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Prostate Cancer Risk and Outcome in WTC Respondents

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Research Aim/Project Overview: The PI should describe the purpose and approach to the project, including the outcomes, methodology and related analyses. Include a discussion of the challenges, successes and lessons learned. Describe the collaborations/partnerships and the role of each external partner.

An excess incidence of prostate cancer has been identified among World Trade Center (WTC) rescue and recovery workers included in the WTC Health Program (WTCHP) at Mount Sinai in New York. An excess has also been reported among WTC-exposed firefighters included in a separate program. It is unclear whether the excess is associated with WTC-related exposures or represents an artifact. Although no environmental agents are established prostate carcinogens, the understanding of the etiology and pathogenesis of prostate cancer – the most common cancer in US men – is limited, and the investigation of high-risk populations might offer new clues. Conversely, the finding that excess risk among WTCHP participants is not attributable to WTC related exposures, but to other factors (for example over-diagnosis because of enhanced surveillance) would represent an important reassuring message to WTCHP members.

The objectives of this project are to elucidate the reasons for the increased incidence of prostate cancer among WTCHP participants and to explore the behavior of these cancers. An analysis of cancer risk according to WTC-related exposures was already planned. This project complemented that analysis by looking at other reasons for an increased prostate cancer risk and investigating whether prostate cancers among WTCHP participants differ from a clinical, epidemiologic and molecular viewpoint from prostate cancers in WTC-unrelated patients diagnosed at Mount Sinai.

The specific aims of the project are:

Aim 1. **To analyze if prostate cancer over-diagnosis occurred among WTCHP because of increased surveillance.** We compared clinical characteristics of prostate cancer tumors diagnosed within WTCHP with those characteristics of an age-matched group of prostate cancers who are not related to WTC. The hypothesis was that over-diagnosis due to screening would result in a large number of early stages prostate cancers among WTCHP. The following items were compared: tumor size, nodal involvement, local and distant metastatic spread, PSA at diagnosis, D'Amico score (which incorporates Gleason score, clinical stage and PSA at diagnosis). This helped dissect whether excess cancer incidence reflects a real increase in risk or is due to over-diagnosis from screening.

Aim 2. **To investigate markers of inflammation in prostate cancer samples from WTCHP patients and matched prostate cancers not related to WTC.** Inflammation is known to promote both tumor development and progression. We analyzed molecular markers of inflammation and immunity in archival prostate cancer samples from WTCHP patients, and matched controls, to (i) determine if tumors in WTCHP patients arise in a different "inflammatory" microenvironment and (ii) correlate these markers with clinical outcomes. Differentially expressed genes between the two groups were examined, along with relevant immune gene sets.

Aim 3. **To compare DNA methylation as a marker of tumor aggressiveness in WTCHP prostate cancer patients and in matched prostate cancers not related to WTC.** DNA global methylation studies indicate low methylation status in more aggressive tumors. We analyzed DNA methylation in prostate tissue samples from WTCHP patients and from a group of age and

stage matched prostate cancers not related to WTC. The hypothesis was that there would be a different DNA methylation pattern (thus different tumor aggressiveness) in WTC prostate cancer patients in comparison to other prostate cancers not WTC related.

This project represents the first in-depth analysis of cancer among WTC rescue and recovery workers. It addresses a potentially important health problem in this population; its results could have practical implications on the surveillance and clinical management of prostate cancer, the most common cancer among male WTCHP members. The project has generated novel data on biomarkers of prostate cancer aggressiveness that could be used to make decisions on clinical treatment.

The WTCHP Responders who participated (as employees or volunteers) in the rescue, recovery and cleanup efforts at the WTC sites have been enrolled at Mount Sinai in the World Trade Center Health Program (WTCHP), which is funded under the James Zadroga 9/11 Health and Compensation Act of 2010, on the basis of eligibility criteria including type of duties, site location and dates and hours worked. The medical protocol for the monitoring program includes self-administered physical and mental health questionnaires as well as a physical examination, laboratory tests, spirometry and a chest radiograph. Over 27,000 responders have had a least one monitoring visit in the WTCHP and have consented to aggregation of their data. About 85% are men. A total of 17,781 male responders have consented to have their records used for medical research. Over one third of male WTCHP members belong to minorities; policemen and other protective service workers represent the largest occupational group. One important characteristic is the high proportion of never smokers; about 20% experienced high or very high WTC-related exposure, as defined in previous studies.

For Aim 1, the study sample consisted of responders enrolled in the WTCHP clinics between July 16, 2002 and December 31, 2013. Cases that were diagnosed in the first 6 months were excluded. There were 442 self-reported cases of prostate cancer, of which 340 were confirmed by cancer registries and medical records.

For Aims 2 and 3, tumor samples were obtained from the pathology archives of Mount Sinai for 15 WTC cases. A series of 14 prostate cancer patients diagnosed and treated at Mount Sinai, who were not part of WTCHP served as a control group.

Methodology and Analyses:
Aim 1- Clinical and demographic information were collected for cases diagnosed among WTC responders. WTC-related environmental exposure information was obtained from questionnaires administered to cohort member at the first visit. Exposure was categorized into four mutually exclusive groups to reflect the intensity and duration of exposure to the dust, smoke, and debris. Group assignment was based on the total time spent working at the WTC site, exposure to the cloud of debris from the collapse of the WTC buildings, and work on the pile of debris. The group at very high exposure encompasses those who worked more than 90 days, were exposed to the dust cloud, and worked on the pile. High exposure was assigned to those who were exposed to the dust cloud, but either worked less than 90 days or did not work on the pile. Because only five participants were classified in the Very High exposure group, the High and Very High exposure groups were combined for analysis. Intermediate exposure comprises those who were not exposed to the dust cloud and either worked between 40 and 90 days or worked on the pile. Low exposure was assigned to those who worked less than 40 days, were not exposed to the dust cloud, and did not work on the pile. Duration of work (continuously) and location of work (on the debris pile, not on the debris pile) at the 9/11 site were also tested for associations.

In the first set of analyses, standardized rate ratios for prostate cancer were calculated for the years of NYSCR-confirmed WTC-respondent cases (2002-2010) compared to the New York state population (SEER*Stat), using the direct standardization method, for five-year age groups and race, using the 2000 US reference population. To reduce the possibility of enrollment bias, only cases diagnosed after enrollment in the WTCHP were included. Therefore those who were diagnosed after September 11, 2001 and before enrollment in WTCHP were excluded. The number of men enrolled in the WTCHP each year served as WTC respondent denominators.

The second set of analyses focuses on characteristics of the WTC prostate cases according to exposure levels. To determine whether bias exists within the cohort based on missing clinical or exposure level data, sensitivity analyses were conducted for each missing variable separately using Pearson's chi-square (or Fisher's exact test for counts < 5). Multivariable logistic regression models adjusting for age at diagnosis (continuous) and race were then used to determine whether clinical indicators of advanced cancer were associated with either: exposure level (categorical), duration of work (continuous), or work on debris pile (dichotomous). The clinical indicators of advanced cancer included: Gleason scores, a histopathological diagnosis of two independent pathologists (<7 vs. ≥ 7); clinical stage, a surgical diagnosis for tumor extending beyond the prostate at stages III and IV vs. confined to prostate at stages I and II); and D'Amico risk score, a recurrence score based on prostate-specific antigen (PSA) before diagnosis, clinical stage, and Gleason score, (high-risk of recurrence after treatment vs. intermediate to low risk of recurrence).

Aim 2- We hypothesized that prostate cancers detected in WTCHP members arise in a more "inflammatory microenvironment" compared with controls, and that these markers of inflammation are associated with poor clinical outcomes. We utilized tumor samples of WTC patients and controls collected at Mount Sinai tumor bank. We sought consent of WTC prostate cancer patients to have their tumor sample retrieved; a comparable number of samples were retrieved from the Mount Sinai tissue bank from prostate cancer patients frequency matched to cases on age, race/ethnicity and year of diagnosis. To characterize the inflammatory microenvironment in these samples, Nanostring was used for a set of 500 inflammatory and immunity genes [Martinez et al., 2006; Verreck et al., 2006]. Differences in gene expression profiling were evaluated in cancer samples in WTCHP patients and controls using Fisher's exact tests for categorical variables and two sample t-tests for continuous variables (Mann-Whitney U test was used when distributions were not normal). Differential gene expression of microarray data was conducted using two sample t-tests with the Benjamini and Hochberg false discovery rate (FDR) [Benjamini & Hochberg, 1995] of 10% used to account for multiple testing. Predictive modeling was be conducted using a variety of statistical techniques including discriminant analysis, k-nearest neighbor, and support vector machines and leave-one-out cross validation methods.

Aim 3- whole genome DNA methylation was performed on the same 29 patients' tissue blocks that were utilized for Aim 2. A window-based approach that looks for regions of consistent difference between the 2 groups was utilized to identify sites that show significant differences in mean methylation between WTCHP and non-WTC prostate cancer cases. For each probe, a T-test p-value was generated and a 1 kb sliding window analysis was performed by combining individual p-values in a combined p-value, using Fisher's method. Out of 733,644 probes, 20,381 that were significant at .0001 Bonferroni corrected p-values, with a case-control beta value >10% were selected. A Likelihood Ratio Test was performed on these probes by comparing a test model including sample group against a null model without sample group. Analyses incorporated covariates of gender, age and Gleason score to remove effects of these

covariates. Individual LRT p-values were combined and significant probes with Bonferroni corrected p-values <.0001 were selected. Significant probes were then annotated with Refseq genes, CPg features and GTex Prostate expression dataset.

Include a discussion of the challenges, successes and lessons learned. Describe the collaborations/partnerships and the role of each external partner.

The main challenge of this project was the ability to reach patients to obtain consent to retrieve their clinical information and tissue blocks. Although we sent each case an invitation letter, there were several "return to sender" designations, indicating an incorrect or nonexistent address, or the participant no longer resides there and has not provided a forwarding address. Other times the participant was deceased. We usually follow-up the letter with a telephone call, but sometimes there was no telephone number provided/disconnected numbers/ wrong telephone numbers provided.

Solutions: we have contacted the patients multiple times by letter and telephone, as well as by email if available. We have also searched the patient on people finder to obtain correct addresses/new addresses. We are calling participants over the phone to make sure that they received the letter/consent form in the mail and to answer any questions about the study. Often the patient has misplaced the consent that was mailed; we re-sent it by mail as requested.

Another challenge is that some hospitals where the patients were treated have since been closed down.

Solution: We are tracking down where the samples were sent to be stored after the hospital closure (usually Iron Mountain), and contact the structure for appropriate retrieval and shipping.

Translation of Research Findings: The PI should describe how the findings will be translated and how they will be used to inform policy or promote, enhance or advance the impact on public health practice. This section should be understandable to a variety of audiences, including policy makers, practitioners, public health programs, healthcare institutions, professional organizations, community groups, researchers and other potential end users. The PI should also provide a discussion of any research findings that informed policy or practice during the course of the project period. If applicable, describe how the findings could be generalized and scaled to populations and communities outside of the funded project.

Key Findings- The age adjusted rate of prostate cancer cases per 100,000 men diagnosed six months after enrollment between 2002-2010 for the NYS population was 261.3 (95% CI: 259.9, 262.8) and 431 .6 (95% CI: 394.8, 603.7) for WTC respondents (standardized rate ratio 1.65; p=0.01). Age specific rate ratio estimates were significantly higher than expected for those in age group 30-49 years (2.28; 95% CI: 1.51, 3.43), 70-74 years (2.05; 95% CI: 1.03, 4.10) and 80-84 years (5.65; 95% CI: 1.41, 22.58). When prostate cancer cases were not restricted to those diagnosed after enrollment (all NYSCR confirmed cases for 2002-2010, n=208), the standardized rate ratio was 2.20; p<0.001.

Among WTC respondents with data available on exposure levels (60%, n=205), 39 (19%) had High or Very High exposures and 129 (63%) had Intermediate exposures. Both 3-level exposure level and working on the Ground Zero "pile" showed progressively higher ORs across clinical indicators of prostate cancer aggressiveness, although associations were statistically significant for clinical stage at the high exposure level only. A dose-response relationship was observed among three-levels of exposure across all indicators of prostate cancer aggressiveness. In stratified analyses by SOC-coded occupation, a positive relationship was found for advanced clinical stage (III or IV) among protective workers (SOC: 33-0000) (OR: 3.81; 95% CI: 0.31, 42.03) and construction workers (OR: 12.58; 95% CI: 0.74, 213.01),

although this was not consistent for alternative indicators, Gleason score and D'Amico risk classification. No association was observed between these indicators of prostate cancer aggressiveness and year of diagnosis.

The study on gene-expression profile, conducted on a subset of the population, suggests that WTC cases have a distinct pattern that includes overexpression of specific inflammatory and innate immunity genes (Figure 1).

Similarly, there is a specific DNA methylation profile that is exclusive to the WTC cases (Figure 2). We are currently in the process of combining the results of the gene expression profile with those of the methylation study, to assess if we can identify a specific behavior that is associated with being a WTC case.

Discussion and interpretation of findings:
It has been recognized that prostate cancers are diagnosed more frequently in heavily screened populations compared to other populations. However, we have found three key aspects that are worth discussion. Firstly, cancer incidence was significantly higher for WTC respondents in the youngest and two of the oldest age groups only. We would expect to find high incidence rates in across all age groups or particularly for all older age groups if an increase in surveillance was expected, particularly men 55-69 years old when PSA testing is recommended to begin by the American Urological Association (AUA). Secondly, only half of WTC respondent prostate cancer cases had one or no PSA visit prior to diagnosis, an information that does not support the concept of increased surveillance. Thirdly, the aggressive characteristic of prostate cancer cases in this cohort and lack of correlation between exposure and the number of PSA surveillance visits weighs against the argument that an observed increased incidence of prostate cancer may be a consequence of increased PSA screening and surveillance among WTC respondents.

Exposures in the 9/11 aftermath included known and suspected carcinogens. Personal protective equipment was not worn for the majority of workers and individuals in the area and our study has found significant association of higher clinical stage with the highest exposure level that includes exposure to the 9/11 dust cloud. The dust cloud that resulted after the collapse of the buildings, exposed respondents to soot, benzene, WTC dust and smoke, which contained asbestos, silica, cement dust, glass fibers, heavy metals, polycyclic aromatic hydrocarbons (PAHs), phthalates, polychlorinated biphenyls, and polychlorinated dibenzofurans, and dioxins from the burning and collapse of the planes and the towers. Other suspected carcinogens, such as fine particulate matter (PM2.5) were elevated above that of normal levels of 2 to 6 times higher in some areas. The first of the WTC towers was 75% coated with half an inch of asbestos (up to the 40^{th} floor), and several buildings from which dust was sampled at least three months post-9/11 contained the highest concentration of asbestos dust compared to other buildings farther away from the WTC disaster site. Although a nationwide Finnish study reported an increased incidence of prostate cancer (SIR 1.21, 95% CI 1.09-1.34) among asbestos construction workers, it has not yet been established whether any of these, or other, suspected carcinogens are responsible for the increase in prostate cancer observed in the WTC population.

WTC respondents, like many employed populations, were substantially healthier than the general population at the time of beginning their service at the WTC site, and were therefore at lower risk of cancer than the general U.S. population, which includes persons who are chronically ill, hospitalized, or otherwise unemployable. Despite this healthy worker effect, our study has found a possible increase in prostate cancer incidence in comparison to the previous

study conducted in 2002-2008. The two largest occupational subgroups in this study included respondents in occupations that required physical and mental fitness: protective services and construction. This study is concordant with larger studies examining prostate cancer incidence among these particular occupations. Statistically significant 2.5- to- 4-fold prostate cancer risks were observed for police officers, and an approximately 1.5-fold risk for firefighters. The rate was higher particularly among men aged < 50 years in five Nordic countries and a California study reported a 1.4-fold risk for firefighters aged 45-59 years. A Swedish cohort study found increased prostate cancer incidence among concrete workers SIR 1.08; 95%CI 1.01 to 1.16 and a large study of all Nordic countries found the risk to be 1.10 (95%CI: 1.06, 1.14) among military workers. Those studies did not contain analyses concerning prostate cancer aggressiveness in the respective populations.

Both protective service and construction work constituted 58.8% of WTC respondent prostate cancer cases and it may be postulated that prostate cancer aggressiveness may be related to other occupation-related exposures rather than WTC-related exposures, although it is more likely that WTC-related exposures were higher in dosage. In models adjusting for both occupation and exposure level, the associations according to occupation were attenuated (OR 0.20 95% CI: 0.05, 0.85 for protective service occupations and OR 0.37 95% CI: 0.09, 1.50 for construction) while the associations were stronger when exposure was considered.
Strengths: In addition to the utilization of varying exposure methodologies, this study also reports prostate cancer incidence in a relatively healthy, mostly non-smoking population of diverse ethnic background. We also utilize clinical assessments of prostate cancer aggressiveness to determine the associations with exposure. The findings did not suggest an inconsistent dose-response effect with exposure for clinical state of prostate cancer and the finding is biologically plausible with what is known about prostate cancer and the exposures involved.

This study is also the first to address the biology of prostate cancer in WTC responders.
Limitations: Due to the relatively small population, the range and breadth of the associations did not have enough statistical power for precision, particularly for subgroups of occupations. Associations for D'Amico scores and exposure may be slightly underestimated due to the larger number of missing exposure levels for those with the highest score. Secondly, detailed medical information on clinical stage was missing for some cases and appears to be influenced by employment status for the most part. This may be due to a lack of outreach in those who are retired, or unemployed. However, a higher proportion of unemployed respondents had a higher number of visits for PSA screening. Further, sensitivity analysis revealed no difference in exposures between participants with missing and non-missing clinical characteristics. As with most solid tumors, the time period of 12 years from 9-11 may likely have been too short to have captured all the prostate cancer cases occurring post-exposure. There was no association or pattern of decreased cancer aggressiveness with recent year of diagnosis, diminishing the possibility that these cases represent a biased sample of more aggressive cancers due to lead time bias.

Considering the large proportion of grade of prostate cancer in younger men and the long latency for prostate cancer development, continued monitoring is needed to determine alternative causes of elevated prostate cancer in the WTC population. Although associations between WTC and prostate cancer are weak, a dose-response relationship for clinical stage suggests that WTC exposure may have played a role in prostate cancer progression and possibly development.

Public Health Relevance and Impact: This section should address improvements in public health as measured by documented or anticipated outcomes from the project. The PI should consider how the findings of the project related beyond the immediate study to improved practices, prevention or intervention techniques, or informed policy, technology or systems improvements in public health.

Since a higher incidence of prostate cancer has been reported in WTC respondents, this has been the first detailed analysis of clinical aspects of WTC-related cases as well as the first study on the association between WTC exposure and prostate cancer. There remain few confirmed and well established prostate cancer risk factors. Further molecular investigation of prostate cancer tumor characteristics linked to exposures incurred during the September 11,2001 aftermath contributes to a better understanding of prostate cancer development and has novel implications for the possibility of primary prevention of prostate cancer. The finding of a specific DNA methylation and gene expression profile in WTC cases in comparison to non –WTC cases suggests that exposure may have had a role in shaping the cancer biology and aggressiveness in the WTC population. This study is now continuing with Dr. Aaronson, to expand to number of cases tested for markers of inflammation and immunity, in order to obtain firm conclusions on this topic. If the results are confirmed, there will be discussions on how to appropriately tailor the follow-up of the male responders in order to identify these aggressive prostate cancers as early as possible.

Publications: Presentations; Media Coverage: Include information regarding all publications, presentations or media coverage resulting from this CDC funded activity. Please include any additional dissemination efforts that did or will result from the project.

Hashim D, Boffetta P, Galsky M, Oh W, Lucchini R, Crane M, Luft B, Moline J, Udasin I, Harrison D, Taioli E. Prostate cancer characteristics in the World Trade Center cohort, 2002-2013. Eur J Cancer Prev. 2016 Nov 24.

Alpert N, Suarez-Farinas M, Boffetta P, Oh W, Gong Y, Lucchini R, Crane M, Luft B, Moline J, Udasin I, Harrison D, Taioli E. Markers of inflammation in WTC prostate cancer samples. Manuscript in preparation.

Alpert N, Suarez-Farinas M, Boffetta P, Lucchini R, Crane M, Luft B, Moline J, Udasin I, Harrison D, Sharp A, Martin-Trujillo A, Taioli E. DNA-methylation in WTC prostate cancer samples. Manuscript in preparation.

Figures:

Figure 1: Volcano plot of WTC and non WTC cases gene expression

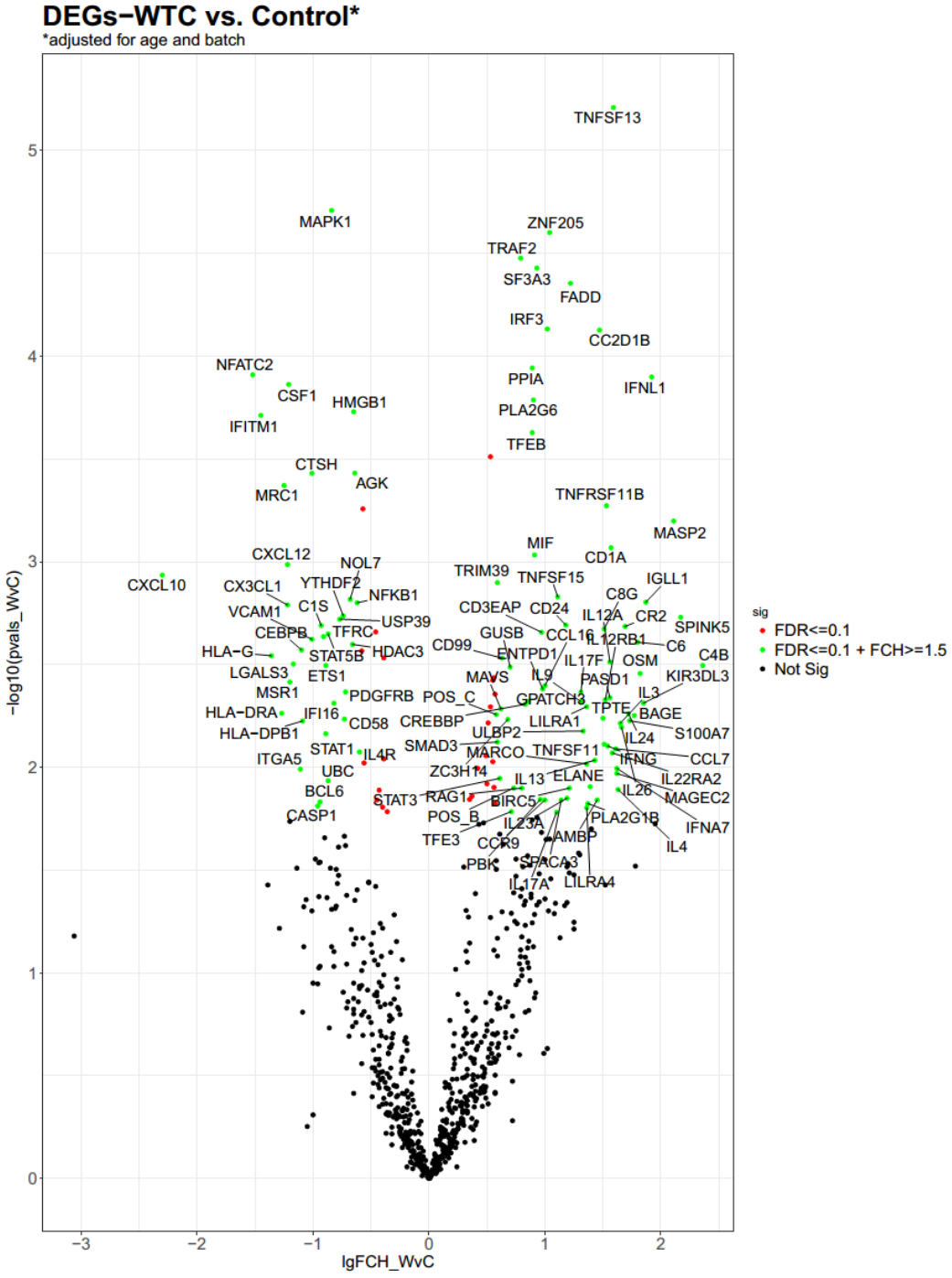

Figure 2: Principal component analysis of DNA methylation data