Reply to Suderman et al.: Importance of accounting for blood cell composition in epigenetic studies

In our paper (1), we primarily questioned whether the statistical approach used by Borghol et al. (2) was justified, because treating neighboring probes as independent can lead to inflated *P* value significance. We are glad to see that in their Letter, Borghol et al. do not dispute this (3). We are also pleased that Borghol et al. concur with our concerns regarding their description of procedures for determining the false-discovery rate (FDR) (1, 3). A thorough description of the FDR approach is of great importance in studies that measure many events such as genome-wide CpG methylation in small cohorts.

We acknowledge the efforts by Borghol et al. (2) to account for individual differences in blood cell composition by comparing their profiles to publically available profiles of isolated B and T cells obtained on different DNA-methylation assay platforms. With that being said, we do not feel that their approach sufficiently addressed this issue and its potential for confounding socioeconomic status (SES)-dependent correlations. Specifically, we note that lymphocytes (which include T and B cells, as well as natural killer cells) typically constitute 20–40% of the leukocytes in most adult whole-blood samples. Most of the remaining cells are granulocytes (typically 40–70%). In studies like ours that use peripheral blood mononuclear cells, granulocytes are removed by centrifugation before DNA extraction. However, in studies of whole blood, granulocytes remain in the sample, and their relatively high prevalence raises concerns that these cells account for a disproportionately large percentage of the DNA-methylation signal, which, in turn, could confound any association it shows with environmental variables like SES. For these reasons, we believe that differences between individuals in granulocyte percentages need to be accounted for, either by physically removing them before analysis or, at the minimum, by statistically adjusting for their prevalence in any correlation analysis. Unfortunately, Borghol et al. did not address the granulocyte issue in their bioinformatics approach.

Last, we also note that Borghol et al. are incorrect in asserting that the array we used was biased toward cancer-related genes (3). This was true for the initial Illumina GoldenGate array but not the Illumina Infinium HumanMethylation27 BeadChip used by us, which contains 27,578 informative CpG sites located in ∼14,000 promoter regions, thus representing more than half of all human genes (4).

Regardless, as we mentioned in our paper (1), despite the differences, both of our studies support a general association of early-life SES and adult DNA methylation. As such, we agree with Borghol et al. that future studies, using appropriate statistics and larger cohorts with well-characterized cellular material, may reveal many more CpG sites associated with early-life SES.

Lucia L. Lam[a], Eldon Emberly[b], Hunter B. Fraser[c], Sarah M. Neumann[a], Edith Chen[d], Gregory E. Miller[d,1], and Michael S. Kobor[a,e,1]

[a]Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute and [e]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada, V5Z 4H4; [b]Department of Physics, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6; [c]Department of Biology, Stanford University, Stanford, CA 94305; and [d]Department of Psychology and Cells to Society (C2S): The Center on Social Disparities and Health, Institute for Policy Research, Northwestern University, Evanston, IL 60208

1 Lam LL, et al. (2012) Factors underlying variable DNA methylation in a human community cohort. *Proc Natl Acad Sci USA* 109(Suppl 2):17253–17260.
2 Borghol N, et al. (2012) Associations with early-life socio-economic position in adult DNA methylation. *Int J Epidemiol* 41(1): 62–74.
3 Suderman M, et al. (2013) Epigenomic socioeconomic studies more similar than different. *Proc Natl Acad Sci USA* 110: E1246.
4 Bibikova M, et al. (2009) Genome-wide DNA methylation profiling using Infinium® assay. *Epigenomics* 1(1):177–200.

Author contributions: L.L.L., E.E., H.B.F., S.M.N., E.C., G.E.M., and M.S.K. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. E-mail: greg.miller@northwestern.edu or msk@cmmt.ubc.ca.