Original Research Article

ISEE Environmental Epidemiology

OPEN

Genome-wide DNA methylation profiles and breast cancer among World Trade Center survivors

Stephanie Tuminello[a], Yibeltal Arega Ashebir[a], Chanel Schroff[b], Sitharam Ramaswami[b], Nedim Durmus[c], Yu Chen[a,d], Matija Snuderl[b], Yongzhao Shao[a,d], Joan Reibman[c,e], Alan A. Arslan[a,d,f,*]

Background: Increased incidence of cancer has been reported among World Trade Center (WTC)-exposed persons. Aberrant DNA methylation is a hallmark of cancer development. To date, only a few small studies have investigated the relationship between WTC exposure and DNA methylation. The main objective of this study was to assess the DNA methylation profiles of WTC-exposed community members who remained cancer free and those who developed breast cancer.

Methods: WTC-exposed women were selected from the WTC Environmental Health Center clinic, with peripheral blood collected during routine clinical monitoring visits. The reference group was selected from the NYU Women's Health Study, a prospective cohort study with blood samples collected before 9 November 2001. The Infinium MethylationEPIC array was used for global DNA methylation profiling, with adjustments for cell type composition and other confounders. Annotated probes were used for biological pathway and network analysis.

Results: A total of 64 WTC-exposed (32 cancer free and 32 with breast cancer) and 32 WTC-unexposed (16 cancer free and 16 with prediagnostic breast cancer) participants were included. Hypermethylated cytosine–phosphate–guanine probe sites (defined as $\beta > 0.8$) were more common among WTC-exposed versus unexposed participants (14.3% vs. 4.5%, respectively, among the top 5000 cytosine–phosphate–guanine sites). Cancer-related pathways (e.g., human papillomavirus infection, cGMP-PKG) were overrepresented in WTC-exposed groups (breast cancer patients and cancer-free subjects). Compared to the unexposed breast cancer patients, 47 epigenetically dysregulated genes were identified among WTC-exposed breast cancers. These genes formed a network, including Wnt/β-catenin signaling genes *WNT4* and *TCF7L2*, and dysregulation of these genes contributes to cancer immune evasion.

Conclusion: WTC exposure likely impacts DNA methylation and may predispose exposed individuals toward cancer development, possibly through an immune-mediated mechanism.

Keywords: World Trade Center; Epigenetics; DNA methylation; Breast cancer

Introduction

Cancer rates among World Trade Center (WTC) responders are elevated.[1–5] The most recent data suggest that the incidence of prostate, thyroid, tonsil, and melanoma cancers are higher than expected and the aging of WTC responders and increasing latency period since 9 November 2001 make it likely that cancer rates will continue to rise.[6] Cancer diagnoses among the WTC-exposed local community member survivors are common, with breast cancer being the most frequently diagnosed subtype.[7,8] WTC-associated cancers, in general, maybe more aggressive than those diagnosed in unexposed individuals,[9–11] with WTC-associated breast cancers more likely to be poorly differentiated and with an aggressive molecular subtype.[12] To date, the biological mechanisms of WTC-associated carcinogenesis remain poorly understood, complicating preventive and therapeutic care. Given the complex mix of carcinogenic agents present in the WTC dust including metals, asbestos, and organic pollutants,[1,5] and the capacity of these chemicals to modify the DNA methylation,[13–22] it has been hypothesized that WTC dust exposure may lead to epigenome-wide changes. More specifically, DNA methylation is a dynamic method of epigenetic regulation sensitive to environmental stimuli.[17] DNA methylation occurs with the addition of a methyl group to the cysteine base at a cytosine–phosphate–guanine (CpG) site.[17,23] DNA methylation of gene promoter sites will often result in the silencing of gene expression.[23] Aberrant DNA methylation is a hallmark of cancer

[a]Department of Population Health, NYU Grossman School of Medicine, New York City, New York; [b]Department of Pathology, NYU Grossman School of Medicine, New York City, New York; [c]Department of Medicine, NYU Grossman School of Medicine, New York City, New York; [d]NYU Perlmutter Comprehensive Cancer Center, New York City, New York; [e]Division of Environmental Medicine, Department of Medicine, NYU Grossman School of Medicine, New York City, New York; and [f]Department of Obstetrics and Gynecology, NYU Grossman School of Medicine, New York City, New York

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Boards of NYU Grossman School of Medicine and Bellevue Hospital (IRB numbers: s17-01207 and i21-00717).

This work was funded through NYU Laura & Isaac Perlmutter Comprehensive Cancer Center Support Inter-Disciplinary Population Research Pilot Grant Program (P30CA016087) and CDC/NIOSH grant 5R21OH012238. The WTC Environmental Health Center and the WTC EHC Data Center are funded by CDC/NIOSH contracts 200 – 2017 – 93327 and 200 – 2017 – 93427. S.T. was supported in part by NYU's T32 Training Program in Healthcare Delivery Science and Population Health Research funded by the Agency for Healthcare Research and Quality (AHRQ) grant HS026120.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.environepidem.com).

*Corresponding Author. Address: Department of Population Health, NYU Grossman School of Medicine, 180 Madison Avenue, New York, NY 10016. E-mail: Alan.Arslan@nyulangone.org (A.A. Arslan).

What this study adds:
This is one of the first epigenome-wide association studies of the WTC-exposed survivors. WTC exposures are associated with specific genome-wide methylation changes that may contribute toward cancer development.

development and progression.[24,25] Global DNA methylation changes, and altered DNA methylation of tumor suppressor and oncogenes, play an important role in early tumorigenesis.[24–26]

To date, few studies have investigated the relationship between WTC exposure and DNA methylation, including our initial two pilot projects.[27,28] Our first pilot study compared global DNA methylation profiles in WTC-exposed cancer-free survivors (n = 18) versus age-matched cancer-free unexposed women (n = 24).[27] Increased global methylation was observed among WTC-exposed subjects. WTC exposure was associated with significant differentially methylated CpG sites affecting several cancer-related pathways.[27] The second pilot study was conducted using peripheral blood samples from WTC-exposed (n = 28) and unexposed women (n = 24) diagnosed with breast cancer, using the same methodology.[28] Again, global hypermethylation among WTC-exposed breast cancer cases was observed compared to unexposed cases. Tumor suppressors and breast cancer-related genes were epigenetically dysregulated and several cancer and immune-related pathways were enriched among the WTC-exposed breast cancer cases.[28] These results suggested that WTC exposure may be linked to genome-wide DNA methylation changes and contribute to cancer development. However, as these were primarily feasibility studies, the interpretation of the data was limited by the small sample size. Although the WTC-exposed and unexposed participants were age matched, other important confounding factors, such as race/ethnicity, smoking status, body mass index (BMI), and education, were not considered. The main objective of the current study was to validate the results of our previous epigenome-wide association studies (EWAS) of WTC-exposed individuals in both cancer-free and breast cancer cases using an independent set of WTC-exposed and unexposed individuals while minimizing prior limitations. To reduce the potential bias from genome-wide testing and to better understand the functional pathways of WTC-associated differential DNA methylation,[29] we augmented the gene set enrichment analysis with network analysis. The newly collected data were analyzed both separately and together with pilot study data to further increase the sample size.

Methods

Study participants and sample collection

After obtaining the institutional review board-approved informed consent, WTC-exposed women were enrolled in the World Trade Center Environmental Health Center (WTC EHC) clinic at Bellevue Hospital in New York City from 16 November 2021 to 15 December 2022. Funded under the James Zadroga 9/11 Health and Compensation Act, the WTC EHC acts as a "center of excellence" for the treatment and surveillance of WTC-affected community members (also called "survivors").[8,30] Patients self-enroll in the program and are required by law to have a "certifiable condition," such as cancer, in addition to WTC exposure.[8,31,32] Descriptive characteristics of the WTC EHC population and diagnosed cancer cases have been previously published.[7] Enrollment into the WTC EHC is ongoing and 1373 breast cancer cases have been diagnosed among the WTC EHC members as of 31 December 2023. During the initial and monitoring visits, participants complete interviewer-administered questionnaires with questions about demographics, occupational and lifestyle factors, medical history, and detailed WTC exposure information.[8,32] Patients are invited for follow-up monitoring visits every 12–18 months.[8] WTC EHC enrollees who were 35–65 years old at the time of blood donation were eligible for this study and were excluded if they were pregnant or breastfeeding, or had been pregnant or breastfeeding in the 6 months before blood donation. A total of 32 WTC-exposed cancer-free women were enrolled in this study. An additional 32 WTC-exposed breast cancer cases were also enrolled postdiagnostically. Breast cancer patients had additional eligibility criteria that they had been diagnosed with invasive ductal carcinoma of the breast and did not have another primary cancer diagnosis, as verified by the medical records. Recently diagnosed (within 5 years) patients with early-stage cancer without a history of systemic (chemotherapy or radiation) treatment were preferentially chosen for participation in the current study to reduce the potential effect of cancer treatments on DNA methylation. Peripheral blood specimens for this study were collected at the time of the patient standard clinical exam and stored at −80 °C.

WTC-unexposed controls were selected from the NYU Women's Health Study (NYUWHS). Between March 1985 and June 1991, 14,274 women were enrolled in the NYUWHS prospective cohort at the Guttman Breast Diagnostic Institute, a mammography screening center in New York City.[33,34] Participants completed a self-administered baseline questionnaire that collected demographic, medical, reproductive, and other health information.[34] Cohort participation required donation of peripheral blood, which was subsequently placed in long-term storage.[34] The inclusion/exclusion criteria of the WTC-unexposed NYUWHS participants were the same as for the WTC-exposed participants. NYUWHS participants were cancer-free at the time of enrollment and blood donation and have been followed up regularly. More than 2000 incident breast cancer cases have been identified in the NYUWHS through active follow-up, either at annual mammographic screening or by mailed questionnaires, with diagnoses confirmed through the state cancer registries of New York, New Jersey, Connecticut, and Florida, and obtaining medical and pathology reports.[35] For the NYUWHS participants who later developed breast cancer, only blood samples collected prediagnostically (within 5 years) were eligible. NYUWHS participants were frequency matched to newly collected samples from WTC EHC participants based on age at blood donation (within 5 years); with 32 WTC EHC exposed cancer-free women matched to 16 NYUWHS cancer-free women, and 32 WTC EHC breast cancer cases matched to 16 NYUWHS prediagnostic breast cancer cases (Tables S1 and Figure S1; http://links.lww.com/EE/A278).

Sample processing, DNA methylation profiling, and differential DNA methylation analysis

All samples were sent to the NYU Langone Health Center for Biospecimen Research and Development laboratory for DNA extraction. DNA was recovered from white blood cells using the PicoPure DNA extraction kit (Thermo Fisher Scientific, Boston, Massachusetts), and then subjected to bead purification with the Sphere quality control kit (Thermo Fisher Scientific, Boston, Massachusetts). DNA purity and quantity were assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). To improve base-pair resolution, DNA was bisulfite converted using the EZ-96 DNA methylation kit (Zymo Research, Irvine, California). After bisulfite conversion, each sample was whole-genome amplified, enzymatically fragmented, and purified.

DNA was then hybridized to the Infinium MethylationEPIC array (Illumina, San Diego, California), which allows to

Environmental Epidemiology (2024) 8:e313

Received 12 February, 2024; Accepted 8 May, 2024

Published online 4 June 2024

DOI: 10.1097/EE9.0000000000000313

Table 1.

Descriptive characteristics of the WTC-exposed versus unexposed participants

	Cancer-free participants		Breast cancer cases	
	WTC exposed (n = 32)	WTC unexposed (n = 16)	WTC exposed (n = 32)	WTC unexposed[a] (n = 16)
Age at sample donation (yrs); mean (standard deviation)	58.7 (6.4)	55.5 (4.4)	56.9 (6.6)	54.7 (5.7)
Race/ethnicity, n (%)				
Asian	1 (3.1%)	0 (0.0%)	4 (12.5%)	0 (0.0%)
Hispanic	0 (0.0%)	3 (18.8%)	4 (12.5%)	0 (0.0%)
Non-Hispanic Black	6 (18.8%)	1 (6.2%)	14 (43.8%)	8 (50.0%)
Non-Hispanic White	15 (46.9%)	11 (68.8%)	9 (28.1%)	5 (31.2%)
Other/unknown	10 (31.2%)	1 (6.2%)	1 (3.1%)	3 (18.8%)
Smoking status, n (%)				
Ever	12 (37.5%)	6 (37.5%)	6 (18.8%)	8 (50.0%)
Never	16 (50.0%)	10 (62.5%)	26 (81.2%)	8 (50.0%)
Unknown	4 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
BMI category, n (%)				
Healthy (18.5 ≤ BMI < 25)	7 (21.9%)	6 (37.5%)	4 (12.5%)	4 (25.0%)
Obese (BMI ≥ 30)	11 (34.4%)	4 (25.0%)	10 (31.2%)	5 (31.2%)
Overweight (25 ≤ BMI < 30)	12 (37.5%)	4 (25.0%)	8 (25.0%)	7 (43.8%)
Underweight (BMI < 18.5)	0 (0.0%)	2 (12.5%)	1 (3.1%)	0 (0.0%)
Unknown	2 (6.2%)	0 (0.0%)	9 (28.1%)	0 (0.0%)
Education level, n (%)				
≤High School/vocational school	4 (12.5%)	8 (50.0%)	4 (12.5%)	8 (50.0%)
≥College	21 (65.6%)	7 (43.8%)	22 (68.8%)	4 (25.0%)
Unknown	7 (21.9%)	1 (6.2%)	6 (18.8%)	4 (25.0%)

[a]Blood sample collected from breast cancer cases prediagnostically.

determine the DNA methylation status of 866,562 CpG probe sites. To avoid batch effects, all samples (WTC-exposed and unexposed cancer-free samples, as well as WTC-exposed and unexposed breast cancers) were run on the same Illumina BeadChip array batch. The genome-wide methylation profiles of WTC-exposed versus unexposed cancer-free women and WTC-exposed versus unexposed breast cancer cases were directly compared. All statistical analyses, modeling, and visualization were performed using the open-source, statistical software R.[36] The R package "minfi" was used to process and analyze methylation data.[37] Using function "detectionP," *P* values per CpG probe sites were computed. Probes were quantile normalized and the background adjusted, using functions "preprocessQuantile" and "preprocessNoob," and used for differentially methylated probes analysis via linear regression for each CpG site. To control for overall type I error, *P* values were adjusted for multiple comparisons using a false discovery rate procedure, and an false discovery rate below 5% (*q* value) was considered statistically significant. The potential confounding of cell composition effects is widely recognized.[29] To account for this, cell type proportions (e.g., CD8T, CD4T, natural killer, B-cell, monocytes, granulocytes) were estimated using the R packages "minfi" and "FlowSorted.Blood.EPIC."[37,38] This allowed for adjustment of results by immune cell fraction per deconvolution, as described in more detail by Houseman et al.[29] Linear regression models for the top 5000 differentially methylated CpG sites were also adjusted for age at blood donation, race/ethnicity, smoking history, BMI, and education.

Functional analyses: gene set pathways and network analysis

Probes were annotated using the HumanMethylation850 manifest provided by the manufacturer (MethylationEPIC_v-1-0_B4; Illumina). Differentially methylated genes were compared against lists of known tumor suppressor genes.[39–41] Genes associated with the top 5000 differentially methylated probes were included in the pathway analysis. The R packages "missMethyl" and "clusterProfiler" were utilized.[42,43] Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway overrepresentation analysis was carried out using the "enrichKEGG" function to determine whether genes belonging to specific KEGG pathways were overrepresented among differentially methylated genes. Results were corrected by Benjamini–Hochberg multiple-comparison correction (*q* value cutoff = 0.05). The top 20 pathways were displayed using a dot plot. The "browseKEGG" function was used to further visualize enriched pathways.

Genes found in the top 20 enriched KEGG pathways were used for network analysis. Network analysis goes beyond gene set overrepresentation analysis by allowing for the investigation of candidate genes while incorporating biological knowledge regarding gene interactions. The STRING 2023 database of protein–protein association networks was used,[44] which can be found at https://string-db.org. Gene networks were further explored and visualized using Cytoscape[45] software, version 3.10.1.

Combined analysis with previous pilot data

We have completed previous pilot EWAS studies of WTC-exposed cancer-free women and WTC-exposed breast cancers (Figure S1; http://links.lww.com/EE/A278). Both projects have been described in more detail elsewhere. To consider our data with previous pilot data (separately, for each comparison group: cancer-free and breast cancer), the top 10,000 CpG sites found to be differentially methylated in the original pilot analyses, or "phase 1," (either cancer-free[27] or breast cancer[28]) were compared against the top 10,000 differentially methylated CpG sites reported here, "phase 2." In total, there were 1336 CpG sites in common between both phases of the cancer-free EWAS projects, and 2180 CpG sites in common for both phases of the breast cancer EWAS projects. Next, all data from cancer-free women (n = 50 WTC exposed, n = 40 WTC unexposed) and data from breast cancer cases (n = 60 WTC exposed, n = 40 WTC unexposed) were combined. Combined data were then analyzed for the overlapping CpG sites (1336 for cancer-free women and 2180 for breast cancer cases) adjusting for phase and used for pathway analysis as described above.

Table 2.

Global DNA methylation; number of CpG probes hypomethylated and hypermethylated per group, cancer-free comparison

	Cancer-free comparison	
	Methylation category	
	Hypo ($\beta < 0.2$)	Hyper ($\beta > 0.8$)
Top 5000 CpG sites		
WTC exposed	1546 (30.9%)	716 (14.3%)
WTC unexposed	1325 (26.5%)	227 (4.5%)
All 865,859 sites		
WTC exposed	184,261 (21.3)	302,134 (34.9)
WTC unexposed	180,539 (20.9)	292,891 (33.8)

β refers to the florescent intensity at that CpG site, where higher β is indicative of more DNA methylation at that particular CpG probe. Proportions based on all CpG sites: hypo ($\beta < 0.2$), hyper ($\beta > 0.8$), and CpG sites with β in-between ($0.2 < \beta < 0.8$).

Results

In this study, there were 64 WTC-exposed (32 cancer-free and 32 with breast cancer) and 32 WTC-unexposed (16 cancer-free and 16 with breast cancer) participants epigenetically profiled. Clinical and demographic data are reported in Table 1. Among cancer-free participants, WTC-exposed women had a mean age at blood donation of 58.7 years and 55.5 years for unexposed women. For those with breast cancer, the mean age at blood donation was 56.9 years and 54.7 years for WTC-exposed versus unexposed participants, respectively. Across all exposure and cancer groups, participants were mostly non-Hispanic Black (total 30.2%) or White (41.6%), never smokers (62.5%), who fell into the overweight (33.3%) or obese (31.2%) BMI categories. Most participants had reported some college or higher education (56.5%).

Comparison of WTC-exposed versus unexposed cancer-free participants

Across the genome, DNA methylation was found to differ according to the WTC exposure status. Among the top 5000 differentially methylated CpG probe sites, hypermethylated sites (defined as $\beta > 0.8$) were substantially more common among WTC-exposed than unexposed cancer-free participants (14.3% vs. 4.5%), with a less pronounced pattern of increased hypermethylation observed among all 850,000 plus CpG sites. (Table 2 and Figure S2; http://links.lww.com/EE/A278).

When comparing against an established list of tumor suppressor genes,[39] important tumor suppressor genes were found to be epigenetically dysregulated among WTC-exposed cancer-free participants. *NF1* and *TSC1* are both tumor suppressor genes and their promoters were found to be statistically significantly hypermethylated among WTC-exposed cancer-free survivors. Other tumor suppressors were also found to be differentially methylated as reported in Table 3. Gene set overrepresentation analysis was conducted to explore the functional pathways of WTC-associated DNA methylation alterations. For the WTC-exposed cancer-free subjects, the top enriched pathways included endocytosis, axon guidance, and others (top 20 pathways reported in Figure 1). The endocytosis and axon guidance pathways were overrepresented when looking at the top 250,000 unadjusted CpG sites, as well as the top 5000 adjusted CpG sites (Figure 1 and Figure S3; http://links.lww.com/EE/A278). Merging with previous pilot data increased the sample size to 50 WTC-exposed versus 40 unexposed cancer-free women. Notably, the axon guidance pathway remained enriched in the merged, larger sample size analysis, although not all top CpG sites were found to be statistically significant after adjustment for important confounders (Figure 2).

Comparison of WTC-exposed versus unexposed breast cancers

Increased global hypermethylation was observed among WTC-exposed breast compared to unexposed breast cancer (24.3% vs. 6.6%). A pattern of increased hypermethylation among WTC-exposed participants was consistently observed among all 850,000 plus CpG sites (Table 4 and Figure S4; http://links.lww.com/EE/A278).

Similarly, comparing WTC-exposed and unexposed breast cancers, WTC exposure was associated with differential methylation of several tumor suppressor genes. Notably, *TP53*, a tumor suppressor with high breast cancer penetrance, was observed to have increased DNA methylation among WTC-exposed breast

Table 3.

Methylation status of known tumor suppressor genes; WTC-exposed versus unexposed cancer-free women among top 5000 sites after adjustment

Gene	Probe ID	β WTC exposed vs. WTC unexposed	*P* value	Region
APC	cg08571859	–0.027	<0.001	Promoter
CARS	cg03340153	0.048	<0.001	Gene
CBFA2T3	cg04447835	0.053	<0.001	Unclassified
FH	cg17788938	–0.024	<0.001	Promoter
FOXP1	cg13240253	0.075	<0.001	Not annotated
NF1	cg14933266	0.023	<0.001	Promoter
NOTCH1	cg23457546	0.048	<0.001	Not annotated
NPM1	cg01206923	–0.062	<0.001	Unclassified
PML	cg26861525	–0.030	<0.001	Promoter
PTEN	cg04743007	–0.061	<0.001	Not annotated
RB1	cg16153267	–0.040	<0.001	Promoter
RUNX1	cg23191157	–0.054	<0.001	Not annotated
SDHB	cg10987625	–0.031	<0.001	Promoter
SMARCB1	cg10117496	0.040	<0.001	Not annotated
TCF3	cg10274664	0.051	<0.001	Not annotated
TSC1	cg11295002	0.042	<0.001	Promoter
TSC2	cg14985030	0.033	<0.001	Not annotated
WRN	cg02994645	–0.050	<0.001	Not annotated

Differentially methylated probes/genes were compared against a list of known tumor suppressor genes (Walker et al, 2012); where gene is associated with multiple CpG probes, top CpG probe was selected; β and *P* value from regression model adjusted for confounders (cell type proportions [CD8T, CD4T, natural killer, B-cell, monocytes, granulocytes] age at blood donation, race/ethnicity, smoking history, BMI, and education).

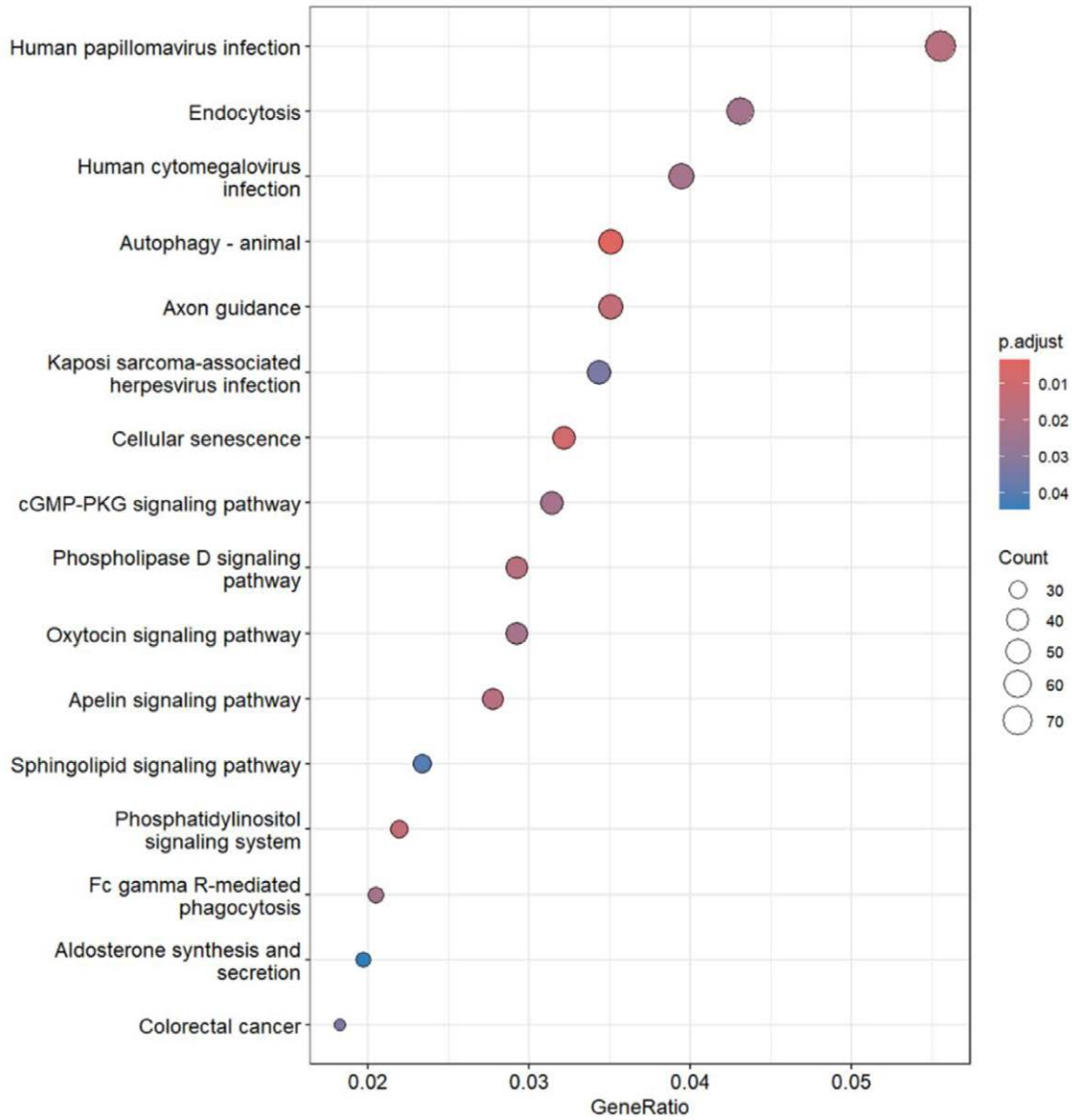

Figure 1. Overrepresentation analysis for WTC-exposed versus unexposed cancer-free women (top significant 5000 CpG sites after adjustment). The *y* axis shows the probe sets with significant overlap with the reference probe sets/genes from the KEGG database. The *x* axis shows the ratio of the number of differentially expressed probe sets/genes to the total number of genes included in the pathway gene set from the reference KEGG pathway database. The dot sizes are proportional to the number of overlapping probe sets/genes. The dot colors show the *P* value adjusted for the false discovery rate. Top 5000 CpG sites associated with 3297 unique genes (all statistically significant, P or $q < 0.05$); top 5000 CpG sites adjusted for race/ethnicity, age at sample donation, smoking status, BMI, education, white blood cell type composition (CD8T, CD4T, B-cell, mono).

cancer cases compared to unexposed cases. *ATM* and *NF2*, moderate breast cancer penetrance genes, were also observed to be dysregulated, among other tumor suppressors (Table 5).

Differentially methylated genes associated with WTC-exposed breast cancers were also found to have an overrepresentation of genes from the endocytosis and axon guidance pathways, among other pathways related to cancer (colorectal cancer, nonsmall cell lung cancer, glioma, and endometrial cancer pathways), endocrine system disruption (oxytocin signaling pathway, thyroid hormone signaling pathway, aldosterone synthesis and secretion, GnRH secretion, endocrine, and other factor-regulated calcium reabsorption), and cholesterol homeostasis and lipid metabolism (phospholipase D signaling pathway) (Figure 3). Similar results were observed when the number of genes for overrepresentation analysis was expanded to the top unadjusted 250,000 CpG probes (Figure S5; http://links.lww.com/EE/A278). In the combined analysis (60 WTC-exposed breast cancer cases and 40 unexposed prediagnostic breast cancer cases), both the endocytosis and axon guidance pathways were still observed to be enriched among WTC-exposed breast cancer cases (Figure 4). These two pathways are illustrated in detail in Figures S6 and S7; http://links.lww.com/EE/A278.

Among WTC-exposed survivors with breast cancer, epigenetically dysregulated genes belonging to cancer-related functional pathways formed complex networks. These networks could be subgrouped to investigate biological relevance. Of the 308 genes found among the top 20 overrepresented pathways, 47 genes clustered together likely due to their shared relevance for immune system regulation and carcinogenesis. This included *TP53, NOTCH1, SMAD3, HDAC1, MAPK7, IGF2R, MSH3, TCF7L2, JAK3*, and *WNT4*, among others (Figure 5).

Discussion

Increasing evidence suggests that WTC exposure is associated with long-term DNA methylation changes that play a role in carcinogenesis. The results of the current study support this hypothesis. A pattern of increased global DNA methylation

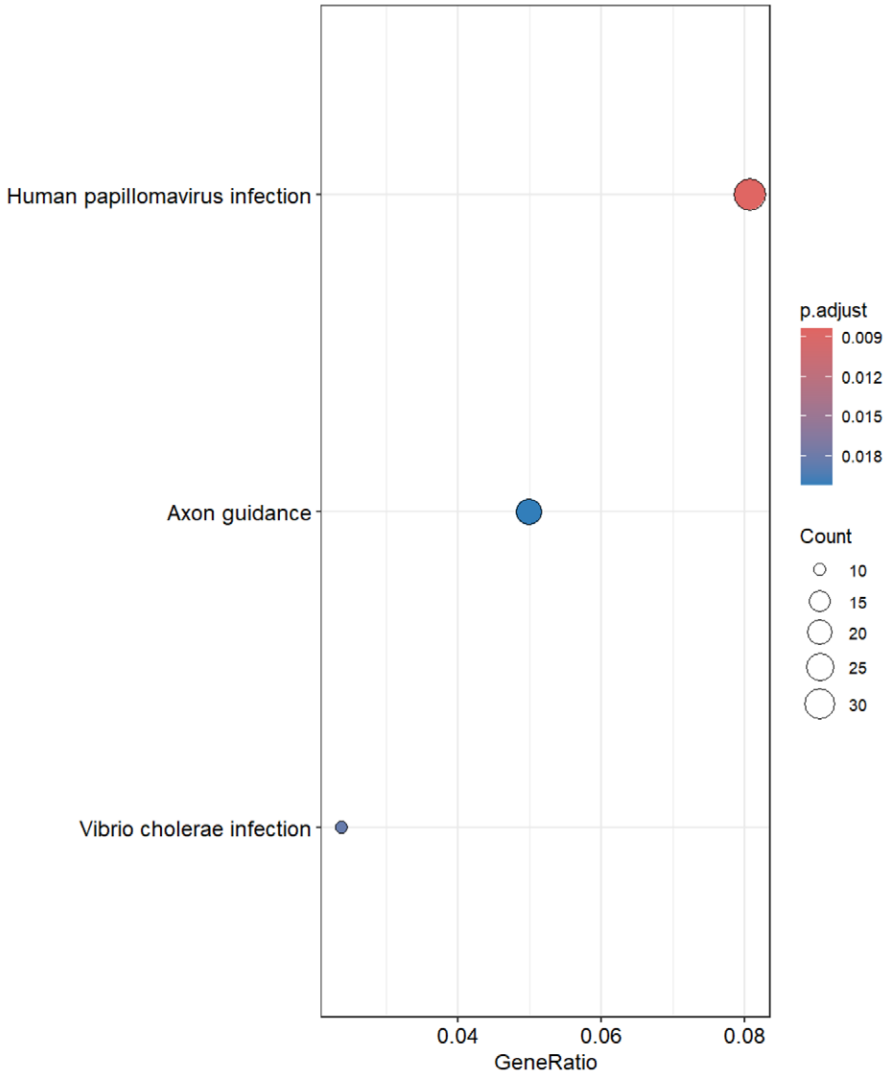

Figure 2. Overrepresentation analysis for WTC-exposed versus unexposed cancer-free women (merged analysis). The *y* axis shows the probe sets with significant overlap with the reference probe sets/genes from the KEGG database. The *x* axis shows the ratio of the number of differentially expressed probe sets/genes to the total number of genes included in the pathway gene set from the reference KEGG pathway database. The dot sizes are proportional to the number of overlapping probe sets/genes. The dot colors show the *P* value adjusted for the false discovery rate. The 1136 overlap (phase 1 and 2) CpG sites associated with 992 unique genes (only 75 sites, 66 genes $P < 0.05$ after adjustment).

Table 4.

Global DNA methylation; number of CpG probes hypomethylated and hypermethylated per group, breast and prediagnostic breast cancer comparison

	Breast and prediagnostic breast cancer comparison	
	Methylation category	
	Hypo ($\beta < 0.2$)	Hyper ($\beta > 0.8$)
Top 5000 CpG sites		
WTC exposed	1871 (37.4%)	1214 (24.3%)
WTC unexposed	1705 (34.1%)	328 (6.6%)
All 865,859 sites		
WTC exposed	184,498 (21.3)	301,626 (34.8)
WTC unexposed	179,232 (20.7)	289,457 (33.4)

β refers to the florescent intensity at that CpG site, where higher β is indicative of more DNA methylation at that particular CpG probe. Proportions based on all CpG sites: hypo ($\beta < 0.2$), hyper ($\beta > 0.8$), and CpG sites with β in-between ($0.2 < \beta < 0.8$).

was consistently observed for both cancer-free and breast cancer-diagnosed WTC-exposed participants, consistent with prior pilot studies.[27,28] Moreover, site-specific DNA methylation changes were observed. These changes between WTC-exposed and unexposed subjects remained statistically significant after adjustment for confounding factors. Important tumor suppressor genes were found to be dysregulated among WTC-exposed cancer-free women, including *NF1*, *NOTCH1*, *PTEN*, *RUNX1*, and among WTC-associated breast cancers, *NOTCH1*, *PML*, *WRN*, *ATM*, *TP53*, the last two of which are breast cancer penetrance genes. These genes have also been observed to be differentially methylated in our pilot data.[27,28] The finding of differentially methylated sites in WTC-exposed cancer-free participants as well as those with breast cancer suggests that cancer-predisposing epigenetic changes may play a role in early WTC-associated carcinogenesis. These findings were observed in the DNA from peripheral blood. Blood-based DNA methylation is easily accessible, stable, and likely corresponds to what is happening on the tissue level.[46] Future studies should validate these findings using WTC-exposed tumor tissues.

A challenge of EWAS studies is to highlight biologically meaningful results. To do this we employed complementary strategies, performing gene set overrepresentation analysis, and then using these results to inform subsequent network analysis. This is a

Table 5.

Methylation status of known tumor suppressor genes; WTC-exposed breast cancer cases versus unexposed prediagnostic breast cancer cases

Gene	Probe ID	β WTC exposed vs. WTC unexposed	*P* value	Region
ATM[a]	cg19288979	−0.04	<0.001	Promoter
FH	cg17788938	−0.03	<0.001	Promoter
FOXP1	cg17872570	−0.04	<0.001	Unclassified
NF2[a]	cg16293088	−0.02	<0.001	Promoter
NOTCH1	cg23457546	0.08	<0.001	Not annotated
PML	cg08066123	−0.03	<0.001	Promoter
SMARCA4	cg05978485	0.05	<0.001	Gene
TCF3	cg16524139	−0.04	<0.001	Unclassified
TP53[b]	cg06365412	0.05	<0.001	Not annotated
WRN	cg02994645	−0.07	<0.001	Not annotated

Differentially methylated probes/genes were compared against a list of known tumor suppressor genes (Walker et al);[39] Where gene is associated with multiple CpG probes, top CpG probe was selected; β and *P* value from regression model adjusted for confounders (cell type proportions [CD8T, CD4T, natural killer, B-cell, monocytes, granulocytes] age at blood donation, race/ethnicity, smoking history, BMI, and education).

[a]Moderate breast cancer penetrance gene.

[b]High breast cancer penetrance gene.

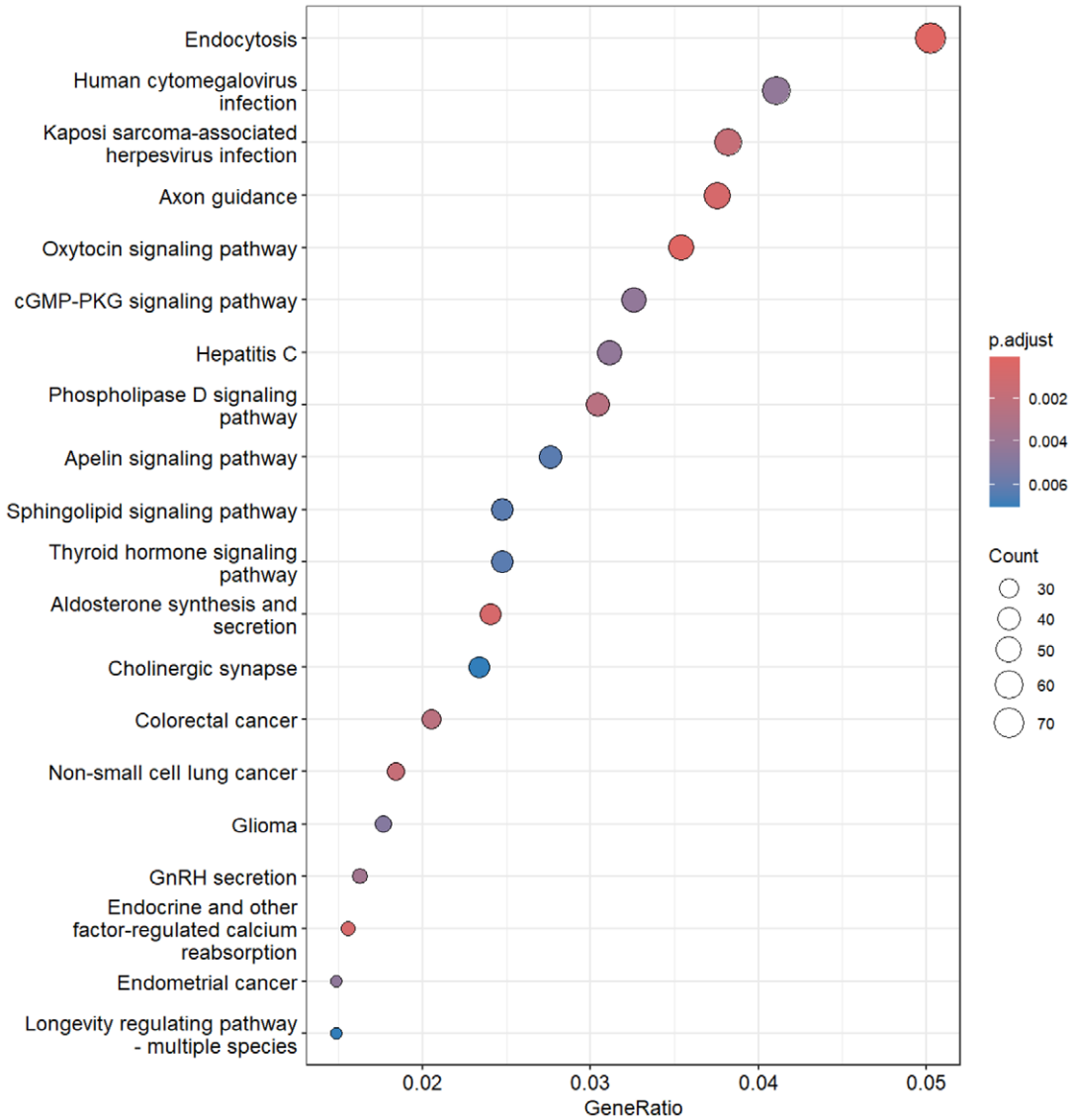

Figure 3. Overrepresentation analysis for WTC-exposed (WTC EHC) versus unexposed (NYUWHS) women with breast cancer (top significant 5000 CpG sites after adjustment). The *y* axis shows the probe sets with significant overlap with the reference probe sets/genes from the KEGG database. The *x* axis shows the ratio of the number of differentially expressed probe sets/genes to the total number of genes included in the pathway gene set from the reference KEGG pathway database. The dot sizes are proportional to the number of overlapping probe sets/genes. The dot colors show the *P* value adjusted for the false discovery rate. Top 5000 CpG sites associated with 3267 unique genes (all statistically significant, *P* or $q < 0.05$).

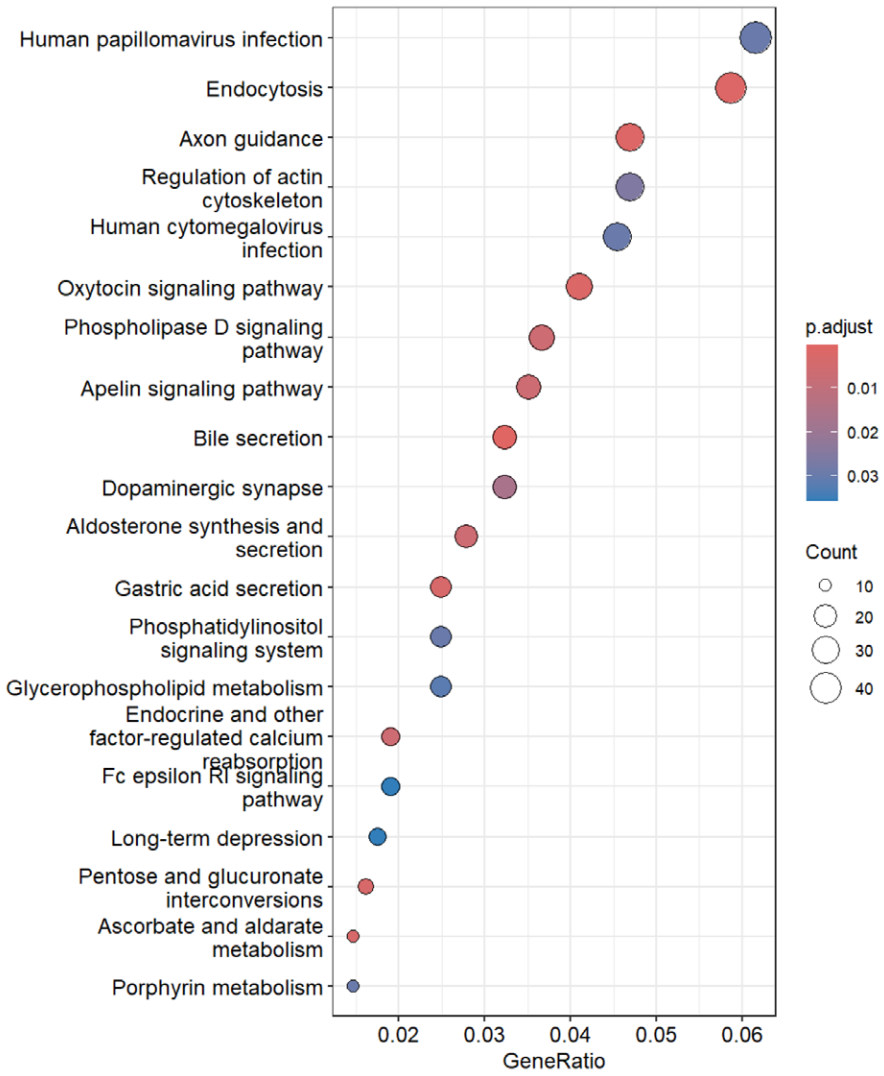

Figure 4. Overrepresentation analysis for WTC-exposed (WTC EHC) versus unexposed (NYUWHS) breast cancer cases (merged analysis). The *y* axis shows the probe sets with significant overlap with the reference probe sets/genes from the KEGG database. The *x* axis shows the ratio of the number of differentially expressed probe sets/genes to the total number of genes included in the pathway gene set from the reference KEGG pathway database. The dot sizes are proportional to the number of overlapping probe sets/genes. The dot colors show the *P* value adjusted for the false discovery rate. The 2180 overlap (phase 1 and 2) CpG sites associated with 1577 unique genes (only 142 sites, 114 genes, $P < 0.05$).

valid approach as demonstrated by Kuan et al[47] in their assessment of DNA methylation profiles among WTC responders with varying levels of WTC exposure. Like Kuan et al, we found that cancer-related pathways were overrepresented, such as the human papillomavirus infection pathway involves many genes related to innate and adaptive immunity, as well as cancer-related genes, such as *mTOR* and *p53*,[48] especially among WTC-exposed breast cancer cases. Genes belonging to overrepresented pathways formed complex protein–protein networks, which we then clustered for further analyses. One cluster was of particular interest and is highlighted here. Among the 47 linked genes were those related to the Wnt/β-catenin pathway, a highly conserved family of proteins critical for tissue homeostasis.[49] Deregulation of Wnt/β-catenin signaling can lead to cancer development, possibly by disrupting cancer immune surveillance.[49] *WNT4* and *TCF7L2,* specifically, were part of this network. Another network gene was *JAK3,* part of the JAK-STAT signaling pathway, which is critical for tumor cell recognition and immune system escape.[50] Also included in this network were other immune-related genes, such as *HDAC1,* an epigenetic regulator of the immune system,[51] and *IGF2R,* a key factor in the regulation of T helper 17 and regulatory T cells.[52] Interestingly, proinflammatory T helper 17 cells have previously been found to be upregulated in WTC-exposed patients as well as rats directly exposed to WTC dust.[9] These genes were found to be in a network with *TP53*, the most commonly dysregulated tumor suppressor gene in human cancers, and its target gene *NOTCH1*.[53] Other cancer-related genes, including tumor suppressor *SMAD3*,[54] and proto-oncogenes *MAPK7* and *MSH3*,[55,56] were also in this network. Of note, *WNT* signaling is an important component of the axon guidance pathway (as demonstrated in Figure S6; http://links.lww.com/EE/A278). The axon guidance pathway is consistently overrepresented among WTC-associated differentially methylated genes. This was found in the cancer-free comparison and breast cancer comparison, both before and after adjustment, and in the combined analyses with "phase 1" pilot data as well. Taken together, this is preliminary evidence that WTC exposure may impact immune system surveillance and tumor immune escape, predisposing WTC-exposed individuals to cancer development.

The study had several limitations. Despite being larger than the previous EWAS studies of WTC-exposed individuals, especially in our combined analyses, the sample sizes were still

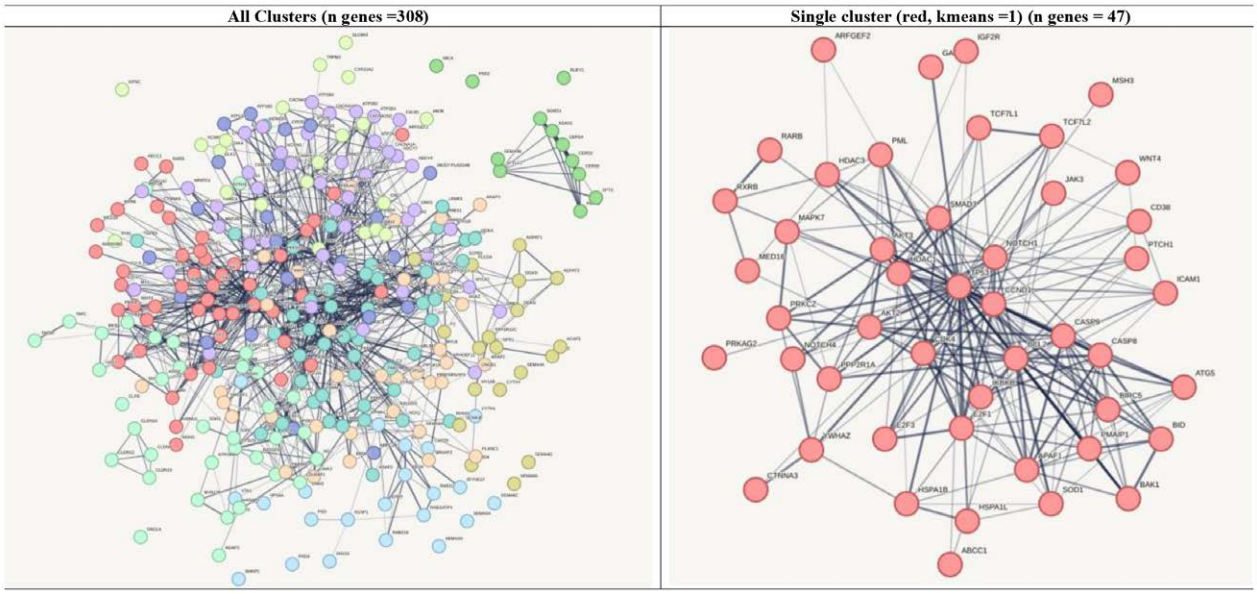

Figure 5. Network analysis for WTC-exposed (WTC EHC) breast cancer cases versus unexposed (NYUWHS) prediagnostic breast cancer cases (308 genes from top 20 overrepresented pathways). Line thickness indicates the strength of data support; medium confidence = 0.400. Colors indicate *k* means clustering set (n = 10).

relatively small. As the cost of global DNA methylation profiling continues to decrease, larger EWAS studies should become more feasible. While we were able to adjust for important confounders, including race/ethnicity, BMI, smoking status, and educational attainment, other potential sources of confounding likely remain. Differences in sample storage time between WTC exposed versus unexposed blood samples is another possible source of bias, although DNA methylation at CpG sites had been shown to be stable under long-term storage.[57] The fact that only prediagnostic breast cancer samples were available for the WTC unexposed women is an additional limitation given that WTC-exposed samples were postdiagnostic, which we attempted to minimize by enrolling only those who developed cancer soon after sample donation, and thus likely had preclinical disease at the time their blood was collected.[46,58] Nevertheless, secular trends in DNA methylation could have impacted results. Also, the WTC disaster was a traumatic event, and stress is known to impact epigenetic regulation.[59,60] Based on the data available, we cannot exclude the possibility that some of the observed differences in DNA methylation between WTC-exposed versus unexposed women could be due to acute and/or chronic stress. Adjustment for multiple testing, gene set overrepresentation analysis, and network analysis all helped to further minimize biases, although there are limitations to these methods. The overrepresentation approach does not take into account all the genes that did not make the list of candidate genes, and so the results are highly dependent on the cutoff used in constructing this list.[61] It is worth noting, however, that several classes of gene pathways reported as overrepresented among WTC-exposed breast cancer cases have been consistently observed, both in our previous pilot studies of WTC exposure among cancer-free women[27] and breast cancer cases,[28] but also in an analysis of the DNA methylation profiles of WTC-exposed versus unexposed prostate cancer cases.[62] These included immune system-related gene pathways, as well as those related to cancer and cell mobility alterations.[63] Interestingly, the endocytosis pathways were consistently observed to be dysregulated among WTC-exposed individuals. Endocytosis refers to the process of cellular digestion but is also known to be coupled with more complex cellular behaviors such as motility.[64] Despite a limited number of studies investigating DNA methylation changes with WTC exposure, the results reported here are consistent with prior data, as summarized in our recent review of WTC exposure, DNA methylation changes, and cancer.[63] This work adds to the growing body of literature pointing to direct dysregulation of cancer genes and pathways, including cellular and immune system dysregulation, as important functional consequences of WTC exposure.

Of note, DNA methylation can play diverse roles in gene regulation. The hypothesized relationship is that promoter hypermethylation of genes will downregulate gene expression, but this is not always necessarily the case.[65] Many of the observed differentially methylated CpG sites had decreased DNA methylation among WTC-exposed women, which could lead to greater gene expression. Toward this end, future studies incorporating gene expression data are warranted. Studies using tumor tissue are also needed to compare the epigenetic changes observed in blood and at the tumor tissue level. Finally, we present a description of the differentially methylated CpG sites associated with WTC exposure, but there are advantages to looking at differentially methylated regions instead, as often it is whole regions, and not just specific sites, that are involved in gene transcription regulation.[66] In the future, focusing on WTC-associated differentially methylated regions could improve the understanding of the functional consequences of complex WTC exposures.

Conclusion

WTC exposure is associated with global and site-specific DNA methylation changes. This was observed among cancer-free WTC-exposed survivors as well as those with breast cancer. Several cancer-related genes and pathways appear to be impacted, and, specifically, WTC exposure may compromise the ability of the immune system to identify and eliminate cancer cells contributing to cancer immune evasion. Future research is needed to validate these findings and to determine whether DNA methylation-based biomarkers can be useful for cancer screening and management of WTC-exposed persons.

Conflicts of interest statement

The authors declare no conflicts of interest.

Acknowledgments

We would like to thank all the enrollees of the WTC EHC and NYUWHS cohorts and participants of this study. We also would like to thank Leigh Wilson, Michelle Hyde, and the entire WTC EHC Data Center for assistance with data and sample collection. We would like to acknowledge healthcare providers at the WTC EHC Clinic at Bellevue Hospital, Dr. Maria C. Crisanti, Dr. Rebecca Florsheim, Angela Torchia, and others. We also extend our thanks to all of our colleagues at the NYU Center for Biospecimen Research and Development, as well as at the NYU Genome Technology Center for help with sample processing and analyses.

References

1. Lioy PJ, Weisel CP, Millette JR, et al. Characterization of the dust/smoke aerosol that settled east of the World Trade Center (WTC) in lower Manhattan after the collapse of the WTC 11 September 2001. *Environ Health Perspect*. 2002;110:703–714.
2. Zeig-Owens R, Webber MP, Hall CB, et al. Early assessment of cancer outcomes in New York City firefighters after the 9/11 attacks: an observational cohort study. *Lancet*. 2011;378:898–905.
3. Solan S, Wallenstein S, Shapiro M, et al. Cancer incidence in World Trade Center rescue and recovery workers, 2001-2008. *Environ Health Perspect*. 2013;121:699–704.
4. Li J, Cone JE, Kahn AR, et al. Association between World Trade Center exposure and excess cancer risk. *JAMA*. 2012;308:2479–2488.
5. Yiin LM, Millette JR, Vette A, et al. Comparisons of the dust/smoke particulate that settled inside the surrounding buildings and outside on the streets of southern New York City after the collapse of the World Trade Center, September 11, 2001. *J Air Waste Manag Assoc*. 2004;54:515–528.
6. Li J, Yung J, Qiao B, et al. Cancer incidence in World Trade Center rescue and recovery workers: 14 years of follow-up. *J Natl Cancer Inst*. 2022;114:210–219.
7. Durmus N, Shao Y, Arslan AA, et al. Characteristics of cancer patients in the World Trade Center Environmental Health Center. *Int J Environ Res Public Health*. 2020;17:E7190.
8. Shao Y, Durmus N, Zhang Y, et al. The development of a WTC Environmental Health Center pan-cancer database. *Int J Environ Res Public Health*. 2021;18:1646.
9. Gong Y, Wang L, Yu H, et al. Prostate cancer in World Trade Center responders demonstrates evidence of an inflammatory cascade. *Mol Cancer Res*. 2019;17:1605–1612.
10. Hashim D, Boffetta P, Galsky M, et al. Prostate cancer characteristics in the World Trade Center cohort, 2002-2013. *Eur J Cancer Prev*. 2018;27:347–354.
11. van Gerwen M, Cerutti JM, Rapp J, Genden E, Riggins GJ, Taioli E. Post-9/11 excess risk of thyroid cancer: surveillance or exposure? *Am J Ind Med*. 2021;64:881–884.
12. Arslan A, Zhang Y, Durmus N, et al. Breast cancer characteristics in the population of survivors participating in the World Trade Center Environmental Health Center Program 2002–2019. *Int J Environ Res Public Health*. 2021;18:7555.
13. Arita A, Costa M. Epigenetics in metal carcinogenesis: nickel, arsenic, chromium and cadmium. *Metallomics*. 2009;1:222–228.
14. Brocato J, Costa M. Basic mechanics of DNA methylation and the unique landscape of the DNA methylome in metal-induced carcinogenesis. *Crit Rev Toxicol*. 2013;43:493–514.
15. Das DN, Ravi N. Influences of polycyclic aromatic hydrocarbon on the epigenome toxicity and its applicability in human health risk assessment. *Environ Res*. 2022;213:113677.
16. Kuzmina NS, Luong TM, Rubanovich AV. Changes in DNA methylation induced by dioxins and dioxin-like compounds as potential predictor of disease risk. *Russ J Genet*. 2020;56:1180–1192.
17. Martin EM, Fry RC. Environmental influences on the epigenome: exposure-associated DNA methylation in human populations. *Annu Rev Public Health*. 2018;39:309–333.
18. Martinez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. *Epigenetics*. 2011;6:820–827.
19. Ravegnini G, Sammarini G, Hrelia P, Angelini S. Key genetic and epigenetic mechanisms in chemical carcinogenesis. *Toxicol Sci*. 2015;148:2–13.
20. Ruiz-Hernandez A, Kuo CC, Rentero-Garrido P, et al. Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence. *Clin Epigenetics*. 2015;7:55.
21. Salemi R, Marconi A, Di Salvatore V, Franco S, Rapisarda V, Libra M. Epigenetic alterations and occupational exposure to benzene, fibers, and heavy metals associated with tumor development (review). *Mol Med Rep*. 2017;15:3366–3371.
22. Zhu Y, Costa M. Metals and molecular carcinogenesis. *Carcinogenesis*. 2020;41:1161–1172.
23. Moore LD, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology*. 2013;38:23–38.
24. Liang WW, Lu RJH, Jayasinghe RG, et al; Clinical Proteomic Tumor Analysis Consortium. Integrative multi-omic cancer profiling reveals DNA methylation patterns associated with therapeutic vulnerability and cell-of-origin. *Cancer Cell*. 2023;41:1567–1585.e7.
25. Baylin SB, Jones PA. A decade of exploring the cancer epigenome — biological and translational implications. *Nat Rev Cancer*. 2011;11:726–734.
26. Lakshminarasimhan R, Liang G. The role of DNA methylation in cancer. *Adv Exp Med Biol*. 2016;945:151–172.
27. Arslan AA, Tuminello S, Yang L, et al. Genome-wide DNA methylation profiles in community members exposed to the World Trade Center disaster. *Int J Environ Res Public Health*. 2020;17:5493.
28. Tuminello S, Zhang Y, Yang L, et al. Global DNA methylation profiles in peripheral blood of WTC-exposed community members with breast cancer. *Int J Environ Res Public Health*. 2022;19:5104.
29. Houseman EA, Kim S, Kelsey KT, Wiencke JK. DNA methylation in whole blood: uses and challenges. *Curr Environ Health Rep*. 2015;2:145–154.
30. Azofeifa A, Martin GR, Santiago-Colón A, Reissman DB, Howard J. World Trade Center Health Program — United States, 2012–2020. *MMWR Surveill Summ*. 2021;70:1–21.
31. Reibman J, Levy-Carrick N, Miles T, et al. Destruction of the World Trade Center towers. lessons learned from an environmental health disaster. *Ann Am Thorac Soc*. 2016;13:577–583.
32. Reibman J, Liu M, Cheng Q, et al. Characteristics of a residential and working community with diverse exposure to World Trade Center dust, gas, and fumes. *J Occup Environ Med*. 2009;51:534–541.
33. Kato I, Akhmedkhanov A, Koenig K, Toniolo PG, Shore RE, Riboli E. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. *Nutr Cancer*. 1997;28:276–281.
34. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, et al. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. *J Natl Cancer Inst*. 1995;87:190–197.
35. *NYU Women's Health Study Cohort Characteristics*. NYU Langone Health. Available at: https://med.nyu.edu/departments-institutes/population-health/divisions-sections-centers/epidemiology/nyu-womens-health-study/cohort-characteristics. Accessed 30 December 2023.
36. *R: The R Project for Statistical Computing*. R Core Team. Available at: https://www.r-project.org/. Accessed 13 November 2022.
37. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*. 2014;30:1363–1369.
38. Salas LA, Koestler DC, Butler RA, et al. An optimized library for reference-based deconvolution of whole-blood biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. *Genome Biol*. 2018;19:64.
39. Walker EJ, Zhang C, Castelo-Branco P, et al. Monoallelic expression determines oncogenic progression and outcome in benign and malignant brain tumors. *Cancer Res*. 2012;72:636–644.
40. Catana A, Apostu AP, Antemie RG. Multi gene panel testing for hereditary breast cancer - is it ready to be used? *Med Pharm Rep*. 2019;92:220–225.
41. Shiovitz S, Korde LA. Genetics of breast cancer: a topic in evolution. *Ann Oncol*. 2015;26:1291–1299.
42. Phipson B, Maksimovic J, Oshlack A. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. *Bioinformatics*. 2016;32:286–288.
43. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS J Integr Biol*. 2012;16:284–287.
44. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023;51:D638–D646.
45. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13:2498–2504.
46. Locke WJ, Guanzon D, Ma C, et al. DNA methylation cancer biomarkers: translation to the clinic. *Front Genet*. 2019;10:1150.

47. Kuan PF, Mi Z, Georgopoulos P, Hashim D, Luft BJ, Boffetta P. Enhanced exposure assessment and genome-wide DNA methylation in World Trade Center disaster responders. *Eur J Cancer Prev*. 2019;28:225–233.
48. *KEGG PATHWAY: Human Papillomavirus Infection - Homo Sapiens (Human)*. Kanehisa Laboratories. Available at: https://www.genome.jp/pathway/hsa05165. Accessed 16 April 2024.
49. Liu J, Xiao Q, Xiao J, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. *Signal Transduct Target Ther*. 2022;7:1–23.
50. Owen KL, Brockwell NK, Parker BS. JAK-STAT signaling: a double-edged sword of immune regulation and cancer progression. *Cancers (Basel)*. 2019;11:2002.
51. Hull EE, Montgomery MR, Leyva KJ. HDAC inhibitors as epigenetic regulators of the immune system: impacts on cancer therapy and inflammatory diseases. *Biomed Res Int*. 2016;2016:8797206.
52. DiToro D, Harbour SN, Bando JK, et al. Insulin-like growth factors are key regulators of T helper 17 regulatory T cell balance in autoimmunity. *Immunity*. 2020;52:650–667.e10.
53. Lefort K, Mandinova A, Ostano P, et al. Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKα kinases. *Genes Dev*. 2007;21:562–577.
54. Millet C, Zhang YE. Roles of Smad3 in TGF-β signaling during carcinogenesis. *Crit Rev Eukaryot Gene Expr*. 2007;17:281–293.
55. Gavine PR, Wang M, Yu D, et al. Identification and validation of dysregulated MAPK7 (ERK5) as a novel oncogenic target in squamous cell lung and esophageal carcinoma. *BMC Cancer*. 2015;15:454.
56. Miao Z, Wu L, Lu M, et al. Analysis of the transcriptional regulation of cancer-related genes by aberrant DNA methylation of the cis-regulation sites in the promoter region during hepatocyte carcinogenesis caused by arsenic. *Oncotarget*. 2015;6:21493–21506.
57. Li Y, Pan X, Roberts ML, et al. Stability of global methylation profiles of whole blood and extracted DNA under different storage durations and conditions. *Epigenomics*. 2018;10:797–811.
58. Bassett LW, Liu TH, Giuliano AE, Gold RH. The prevalence of carcinoma in palpable vs impalpable, mammographically detected lesions. *AJR Am J Roentgenol*. 1991;157:21–24.
59. Harvanek ZM, Fogelman N, Xu K, Sinha R. Psychological and biological resilience modulates the effects of stress on epigenetic aging. *Transl Psychiatry*. 2021;11:601.
60. Zannas AS. Epigenetics as a key link between psychosocial stress and aging: concepts, evidence, mechanisms. *Dialogues Clin Neurosci*. 2019;21:389–396.
61. Irizarry RA, Wang C, Zhou Y, Speed TP. Gene set enrichment analysis made simple. *Stat Methods Med Res*. 2009;18:565–575.
62. Yu H, Tuminello S, Alpert N, et al. Global DNA methylation of WTC prostate cancer tissues show signature differences compared to non-exposed cases. *Carcinogenesis*. 2022;43:528–537.
63. Tuminello S, Nguyen E, Durmus N, et al. World Trade Center exposure, DNA methylation changes, and cancer: a review of current evidence. *Epigenomes*. 2023;7:31.
64. Schmid SL, Sorkin A, Zerial M. Endocytosis: past, present, and future. *Cold Spring Harb Perspect Biol*. 2014;6:a022509.
65. Itai Y, Rappoport N, Shamir R. Integration of gene expression and DNA methylation data across different experiments. *Nucleic Acids Res*. 2023;51:7762–7776.
66. Smith RG, Pishva E, Shireby G, et al. A meta-analysis of epigenome-wide association studies in Alzheimer's disease highlights novel differentially methylated loci across cortex. *Nat Commun*. 2021;12:3517.