between duplicate ARG2 measurements. Cases and controls were similar with regard to sex, race/ethnicity, gestational age, birth weight, and birth order (**Table 1**). Additional data on distribution of ARG2 in relationship with these covariates are presented in Supplementary Tables 1 and 2. Compared with controls, ALL cases were more likely to be delivered by cesarean section (39% vs 28%, p=0.02) and were older at neonatal blood collection (median: 29 vs. 27 hours, p=0.01). In addition, among controls, calendar age of the card was negatively correlated with ARG2 level (Spearman correlation coefficient = -0.20, p=0.0001). The multivariable analysis suggested that the risk of childhood ALL increased by more than two-fold in subjects whose level of ARG2 at birth was in the 3rd or 4th quartile, compared to those whose ARG2 level was in the lowest quartile. The odds ratio was 2.20 (95% confidence interval (CI): 1.21 - 3.99, p=0.01) and

2.28 (95% CI: 1.28 - 4.07, p=0.01) for Q3 and Q4, respectively (Figure 1), with a significant trend: OR = 1.31 (95%CI: 1.10-1.57, p = 0.0021). This relationship did not change when samples were not adjusted for protein extraction levels (OR = 1.34; 95%CI:1.06-1.69, no correction for protein concentration). ARG2 levels were higher for children born with cesarean section among cases (P = 0.06 by Wilcoxon rank test) and controls (P = 0.56), and the case-control relationship with ARG2 was slightly weakened by removing all subjects with cesarean section birth, OR = 1.21(95%CI 0.98-1.52, p = 0.07, trend test) (Supplementary Table 3) while the full dataset retained significance when adjusting for cesarean birth (Figure 1 and Supplementary Table 4). When the analysis was restricted to B cell only (100 cases), ARG2 retained significance, OR=1.22(95%CI 1.00-1.49, P = 0.05, Supplementary Table 5). We therefore found that the higher neonatal ARG2 levels associated with a ALL later in childhood held true for the most common subtype of ALL: pre-B cell. Arginase has been reported to impact the immune system via its role in depletion of L-arginine, which may impair NO-mediated cytotoxicity, decrease TLR-4 mediated proinflammatory response and suppress T-cell function via downregulation of TCR-CD3ζ.[8] Several studies have shown increased arginase production by myeloid-derived suppressor cells (MDSCs) in adult tumors leading to a suppressed response in the tumor immune microenvironment.[11] Recently, a transitory presence of MDSCs has been identified in newborns, suggesting that these cells have an additional role of regulating immune suppression early in life.[12] In addition, infection susceptibility in newborn mice has been associated with temporal presence of CD71+ immunosuppressive erythroid cells producing ARG2,[13] which could impact the response to early infections known to affect risk of ALL.[4,5] While higher ARG2 may be indicative of greater immunosuppression at birth, it is also possible that it is simply a marker of the level of early infant immune stimulation prior to Guthrie card blood sampling. Therefore, higher ARG2 levels could signify a naïve neonatal immune system due to lower prior microbial exposures,[3] and those subjects with high ARG2 may react

inappropriately to new infections thereby stimulating leukemogenesis.[4,5] Our group previously reported a deficit of IL-10 at birth among children who subsequently developed ALL, suggesting that a child's baseline immune function at birth may affect his/her response to subsequent infectious exposures and leukemia risk.[14] Recently this observation was expanded to additional cytokines.[15] Taken together with the current results, the evidence suggests that a dysregulated immune response around the time of birth may affect the responsiveness of the developing immune system and also provide a growth advantage for a preleukemic clone. Strengths of the study include the low likelihood for selection and information bias (population-based ascertainment of study subjects, no need to contact subjects for participation, preexisting data on important covariates through birth records) and the availability of archived newborn blood specimens for measurement of ARG2 level at birth. While samples that have been stored for longer time appeared to have lower ARG2 levels, our cases and controls were frequency matched on year of birth and hence the duration of sample storage. Nondifferential misclassification regarding ARG2 levels would likely have biased our results towards the null. In summary, the present study showed that a higher ARG2 level at birth was associated with statistically significant increased odds of developing ALL later in childhood. This finding suggests that immune changes related to ARG2 levels are evident long before the clinical manifestation of childhood leukemia. Our results require validation and further investigation in larger studies of neonatal ARG2 that include detailed assessment of early childhood infections and autoimmune diseases. However, this novel finding contributes to increasing evidence for a role of immune dysregulation at birth in the development of childhood ALL.

Disclosure of Conflicts of Interest

The authors do not have any competing financial or other interests in the contents or interpretations presented within this manuscript.

Authorship Contributions

A.B.N. performed experiments, analyzed data, and drafted the manuscript with JW; M.Z., A.D. performed experiments and A.D. conceived the study with J.W.; R.W. planned and conducted statistical analysis and co-wrote the sections on statistical analysis and results; H.H. ascertained and managed Guthrie Cards and laboratory activities; L.M., C.J., K.G., S.K., C.M., P.B. contributed to the analysis and reviewed the manuscript; X.M. and J.W. designed the overall study, planned the analysis, and helped to draft the manuscript.

1. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, 2001–10: a population-based registry study. Lancet Oncol. 2017;18(6):719–731.

2. Hudson MM, Ness KK, Gurney JG, et al. Clinical Ascertainment of Health Outcomes Among Adults Treated for Childhood Cancer. JAMA. 2013;309(22):2371.

3. Greaves M. A causal mechanismn for childhood acute lymphoblastic leukaemia. Nat Rev Cancer. 2018;18(8):471-484.

4. Chang JS, Tsai C-R, Tsai Y-W, Wiemels JL. Medically diagnosed infections and risk of childhood leukaemia: a population-based case–control study. Int J Epidemiol. 2012;41(4):1050–1059.

5. Crouch S, Lightfoot T, Simpson J, Smith A, Ansell P, Roman E. Infectious Illness in Children Subsequently Diagnosed With Acute Lymphoblastic Leukemia: Modeling the Trends From Birth to Diagnosis. Am J Epidemiol. 2012;176(5):402–408.

6. Urayama KY, Buffler PA, Gallagher ER, Ayoob JM, Ma X. A meta-analysis of the association between day-care attendance and childhood acute lymphoblastic leukaemia. Int J Epidemiol. 2010;39(3):718–732.

7. Wang R, Wiemels JL, Metayer C, et al. Cesarean Section and Risk of Childhood Acute Lymphoblastic Leukemia in a Population-Based, Record-Linkage Study in California. Am J Epidemiol. 2017;185(2):96–105.

8. Munder M. Arginase: An emerging key player in the mammalian immune system: REVIEW. Br J Pharmacol. 2009;158(3):638–651.

9. Badurdeen S, Mulongo M, Berkley JA. Arginine depletion increases susceptibility to serious infections in preterm newborns. Pediatr Res. 2015;77(2):290–297.

10. McGovern N, Shin A, Low G, et al. Human fetal dendritic cells promote prenatal T-cell immune suppression through arginase-2. Nature. 2017;546(7660):662–666.

11. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. Nat Immunol. 2018;19(2):108–119.

12. He YM, Li X, Perego M, et al. Transitory presence of myeloid-derived suppressor cells in neonates is critical for control of inflammation. Nat Med. 2018;24(2):224–231.

13. Elahi S, Ertelt JM, Kinder JM, et al. Immunosuppressive CD71+ erythroid cells compromise neonatal host defence against infection. Nature. 2013;504(7478):158–162.

14. Chang JS, Zhou M, Buffler PA, Chokkalingam AP, Metayer C, Wiemels JL. Profound deficit of IL10 at birth in children who develop childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev. 2011;20(8):1736–1740.

15. Søegaard SH, Rostgaard K, Skogstrand K, Wiemels JL, Schmiegelow K, Hjalgrim H. Neonatal inflammatory markers are associated with childhood b-cell precursor acute lymphoblastic Leukemia. Cancer Res. 2018;78(18):5458-5463.

Table 1. Characteristics of Childhood Acute Lymphoblastic Leukemia Cases and Controls

		Case	Control	p
		n (%)	n (%)	
Total		137	492	
Sex				
	Male	78 *(57)*	283 *(58)*	0.9
	Female	59 *(43)*	209 *(42)*	
Race/ethnicity				
	Hispanic	74 *(54)*	279 *(57)*	0.85
	Non-Hispanic White	41 *(30)*	139 *(28)*	
	Other	22 *(16)*	74 *(15)*	
Gestational age (weeks)				
	<37	13 *(9)*	46 *(9)*	0.88
	37-41	112 *(82)*	397 *(81)*	
	≥42	9 *(7)*	26 *(5)*	
	Unknown	3 *(2)*	23 *(5)*	
Birth weight (grams)				
	<2500	4 *(3)*	19 (4)	0.63
	2500-2999	23 *(17)*	74 *(15)*	
	3000-3499	57 *(42)*	193 *(39)*	
	3500-3999	44 *(32)*	153 *(31)*	
	≥4000	9 *(7)*	53 *(11)*	
Birth order				
	First	55 *(40)*	185 *(38)*	0.8
	Second	46 *(34)*	165 *(34)*	
	Third or higher	36 *(26)*	142 *(29)*	
Plurality				
	Singleton	131 (96)	480 *(98)*	0.23
	Multiple	6 *(4)*	12 *(2)*	
Delivery mode				
	Vaginal delivery	84 *(61)*	354 *(72)*	0.02
	Cesarean delivery	53 *(39)*	138 *(28)*	
Year of birth				
	2000-2002	49 *(36)*	173 *(35)*	0.7
	2003-2005	61 *(45)*	206 *(42)*	
	2006-2009	27 *(20)*	113 *(23)*	
Age at neonatal blood collection (hours)				
	Median (interquartile range)	29 *(25-40)*	27*(23-36)*	0.01
Mother's age at delivery (years)				
	<25	30 *(22)*	149 *(30)*	0.15

25-34	85 *(62)*	269 *(55)*	
≥35	22 *(16)*	74 *(15)*	
Mother's place of birth			
United States	78 *(57)*	262 *(53)*	0.44
Other	59 *(43)*	230 *(47)*	

Figure Legends:

Figure 1. Adjusted odds ratios for quartiles of neonatal Arginase 2 associated with childhood acute lymphoblastic leukemia (ALL) (n=137 cases, n=492 controls). Adjusted for sex, race/ethnicity (non-Hispanic white, Hispanic white and other), birth weight (<2500, 2500-2999, 3000-3499, 3500-3999, ≥4000), birth order (first, second, third or higher), plurality (singleton, multiple), delivery mode (vaginal, cesarean delivery), year of birth, age at neonatal blood collection, mother's age at delivery (<25, 25-34, ≥35), and mother's place of birth (United States, other). Abbreviations: OR: odds ratio; LCL – lower 95% confidence limit; UCL – upper 95% confidence limit.

Supplementary Table 1: ARG2 levels by case and control status, and within quartiles (protein-corrected)

		cases				controls		
ARG2	n	median	mean	range		median	mean	range
overall	137	8.0	12.1	0.83-242.3	492	6.9	11.6	1.0-279.7
Q1	21	3.2	3.0	0.83-4.2	123	3.2	3.2	1.0-4.6
Q2	29	5.7	5.7	4.7-6.7	123	5.7	5.7	4.6-6.9
Q3	44	8.5	8.6	6.9-10.7	123	8.5	8.7	6.9-10.9
Q4	43	14.2	24.3	10.9-242.3	123	16.5	28.6	10.9-279.7

Supplementary Table 2: ARG2 levels (protein corrected) by demographic factors

		ARG2 cases (N = 137)			ARG2 controls (N = 492)		
		median	mean	range	median	mean	range
race	White	8.6	10.8	3.2-42.9	7.2	11.1	1.4-192.9
	Hispanic	8.0	10.0	0.8-45.3	7.5	12.6	1.0-279.7
	Other	7.7	21.4	1.1-242.3	5.7	8.4	1.5-61.9
Sex	Female	7.9	9.3	0.8-39.1	6.8	12.0	1.2-279.7
	Male	8.3	14.1	1.5-242.3	7.1	11.2	1.0-222.2
age at collecti(	mean (SD)						
(hours)							
birthweight (g)	<2500	10.9	10.2	4.8-14.2	7.3	11.1	3.2-61.9
	2500-2999	7.5	10.9	1.1-45.3	6.9	13.8	2.0-279.7
	3000-3499	9.1	16.3	2.6-242.3	6.8	11.9	1.0-49.6
	3500-3999	7.7	8.2	0.83-22.6	6.7	9.2	1.5-49.6
	4000+	7.3	7.6	4.2-13.5	7.9	13.9	2.1-222.2
gestational age	<37	10.7	32.5	4.8-242.3	6.6	15.7	2.0-279.7
	37-41	7.6	9.9	0.83-68.7	6.8	10.7	1.2-192.9
	42+	9.6	9.3	3.5-13.1	7.1	16.9	1.0-192.9
	Unknown	11.8	11.7	11.2-12.0	9.3	11.8	1.6-30.5
Plurality	No	8.0	12.0	0.83-242.3	6.9	11.6	1.0-279.7
	Yes	9.7	14.1	2.5-39.2	7.1	8.3	1.2-25.3

birth order	1	8.0	10.9	1.1-68.7	7.2	13.7	1.5-279.7
	2	8.0	14.1	0.83-242.3	6.9	9.4	1.8-84.4
	3+	8.4	11.2	1.8-45.4	6.3	11.3	1.0-192.9
C-section	No	7.6	9.2	1.1-42.9	6.8	12.7	1.5-279.7
	Yes	9.6	16.6	0.83-242.3	7.2	8.5	1.0-45.5
Mother's age	<25	9.2	10.2	2.6-22.9	6.7	11.9	1.5-192.9
	25-34	8.0	10.6	0.83-68.7	7.0	11.7	1.0-279.7
	35+	7.5	20.3	2.7-242.3	6.9	10.4	1.5-84.4
Mother's birthplace	US	8.0	9.8	1.5-42.9	6.8	10.2	1.5-192.9
	Other	8.4	15.1	0.83-242.3	6.9	13.1	1.0-279.7
Year of birth	2000	7.1	7.5	3.3-15.5	7.7	11.4	1.7-93.0
	2001	7.2	10.0	0.83-68.7	8.7	19.2	1.5-279.7
	2002	10.5	13.9	5.3-45.4	7.9	13.9	3.3-192.9
	2003	9.7	23.8	4.7-242.3	7.5	9.5	2.6-37.6
	2004	9.1	10.6	1.5-39.1	5.5	9.9	1.0-135.7
	2005	6.3	12.4	3.1-42.9	5.8	12.4	1.5-222.2
	2006	7.6	9.5	3.1-42.9	6.0	8.0	1.7-30.5
	2007	6.1	7.6	2.5-30.6	5.9	7.2	2.2-18.7
	2008	7.7	9.6	3.9-20.3	5.9	7.6	1.8-22.6
	2009				4.7	4.7	2.0-7.3

All values are expressed as ng Arginase-2 per ug protein X 10

Supplementary Table 3: Case-control resuts for ARG2 levels while exclude participants delivered by c-section, and quartiles cutoffs based on all controls (n=84 cases, n=354 controls)

ARG2	Case n (%)	Control n (%)	OR	95% CI	p
1st quartile	12 (14.3)	84 (23.7)	1		
2nd quartile	21 (25.0)	95 (26.8)	1.48	0.67-3.31	0.33
3rd quartile	29 (34.5)	84 (23.7)	2.39	1.10-5.17	0.03
4th quartile	22 (26.2)	91 (25.7)	1.77	0.82-3.81	0.14
Trend			1.22	0.98-1.52	0.07

Supplementary Table 4: Case-control resuts for ARG2 levels with all participants adjusted for c-section, and without adjustment (n=137 cases, n=492 controls)

A: with C-section

ARG2	OR	95% CI	p
1st quartile	1		
2nd quartile	1.49	0.79-2.80	0.26
3rd quartile	2.2	1.21-3.99	0.01
4th quartile	2.28	1.28-4.07	0.01
Trend	1.31	1.10-1.57	<0.01

B: without C-section

OR	95% CI	p
1		
1.42	0.76-2.66	0.28
2.17	1.20-3.92	0.01
2.26	1.27-4.01	0.01
1.32	1.11-1.57	<0.01

Adjusted odds ratios for quartiles of neonatal Arginase 2 associated with childhood acute lymphoblastic leukemia (ALL) (n=137 cases, n=492 controls).
Adjusted for sex, race/ethnicity (non-Hispanic white, Hispanic white and other), birth weight (<2500, 2500-2999, 3000-3499, 3500-3999, ≥4000),
birth order (first, second, third or higher), plurality (singleton, multiple), delivery mode (***A*** only: vaginal, cesarean delivery), year of birth, age at neonatal blood collection,
mother's age at delivery (<25, 25-34, ≥35), and mother's place of birth (United States, other).

Supplementary Table 5: Case control results for ARG2 levels for B-cell ALL only (ICD-O-3:9836, case=100, control=492)

ARG2	Case n (%)	Control n (%)	OR	95% CI	p
1st quartile	15(15.0)	123(25.0)	1		
2nd quartile	25(25.0)	123(25.0)	1.82	0.88-3.75	0.11
3rd quartile	34(34.0)	123(25.0)	2.36	1.18-4.71	0.02
4th quartile	26(26.0)	123(25.0)	1.91	0.93-3.93	0.08
Trend			1.22	1.00-1.49	0.05

Adjusted odds ratios for quartiles of neonatal Arginase 2 associated with childhood acute lymphoblastic leukemia (ALL) (n=137 cases, n=492 controls).
Adjusted for sex, race/ethnicity (non-Hispanic white, Hispanic white and other), birth weight (<2500, 2500-2999, 3000-3499, 3500-3999, ≥4000),
birth order (first, second, third or higher), plurality (singleton, multiple), delivery mode (vaginal, cesarean delivery), year of birth, age at neonatal blood collection,
mother's age at delivery (<25, 25-34, ≥35), and mother's place of birth (United States, other).