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LETTER TO THE EDITOR

Social-epigenetic mediators for racial disparities in pulmonary impairment among childhood cancer survivors

Dear Editor

Previous research found that childhood cancer survivors of African ancestry have significantly higher morbidity and mortality than those of European ancestry [1]. However, after adjusting for socio-economic factors, the magnitudes of racial health disparities are either substantially decreased or become statistically non-significant [1], suggesting that social and economic determinants may contribute to racial health disparities. Recently, we conducted epigenome-wide association studies (EWAS) for three key social determinants of health (SDOHs), namely, personal educational attainment, personal income, and neighborhood deprivation among survivors of childhood cancer, where 130 epigenome-wide significant SDOH-CpG associations were identified among European ancestry survivors, and 25 of which were also validated in African ancestry survivors [2]. Notably, many SDOH-associated CpG sites are also associated with tobacco use.

Although pulmonary impairment is an integral part of the overall disease burden, racial disparities in this specific group of conditions have not been documented, and potential underlying mechanistic causal pathways have not been studied. Moreover, other observational studies have shown that blood DNA methylation (DNAm) signature was associated with pulmonary functions [3–5]. In this cross-sectional study, we hypothesized that race and its associated SDOHs might contribute to the risk of pulmonary impairment, evaluated whether SDOH-associated CpG sites were associated with specific parameters of pulmonary function, and further applied mediation analysis to explore the potential mediating role of these DNAm sites for the association between SDOHs and risk of impaired pulmonary functions. The methods are described in **Supplementary Methods** and **Supplementary Table S1**.

The occurrence rates of three adverse pulmonary outcomes, obstructive pulmonary deficit (OPD), pulmonary diffusion deficits (PDD), and restrictive pulmonary deficit (RPD), were compared between African- and European ancestry survivors in the St. Jude Lifetime Cohort (SJLIFE) study [6]. The study population is described in the **Supplementary Results** and **Supplementary Table S2**. In an unadjusted model considering common terminology criteria for adverse event (CTCAE) grade ≥2, the occurrence rates of PDD and RPD were significantly higher in African ancestry survivors than in European ancestry survivors (PDD: 25.2% vs. 18.2%, $P = 0.033$; RPD: 14.2% vs. 7.5%, $P = 0.002$), whereas OPD was comparable between the two race groups (9.8% vs. 13.1%, $P = 0.206$) (**Supplementary Figure S1**). In a multivariable model, adjusting for other covariates but without the inclusion of SDOHs (the base model), race was significantly associated with PDD ($P = 0.024$) and RPD ($P = 0.004$, **Supplementary Table S3**). When SDOHs were added to the model (the full model), the effect of race on pulmonary impairment became non-significant for PDD ($P = 0.183$), slightly attenuated for RPD ($P = 0.006$) and remained non-significant for OPD ($P = 0.434$). Notably, treatment factors, including chest RT and lung surgery, were significantly associated with all three conditions. The effect of current smokers became non-significant for OPD and PDD. Interestingly, BMI was inversely associated with PDD.

Because SDOHs only accounted for the racial disparity in PDD from the above analyses, we further analyzed if SDOH-associated CpG sites could mediate the association between SDOHs and PDD. Among the 130 SDOH-CpG associations identified in our previous EWAS on European ancestry survivors [2], 61 CpGs (29 for educational attainment, 16 for personal income, and 16 for area deprivation index [ADI]) were significantly associated with the risk of PDD after adjusting for multiple comparisons

Abbreviations: EWAS, Epigenome-wide association studies; SDOH, Social determinants of health; DNAm, DNA methylation; OPD, Obstructive pulmonary deficit; PDD, Pulmonary diffusion deficits; RPD, Restrictive pulmonary deficit; SJLIFE, St. Jude Lifetime Cohort; CTCAE, Common terminology criteria for adverse event; ADI, Area deprivation index; FDR, False-discovery rate; CPOX, Coproporphyrinogen oxidase; ACLY, ATP citrate lyase; HPS4, Hermansky-Pudlak syndrome 4; CLDND1, Claudin domain containing 1; ACME, Average causal mediation effects; meQTL, Methylation quantitative trait loci.

($P_{\text{false discovery rate (FDR)}} < 0.050$) (**Supplementary Table S4**), some of which have been previously reported to be associated with health conditions related to pulmonary functions. In the mediation analysis, 17 out of 29 educational attainment-associated CpGs were identified with significant average causal mediation effects (ACME) after adjusting for multiple comparisons. Using a squared pairwise Pearson correlation coefficient r^2 threshold of 0.05, three independent CpGs, cg04180924 (chr3, coproporphyrinogen oxidase [*CPOX*], mediation = 32.9%, $P_{\text{FDR}} = 0.014$), cg11205006 (chr22, *HPS4*, mediation = 19.0%, $P_{\text{FDR}} = 0.024$), and cg27470486 (chr17, ATP citrate lyase [*ACLY*], mediation = 8.6%, $P_{\text{FDR}} = 0.044$), were obtained by top-down pruning the 17 CpGs sorted by estimated ACME in decreasing order. For the final mediation analysis, a combined score (i.e., summation of DNAm levels of the three CpGs with the same direction of association) was used as the mediating variable, and a 48.9% mediation effect for educational attainment on PDD was achieved (**Table 1**). Similarly, the same single mediator, cg08064403, partially mediated the effect of personal income (mediation = 25.9%, $P < 0.001$) and ADI (mediation = 24.1%, $P < 0.001$) on PDD (**Table 1**). None of the SDOH-associated CpG sites was significantly associated with the risk of PDD among African ancestry survivors after adjusting for multiple comparisons because of the small number of African-ancestry survivors, hence similar mediation analysis could not be conducted among African ancestry survivors.

For each CpG mediating the association between SDOHs and pulmonary conditions in European ancestry survivors, a linear regression of expression levels for Illumina-annotated genes against DNAm levels of CpGs was performed. The DNAm levels of four CpGs were negatively correlated with the gene expression levels of Illumina-annotated genes: *ACLY* (cg27470486), which plays a role in lipid synthesis in the lung [7]; Hermansky-Pudlak syndrome 4 (*HPS4*) (cg11205006), which is related to pulmonary fibrosis [8]; *CPOX* (cg04180924 and cg08064403) [9] and claudin domain containing 1 (*CLDND1*) (cg08064403) [10], two smoking-related genes (**Supplementary Figures S2-S3** and **Supplementary Table S5**). Based on these correlations between DNAm and gene expression levels, all four CpGs were deemed as expression quantitative trait methylations.

We leveraged the molecular profiling data of the well-established SJLIFE cohort and provided strong evidence supporting social-epigenetic mediators for racial disparities in pulmonary impairment among childhood cancer survivors. The present study had some limitations. First, the analysis was based on a relatively short follow-up from blood drawn for DNAm detection, so there was no clearly defined temporal association to establish the causality.

TABLE 1 DNA methylation partially mediates the association between social determinants of health factors and risk of pulmonary diffusion deficits in European ancestry

Social determinants of Health	CpG	Chr	Position	HGNC gene	ACME	ADE	Total effect*	Mediation†	P_{FDR}
Educational attainment	cg04180924	chr3	98,553,219	*CPOX*	−0.0137	−0.0280	−0.0417	32.9%	0.014
	cg11205006	chr22	26,479,532	*HPS4*	−0.0058	−0.0246	−0.0304	19.0%	0.024
	cg27470486	chr17	41,917,434	*ACLY*	−0.0029	−0.0307	−0.0336	8.6%	0.044
	Combined methylation score				−0.0214	−0.0224	−0.0438	48.9%	0.002
Personal income	cg08064403	chr3	98,521,413	*CLDND1;CPOX;RP11-227H4.5*	−0.0101	−0.0290	−0.0392	25.9%	<0.001
Area deprivation index	cg08064403	chr3	98,521,413	*CLDND1;CPOX;RP11-227H4.5*	0.0002	0.0007	0.0009	24.1%	<0.001

Abbreviations: Chr, chromosome; HGNC, TheHUGO genome nomenclature committee; ACME, average causal mediation effect; ADE, average direct effect; FDR, false discovery rate.

*Calculated by summing ACME and ADE.

†Calculated by ACME divided by total effect (the sum of ACME and ADE).

Second, we attempted to take advantage of the existing whole-genome sequencing data to search for methylation quantitative trait loci (meQTL), but we did not find any strong meQTL for the genomic regions of interest that could be used in Mendelian randomization for causal inference. Third, air pollution from neighborhoods or occupational exposures and second-hand smoke, which may also contribute to pulmonary impairment, were also not considered due to the lack of data. Lastly, our analysis was based on each individual condition separately, other factors including co-morbidity (e.g., among 209 survivors with PDD, 70 had OPD, 53 had RPD, and 42 had both OPD and RPD), type and stage of primary diagnosis may also confound the results.

In conclusion, the risk of pulmonary impairment among survivors of childhood cancer differs by specific condition (PDD or RPD) and race (African and Europena ancestry). SDOHs may partially explain the observed racial disparity in PDD, potentially through an epigenetic mechanism. Social-epigenetic studies like ours could inform intervention strategies, such as improving social integration and social support to counteract the elevated disease risk for social-economically disadvantaged survivors. The efficacy of this type of intervention can be objectively measured by the improvement of epigenetic markers as an intermediate outcome. Ultimately, we will close the gap of disparity in pulmonary impairment and other health outcomes due to race or social adversity among survivors of childhood cancer.

DECLARATIONS

AUTHORS' CONTRIBUTIONS

ZW and ICH designed and supervised the study; MMH, KKN, KRK and LLR assisted in or provided support for data collection and recruitment of study participants; JE, HM, EP, GN, EW, and JZ supervised sample processing, and/or performed DNA/RNA extractions, carried out the Infinium MethylationEPIC array scanning and RNA sequencing; NS, QD, CC, QL, DKS, ICH, and ZW performed bioinformatic and statistical analysis; NS, QD, ICH, and ZW wrote the first draft of the manuscript. All authors contributed to data interpretation and writing and approved the final manuscript for publication.

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Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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DATA AVAILABILITY STATEMENT

DNA methylation data are accessible at NCBI Gene Expression Omnibus database under the accession number GSE169156 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE169156). Additional clinical data about the study participants in the St. Jude Lifetime Cohort can be accessed via the survivorship portal (http://survivorship.stjude.cloud/).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The SJLIFE study protocol was approved by the Institutional Review Board (IRB) at St. Jude Children's Research Hospital with a reference number (010882). All SJLIFE study participants provided written informed consent. This study was performed in accordance with the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Not applicable.

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SUPPORTING INFORMATION

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