A. OVERALL COVER PAGE

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Human Subjects: NA	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overarching aim of this study is to determine whether WTC exposed subjects have an increased prevalence of CHIP, to test the hypothesis that CHIP-specific mutations will be detected in a subgroup of healthy WTC responders, allowing earlier identification of a subpopulation at higher risk for developing hematologic neoplasms and cardiovascular disease. In Specific Aim 1 we plan to determine the prevalence of CHIP in 350 WTC responders, and to compare it with unexposed controls. In Specific Aim 2 we plan to investigate the association between prevalence of CHIP and cardiovascular biomarkers, risk factors and risk scores in WTC responders. In Specific Aim 3, we plan to investigate the association between prevalence of CHIP and quantitative measures of WTC exposure.
The integration of the results would lead to the identification of WTC responders at high risk of CHIP-related conditions, who might be subjects to enhanced medical surveillance.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Results will be communicated to the WTC Responders enrolled at Stony Brook Clinical Center of Excellence.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

Prevalence of Chronic Hematopoiesis of Indeterminate Potential (CHIP) Among WTC Responders

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Project Title: Prevalence of Chronic Hematopoiesis of Indeterminate Potential Among WTC Responders.

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List of Abbreviations

World Trade Center (WTC)

Clonal hematopoiesis of indeterminate potential (CHIP)

Lymphoid-CHIP (L-CHIP)

Myeloid-CHIP (M-CHIP)

Body Mass Index (BMI)

Whole Exome Sequencing (WES)

Cardiovascular disease (CVD)

Institutional Review board (IRB)

Confidence Interval (CI)

Standardized Incidence Ratio (SIR)

Acute Myeloid Leukaemia (AML)

Abstract (550 words or less)

Hematologic neoplastic and pre-neoplastic conditions are a heterogeneous group of diseases that arise from acquired genetic and epigenetic alterations in hematopoietic precursor cells. The presence of such alterations can be detected even prior to overt hematological manifestations due to increasing availability of molecular testing among individuals without over neoplastic or pre-neoplastic conditions. This finding has been defined as clonal hematopoiesis of indeterminate potential (CHIP). CHIP mutations can originate in lymphoid cells (L-CHIP) and in myeloid cells (M-CHIP). Despite the absence of detectable hematologic disorders, mutations characteristically associated with hematologic neoplasms were identified in 6 percent of individuals aged 60 years or more and associated with certain HLA types; these individuals are also at increased risk of CVD. The overarching goal of this study was to measure the prevalence of CHIP mutations in a population of WTC rescue and recovery workers, to compared the prevalence to that of subjects not exposed to WTC, and to analyze the association between CHIP mutation status and WTC exposure and other characteristics of study subjects.

We analyzed samples from 357 responders recruited at the WTC Clinical Center of Excellence at Stony Brook University, of whom 130 (36.4%) were positive for CHIP mutations and 227 (63.6%) were negative. In particular, 56 responders (15.7%) were positive for M-CHIP mutations and 72 (20.2%) were positive for L-CHIP mutations. M-CHIP mutation status was associated with age ($p<0.001$), smoking status ($p=0.02$) and BMI ($p=0.03$). L-CHIP mutation was not associated with any of the factors under investigation.

Although the prevalence of responders with cardiovascular disease was higher in responders with both M-CHIP ($p=0.2$) and L-CHIP ($p=0.4$) mutations, the difference was not statistically significant. Among cardiovascular and metabolic biomarkers, carriers of M-CHIP mutations had a higher level of lymphocytes ($p=0.03$) and erythrocytes ($p=0.04$) compared to non-carriers, while no differences were observed among carriers of L-CHIP mutations. Estimated WTC exposure was not associated with either M-CHIP or L-CHIP mutation status.

The prevalence of M-CHIP mutations was higher in WTC responders included in this analysis than in other populations. There are limited data in the literature on the prevalence of L-CHIP mutations. Factors associated with prevalence of M-CHIP mutations are consistent with those identified in previous studies. The lack of association with WTC exposure might be explained by low statistical power in separating exposure level of responders. In conclusion, WTC responders have a high prevalence of M-CHIP mutations which, however, does not seem to be associated with indicators of WTC exposure.

Section 1
Significant or Key Findings

Findings are presented separately for each Specific Aim

Specific Aim 1: To compare the prevalence of CHIP-related mutations in WTC responders and unexposed controls. *We will test the hypothesis that the prevalence of CHIP-associated mutations in WTC responders is higher than in unexposed controls. Using WES we will measure the prevalence of CHIP-associated mutations in blood samples of 350 WTC responders and compare it with the prevalence among 700 age-, sex and race-matched unexposed controls from several population-based studies included in the database of Genotypes and Phenotypes (dbGaP), and conduct exploratory analyses on mutations in specific genes and on the interaction between CHIP and major HLA types.*
We analyzed samples from 357 responders recruited at the WTC Clinical Center of Excellence at Stony Brook University, of whom 130 (36.4%) were positive for CHIP mutations and 227 (63.6%) were negative. In particular, 56 responders (15.7%) were positive for M-CHIP mutations and 72 (20.2%) were positive for L-CHIP mutations. M-CHIP mutation status was associated with age ($p<0.001$), smoking status ($p=0.02$) and BMI ($p=0.03$). L-CHIP mutation was not associated with any of the factors under investigation.

Specific Aim 2: To investigate the association between cardiovascular biomarkers and risk scores, and CHIP-related mutations in WTC responders. *We will use the WES-based analysis of CHIP conducted in Aim 1 and data on cardiovascular biomarkers and components of CVD risk scores generated during WTC medical surveillance visits to test in the sample of 350 WTC responders enrolled in Aim 1the hypothesis that WTC responders with CHIP-related mutations have the highest risk for CVD.*
Although the prevalence of responders with cardiovascular disease was higher in responders with both M-CHIP ($p=0.2$) and L-CHIP ($p=0.4$) mutations, the difference was not statistically significant. Among cardiovascular and metabolic biomarkers, carriers of M-CHIP mutations had a higher level of lymphocytes ($p=0.03$) and erythrocytes ($p=0.04$) compared to non-carriers, while no differences were observed among carriers of L-CHIP mutations. Estimated WTC exposure was not associated with either M-CHIP or L-CHIP mutation status.

Specific Aim 3: To explore the association of CHIP-related mutations in WTC responders and WTC exposure. *We will use results on CHIP generated in Aim 1 and WTC exposure data derived from details assessment of WTC-related activities to test the hypothesis that responders with higher WTC-*

related exposure have a higher prevalence of CHIP-associated mutations than responders with lower WTC-related exposure. The work will be done in the sample of 350 WTC responders enrolled in Aim 1. The prevalence of M-CHIP mutations was higher in WTC responders included in this analysis than in other populations. There are limited data in the literature on the prevalence of L-CHIP mutations. Factors associated with prevalence of M-CHIP mutations are consistent with those identified in previous studies. The lack of association with WTC exposure might be explained by low statistical power in separating exposure level of responders. In conclusion, WTC responders have a high prevalence of M-CHIP mutations which, however, does not seem to be associated with indicators of WTC exposure.

Additional exploratory statistical analyses on interaction between CHIP mutation and behavioral and clinical characteristics are going on.

Translation of Findings

The presence of CHIP is not a disease per se, rather a marker of risk of leukemia, cardiovascular diseases and possibly other conditions. Our study found that WTC rescue and recovery workers have a higher prevalence of this type of mutations compared to unexposed population. On the other hand, there was no strong association with estimated WTC exposure. This latter finding can be explained by (i) error in the definition of WTC exposure, which was based on retrospective data, (ii) a role of factors other than WTC exposure per se in causing CHIP in this population, such as chronic stress. In any case, the higher prevalence of CHIP is an additional argument for maintaining the existing medical monitoring and treatment program for WTC rescue and recovery workers.

Research Outcomes/Impact

The goal of this study is to determine whether WTC exposed subjects have an increased prevalence of clonal hematopoiesis of indetermined potential (CHIP), a condition associated with hematologic neoplasms and cardiovascular disease. Two types of CHIP mutations have been described, myeloid-CHIP (M-CHIP) and lymphoid-CHIP (L-CHIP). We analyzed blood samples from 357 WTC responders recruited at the Clinical Center of Excellence at Stony Brook University.

A total of 130 responders (36.4%) were positive for CHIP mutations, including 56 responders (15.7%) who were positive for M-CHIP mutations and 72 (20.2%) who were positive for L-CHIP mutations. The proportion of responders with M-CHIP mutation is higher than that found in unexposed populations, while limited data are available in the literature on the proportion of subjects with L-CHIP mutation.

The presence of M-CHIP mutation was associated with older age, smoking status, and overweight/obesity, whereas the presence of L-CHIP mutation was not associated with these or other factors under investigation. Some cardiovascular and metabolic biomarkers were higher in carriers of M-CHIP mutations, although the clinical significance of this finding is unclear.

Exposure to WTC, including being exposure to the dust cloud or being present on the day of the attack or the next day, was not associated with either M-CHIP or L-CHIP mutation status.

This is the first study analyzing both M-CHIP and L-CHIP mutations in WTC rescue and recovery workers, and adds to the existing evidence of an increased prevalence of this condition. Specific surveillance programs should be considered to minimize the impact of CHIP among WTC rescue and recovery workers.

Section 2

Scientific Report

Background

Following the terrorist attacks of September 11, 2001, World Trade Center (WTC) responders received intense exposures to known or suspected carcinogens, leading to concern that these people may be at higher risk of developing several types of cancer [1]. Relatively little is known about the molecular mechanisms of environmental risk factors for development of hematologic neoplasms. Based on a four-level classification system of WTC exposure level that includes dust exposure, duration and worked on debris pile [2], an analysis of hematopoietic and lymphoid cancers and exposure suggested a dose-response relationship. A recently updated analysis on leukemia for the years 2002 to 2013 found increase risk for all leukemias (standardized incidence ratio [SIR]: 1.37; 95% confidence interval [CI]: 0.98-1.86) and in particular acute myeloid leukemia (AML) (SIR 3.45; 95% CI: 1.12-8.05) for the age group 35-44 for which rates of this leukemia subtype begin to increase [3]. In addition, WTC responders are at increased risk of cardiovascular diseases (CVD) [4-5].

Hematologic neoplastic and pre-neoplastic conditions are a heterogeneous group of diseases that arise from acquired genetic and epigenetic alterations in hematopoietic precursor cells. The presence of such alterations can be detected even prior to overt hematological manifestations due to increasing availability of molecular testing among individuals without over neoplastic or pre-neoplastic conditions. This finding has been defined as clonal hematopoiesis of indeterminate potential (CHIP) [6]. Despite the absence of detectable hematologic disorders, mutations characteristically associated with hematologic neoplasms were identified in 6 percent of individuals aged 60 years or more and associated with certain HLA types; these individuals are also at increased risk of CVD.

Specific Aims

The Specific Aims and Hypotheses of the study were as follows:

Specific Aim 1: To investigate differences in leukemia-associated genomic alterations in WTC responders with myeloid malignancies compared to matched non-WTC cases. This will be accomplished by performing whole exome sequencing aimed at identifying known and novel mutations (base substitutions, insertion/deletions, and copy number variants) among WTC responders diagnosed with myeloid leukemia or related conditions such as myelodysplastic syndrome, myeloproliferative neoplasm, and polycythemia vera (n=50) and comparing their profile with that of age- sex- and disease type- matched controls from

the tumor biobank at Mount Sinai. We hypothesize that WTC-exposed patients have distinct mutational profiles from non-WTC responders. In an additional exploratory analysis, we will compare the outcome between of the cases included in the proposed research, after stratification by type of disease, taking into account WTC exposure status, clinical characteristics, and molecular genetic profiles.

Specific Aim 2: To determine the prevalence of CHIP in WTC responders, and to investigate its association with WTC exposure. In Sub-Aim 2.1, we will measure the prevalence of CHIP-associated mutations in a sample of 1,400 WTC responders and test the hypothesis that the prevalence is higher than in age-matched historical controls. In Sub-Aim 2.2, we will compare the prevalence of CHIP-associated mutations among WTC responders with respect to WTC exposure level. Specifically, we will test the hypothesis that responders with higher WTC-related exposure have a higher prevalence of CHIP-associated mutations than lower-exposed WTC responders.

Methods

The World Trade Center Health Program (WTCHP) General Responder Cohort (GRC). The WTCHP GRC is comprised of individuals who participated in the rescue, recovery, and/or cleanup efforts at the WTC site after 9/11/2001 on the basis of eligibility criteria, which included type of duties, site location, and dates and hours worked/ volunteered [3, 7]. The present research study is based on a subset of WTCHP GRC participants located in Long Island, who were referred to the WTC Clinical Center located at Stony Brook University and were enrolled in the WTCHP (referred to here as the WTC cohort). The WTC Stony Brook Clinical Center was established in 2002 to monitor and treat WTC-related conditions in individuals who responded to the WTC disaster. Participants with documented WTC response experience were enlisted. The monitoring program protocol included self-administered physical and mental health questionnaires followed by a physical examination, laboratory tests, spirometry, and a chest radiograph. This population has been characterized very carefully and included in previous studies of various health conditions associated with response to the WTC disaster, including post-traumatic stress disorder (PTSD), prostate cancer, cognitive impairment, and COVID-19 [8-11]. Routine monitoring visits, scheduled every 12–18 months, were performed by SBU WTC Clinical Center staff. Compared with the WTCHP GRC as a whole, the WTC cohort includes relatively more law enforcement personnel and men and fewer individuals with a low level of education (i.e. without a high-school degree) [9].

Study Participant Demographics. From the WTCHP GRC members enrolled at the SBU WTC Clinical Center, we randomly selected 350 participants for WES, which, after checking for and removing

duplicates, resulted in 345 unique samples total. Detailed phenotypic characteristics of the study cohort are provided in **Table 1**. Over 90% were male and white; only 7.5% were female, and 9% were non-white. Less than 1% of participants were < 50 years old, 51.6% were 50-59 years old, 36.5% were 60-69 years old, and 11% were 70 or more years old. Among the 345 participants, 4.3% were current smokers, 44.6% were former smokers, and 51% had never smoked.

Sample Collection. All participants who had consented to the research provided blood samples during their annual clinical checkup visits between 2016 and January 2019. Whole blood samples were collected into Vacutainer Plastic K2EDTA (containing ethylenediaminetetraacetic acid) Tubes (BD, 367527) and stored at -80 C until analysis.

Sequencing Analysis. For the 350 WTCHP GRC individuals, deep WES was performed at Azenta Inc (Burlington, MA, USA) using the HiSeq 2500 system (Illumina, San Diego, CA, USA), adhering to standard protocols. The sample quality control (QC), library construction, and sequencing were performed in line with industry quality of process standards. A median 250X coverage was used in sequencing, to allow for ample sensitivity and accuracy in calling CHIP mutations even at low clonal frequencies. At the end of the sequencing procedure, high-quality 150-bp, pair-end read data were generated in standard fastq format.

Data Pre-Processing for Variant Discovery. First, we performed pre-processing and quality control (QC) of raw sequence reads using fastp [12]. Specifically, we trimmed the adapters and filtered out bad reads (low quality, too short or too many unknown bases). Next, we adhered to the Genome Analysis Toolkit (GATK, https://software.broadinstitute.org/gatk) best practices for data pre-processing to generate analysis ready bam files from the fastq files. Briefly, we aligned the sequence reads to the Genome Reference Consortium Human Build GrCh38 using BWA-MEM (https://doi.org/10.48550/arXiv.1303.3997) followed by duplicate marking using Picard (http://broadinstitute.github.io/picard) and base quality score recalibration using GATK. We used these processed bam files for calling germline variants and somatic mutations.

Germline Variant Calling. We called germline variants using HaplotypeCaller in GATK in GVCF mode. Then, we jointly genotyped individual gVCF files for all autosomes. Finally, we filtered variants by variant quality score recalibration in GATK and only included sites with < 20% missing data.

Kinship Analysis. To exclude any genetic duplicates among the 350 WTC cohort members, we performed kinship analysis with germline SNPs using KING software [13] and identified duplicate pairs with kinship coefficient > 0.354. We identified three pairs of duplicates out of which one pair was from the same individual but at different time points. We excluded five of these six samples from analysis and included only the most recent sample collected from the individual with a duplicate pair.

Somatic Variant Calling. After removing the duplicates, to identify somatic mutations within the remaining 345 WTCHP GRC individuals, we performed somatic variant calling on the analysis-ready bam files using the GATK Mutect2 pipeline in tumor-only mode. To exclude likely germline calls and sequencing artifacts, we provided external reference of germline variants from gnomAD and a Panel Of Normals (PON) to Mutect2. To create the PON, we used WES data from the publicly available Genotype-Tissue Expression (GTEx) cohort (phs000424), where we filtered for 70 young individuals (aged ≤ 40 years) as they would be less likely to have CHIP mutations. To identify somatic mutations with high confidence, we applied the orientation bias filter of Mutect2 and then further narrowed down these somatic variants with the PASS filter.

Somatic Variant Filtering for M- and L-CHIP. To identify M- and L-CHIP mutation carriers, we filtered for somatic variants and somatic driver genes known to be associated with myeloid malignancies (M-CHIP) and lymphoid neoplasms (L-CHIP) . Towards this end, we considered 76 somatic driver genes for M-CHIP [14, 15] and 235 [16] driver genes for L-CHIP . From these lists, we first filtered for somatic variants at a Mutect2 Variant Allele Fraction (VAF) > 2%. Next, we filtered the L-CHIP somatic variants further for *pathogenic* (variants curated from cBioPortal) or *putative* (variants that alter canonical protein sequence) [16]. Additionally, to remove likely artifacts, we used the following QC filters for L- and M-CHIP somatic variants.

CHIP Mutation QC Filters. We required the somatic variants at a minimum depth of 20 reads, and a minimum number of 3 reads (supporting the mutant allele) and at least one read in both forward and reverse direction (supporting the reference and mutant alleles). In addition, we excluded somatic variants observed in gnomAD with allele frequency ≥ 0.1% and with an observed frequency > 1% in the analysis cohort (unless previously reported to be involved in hematologic malignancies). Next, we annotated the identified somatic variants for pathogenicity using Combined Annotation Dependent Depletion (CADD) score and excluded variants with a scaled CADD score < 10. Finally, we implemented additional stringent filtering criteria for L-CHIP *putative* mutations. Unlike the L-CHIP *pathogenic* mutations, we required

these variants to have a minimum alternate allele read of 5, maximum VAF of 0.2 and at least two reads in both forward and reverse direction supporting the alternate allele.

There were two exceptions to our filtering strategy. First, a recent study (PMID: 35041928) reported that M-CHIP variants in the *U2AF1* gene cannot be reliably identified in the human GrCh38 reference genome due to unintended replication of the *U2AF1* locus in chromosome 21. To capture any M-CHIP variants in *U2AF1*, we repeated the alignment and somatic variant calling steps using the human genome reference build GrCh37. Second, because a recent study reported that the *ASXL1*-G646Wfs*12 variant with a VAF ≥ 10% was a true CHIP variant [17] and not a sequencing artifact, we checked for this variant in the WTC cohort. However, we did not identify any individuals with CHIP variants in either the *U2AF1* gene or at *ASXL1-G646Wfs*12*. At the end of these analyses, we considered all WTC individuals who harbored at least one known M- or L-CHIP somatic variant as having CHIP.

Prevalence of CHIP-Related Mutations in WTC Responders and Unexposed Controls. We tested the hypothesis that the prevalence of CHIP-associated mutations in WTC responders was higher than that in the unexposed controls. Towards this end, as controls, we used WES data from the 293 healthy control individuals among the Mount Sinai Crohn's and Colitis Registry (MSCCR) cohort. Detailed phenotypic characteristics of the control cohort are provided in **Table 3**. As the median coverage of the WTC cohort was deeper compared to that of the controls, we downsampled the WTC data by using Picard so as to have a cohort average total number of aligned bases in mapped reads passing Illumina's filter similar to the control cohort.

Data on additional risk factors for the study cohort**.** Overall, the data we have collected on risk factors in addition to whole-exome sequencing data on the study cohort include demographics (gender, race/ethnicity), exposure, blood counts (including various immune cell subsets, as listed below), smoking history, body mass index (BMI), mental, cognitive and general health characteristics (including cholesterol and triglyceride levels) assessed at each monitoring visit. These data were available from 294 study subjects, encompassing 15 blood count parameters and 5 lipid measurements, all of which were conducted concurrently with the collection of DNA samples for CHIP analysis. Specifically, we included the following parameters: platelet count, basophil count, lymphocyte count, neutrophil count, segmented neutrophil count, eosinophil count, monocyte count, LMR (ratio lymphocyte/monocyte), white blood cell count (WBC), red blood cell count (RBC), mean volume red blood cell, mean hemoglobin (MCH), mean hemoglobin concentration (MCHC), mean hemoglobin volume (MCV), red cell distribution width (RDW), total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and very low LDL (VLDL).

In addition, PTSD symptoms were assessed using the PTSD Checklist (PCL) [18] , a 20-item self-report measure modified to assess symptoms over the past month. The PCL has excellent psychometric properties, convergent validity and internal consistency [19] . WTC exposure severity was assessed during the intake interview at the WTC Health Program and have been described in detail [20] . Briefly, an exposure severity variable was created using total time spent working at Ground Zero or on the debris pile [20] . Mild cognitive impairment (MCI) was measured using the Montreal Cognitive Assessment (MoCA), a widely used objective multidomain test [21] .

Statistical analysis. To summarize categorical variables, we used counts and percentages and for their analysis used Fisher's exact tests. To summarize the continuous variables we used median and median absolute deviation and for their analysis we used Wilcoxon rank sum tests. For association analyses, as the majority of the participants were Caucasian, we collapsed the race variable into two categories: Caucasian and non-Caucasian/ unknown. We collapsed exposure as a variable into three categories, including very low/low, intermediate and high/very high. We excluded the missing values from the association analysis. We considered a p-value < 0.05 as statistically significant. In these analyses, we defined participants who did not harbor any M and L-CHIP mutations as CHIP negative controls (N=227). We considered several case groups, including (a) CHIP positive (at least one M or L-CHIP mutation (N=118)), (b) M-CHIP positive, (at least one M-CHIP mutation (N=56), (c) L-CHIP positive (at least one L-CHIP mutation (N=74)), (d) *DNMT3A* mutation (at least one mutation on *DNMT3A* gene (N=22)), (e) *TET2* mutation (at least one mutation in *TET2* gene (N=15)), (f) *PPM1D* mutation (at least one mutation in *PPM1D* gene (N=11)), (g) *EEF1A1* mutation at least one mutation in *EEF1A1* gene (N=18)), and (h) *DDX11* mutation (at least one mutation in *DDX11* gene (N=13)). For each case/control comparison, among the characteristics that were significant from marginal associations, we fitted a multivariate logistic regression using case/control as outcome variable and the significant characteristics as covariates.

Results.

To study the prevalence of CHIP mutations in WTC debris exposed first responders, we collected blood and extracted DNA from 350 samples, and then used deep WES at 250X to examine their clonal somatic variants in known driver genes of myeloid (M-CHIP) and lymphoid malignancies (L-CHIP). The study outline is shown in **Figure 1A**. The participants aged 48-90 years (median, 59 years), and had no previous hematologic malignancy diagnosis at the time of enrollment. After filtering samples for duplicates, we analyzed 345 total samples for CHIP, on which we applied rigorous variant QC metrics and filtered for

somatic mutations (Figure 1B). We provide the participant demographics in **Table 1**. Next, we associated CHIP prevalence with age, ancestry, exposure, HLA zygosity , blood counts and other clinical variables. Additionally, we compared the prevalence of CHIP in WTC debris exposed first responders to 293 unexposed controls from the New York City, NY area from the Mt Sinai Crohn's and Colitis Registry (MSCCR) cohort.

Characteristics of L-CHIP and M-CHIP mutations. To identify the WTC cohort members with M/L-CHIP mutations, we filtered for pre-defined M-CHIP mutations in 76 genes (PMID: 33057201, 35835912) and L-CHIP mutations in 235 genes (PMID: 34663986) associated with myeloid and lymphoid malignancy respectively (**Table 2**). Towards this end, we set up a computational analysis pipeline, the steps of which we summarize in **Figure 1B**. After sample QC, 16.23% (56 out of 345) of the WTC cohort participants harbored M-CHIP mutations (71 mutations in 13 genes), while 21.45% (74 out of 345) harbored 85 L-CHIP mutations mutations in 43 genes). The majority (>80%) of the participants who harbored M/L-CHIP mutations carried only a single CHIP mutation (**Figure 2-A,D**). Of the M-CHIP mutations, 39% were non-synonymous, 30% were stop-gain , 23% were frameshift deletions and the rest were frameshift insertions and splicing (**Figure 2B**). On the other hand, for L-CHIP, 87% were non-synonymous, 9% were stop-gain and the rest were frameshift indels (**Figure 2E**). We also observed that >80% of M-CHIP and >90% of L-CHIP mutations had VAF<0.2 (**Figure 2-C,F**).

Consistent with literature on CHIP and aging, the prevalence of M-CHIP mutations increased with age (*p*-value = 2.45e-05). We provide the distribution of the prevalence of M/L-CHIP mutations in different age groups in Table 2. However, we did not observe a similar trend with L-CHIP mutations (*p*-value = 0.098). Top genes associated with M-CHIP mutations were *DNMT3A, TET2, PPM1D* and *ASXL1* (Figure 3A). Among these genes, *TET2* mutations exhibited the highest VAF.

Factors associated with CHIP prevalence. To identify the factors influencing CHIP prevalence, we evaluated the associations between M- and/or L-CHIP mutations and i) clinical variables - age, gender, race, smoking status, cardiovascular diseases (CVD), stroke, body mass index (BMI), Montreal Cognitive Assessment (MoCA), PTSD Checklist (PCL); ii) blood parameters (blood and lipid counts) and iii) HLA zygosity (**Figure 4**). In the association analysis, we first performed univariate analysis using the individual factors. Once we identified significant characteristics that influenced CHIP mutation status, we performed multivariate analysis. From these analyses, we observed that CHIP positive cases (individuals with either M- or L-CHIP mutations) were associated with higher proportion of HLA-DMB homozygosity and older age compared to CHIP negative (individuals with neither M- nor L-CHIP

mutations) cases. Both variables remained significant in the multivariate logistic regression model. Particularly, M-CHIP positive cases were associated with higher proportion of previous-smokers, older age, lower BMI and lower platelet count compared to CHIP negative controls. Smoking, age and platelet count remained significant in the multivariate logistic regression model. L-CHIP positive cases were not associated with the participant characteristics considered in this study.

Next, we looked at associations based on the top frequently mutated M/L-CHIP genes. Participants with *DNMT3A* mutations, the most frequently mutated M-CHIP gene, were associated with older age, lower BMI and lower absolute lymphocytes. Age remained significant in the multivariate logistic regression model. The median age of participants with *DNMT3A* was 66.5, whereas the median age of other M-CHIP positive participants was 61. The median age of CHIP negative controls was 59. *TET2* mutation status, the second most frequently mutated M-CHIP gene, was associated with older age, lower absolute lymphocytes and red blood cell (RBC) counts, and higher segmented neutrophils. Age remained significant in the multivariate logistic regression model. Mutation status of the third most frequently mutated M-CHIP gene, *PPM1D,* was associated with higher proportion of DQA1 homozygosity, lower platelet count and higher mean corpuscular hemoglobin (MCH). None of the variables remained significant in the multivariate logistic regression model.

For L-CHIP, *EEF1A1* mutation status, the most frequently mutated L-CHIP gene, was associated with lower proportion of DPA1 homozygosity. Finally, participants with *DDX11* (DEAD/H-box helicase 11) mutations, the second most frequently mutated L-CHIP gene, were associated higher PCL scores, absolute lymphocytes, mean corpuscular volume (MCV) and lymphocyte monocyte ratio (LMR), and lower MoCA scores and segmented neutrophils. MoCA scores remained significant in the multivariate logistic regression model. The median MoCA score for participants with *DDX11* mutation was 22, which was within the range indicative of mild cognitive impairment. *DDX11* belongs to the DEAD box protein family which is implicated in cellular processes involving alteration of RNA secondary structure. Furthermore, Lovell *et al* (PMID: 10650137) found that DNA helicase activity was altered in brain regions of patients with Alzheimer's disease (AD) which may interfere with base excision repair mechanism, potentially leading to the pathogenesis of neurodegeneration of AD.

Association of M-CHIP and L-CHIP prevalence with WTC exposure The overall prevalence of M-CHIP and L-CHIP in the UK Biobank study was 5.8% and 1.3% [15] and that of M-CHIP in TOPMED is 4.3% [14]. However, these two studies have used slightly different gene lists as well as filtering criteria to define CHIP. Therefore, to understand the influence of WTC debris exposure on CHIP

prevalence, we compared the first responders to healthy control individuals among the Mount Sinai Crohn's and Colitis Registry (MSCCR) cohort (**Table 3**). Since the sequencing depth of both the cohorts were different, we downsampled the WTC sequencing dataset (see Methods) for direct comparison. We observed higher prevalence of M- and L-CHIP mutations in most of the age categories (**Figure 5A and B**) though not significantly different ($p \leq 0.05$) except for the higher prevalence of L-CHIP mutations in the WTC cohort compared to controls for age ≤ 55 ($p=0.04$). Next, we asked whether there was any difference in the number of mutations in the top genes (**Figure 5 C and D**) between the two cohorts. In terms of M-CHIP mutations, we observed a significant difference between the number of TET2 mutations comparing WTC to control ($p=0.02$). Furthermore, in terms of L-CHIP mutations we observed a significantly higher number of mutations of EEF1A1 ($p< 0.0001$) and DDX11 ($p=0.04$) in WTC cohort vs unexposed controls.

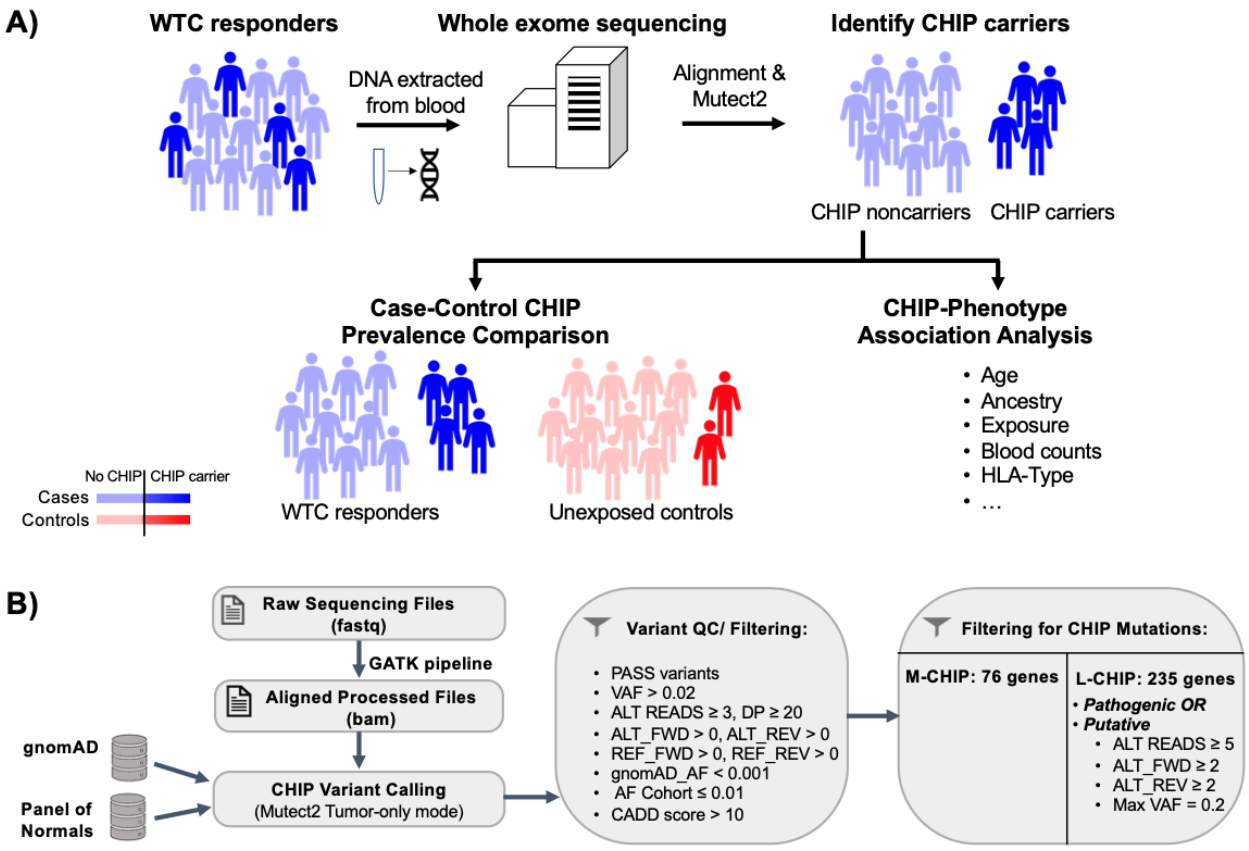

Figure 1. Study design and pipeline A) Study outline **B)** WES data analysis pipeline to identify M/L-CHIP mutations. .

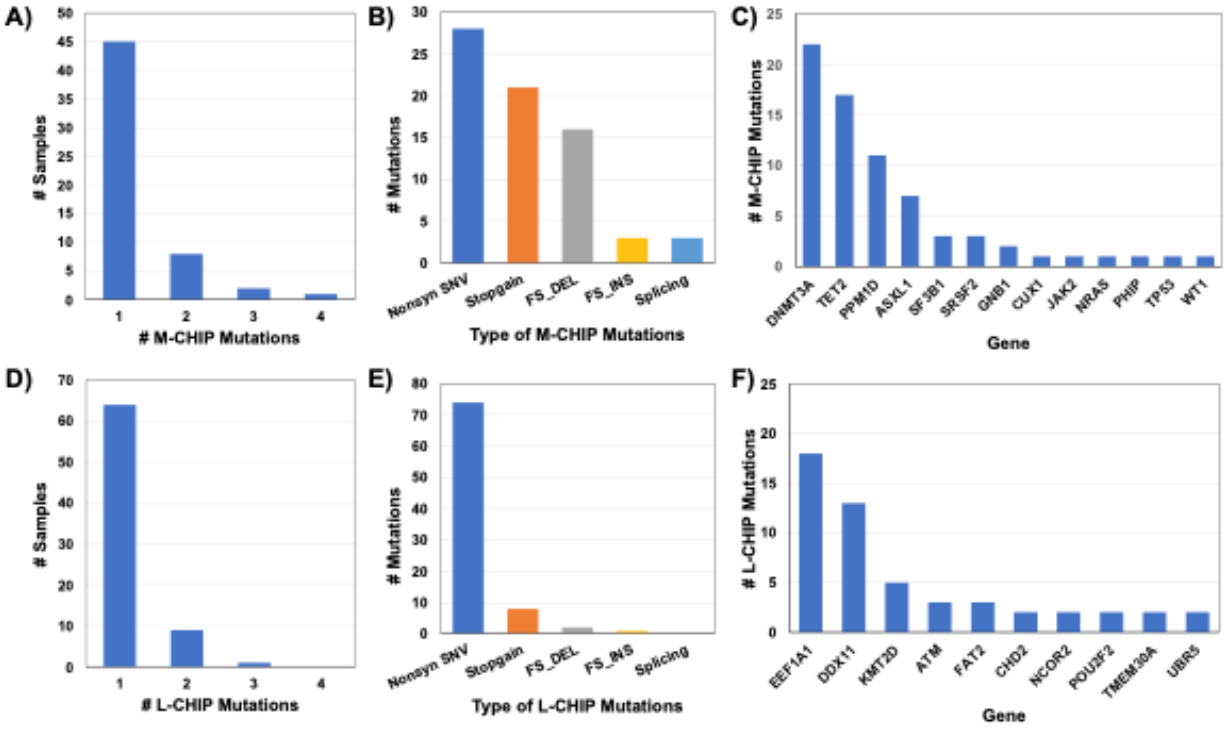

Figure 2. Characteristics of M-CHIP and L-CHIP mutations in 345 WTC-debris-exposed first-responders. A) Number of samples with 1,2, 3 and 4 M-CHIP mutations, **B)** Number of different types of M-CHIP mutations, **C)** Histogram of the variant allele fraction (VAF) of the M-CHIP mutations, **D)** Number of samples with 1,2, 3 and 4 L-CHIP mutations **E)** Number of different types of M-CHIP mutations, and **F)** Histogram of the variant allele fraction of L-CHIP mutations.

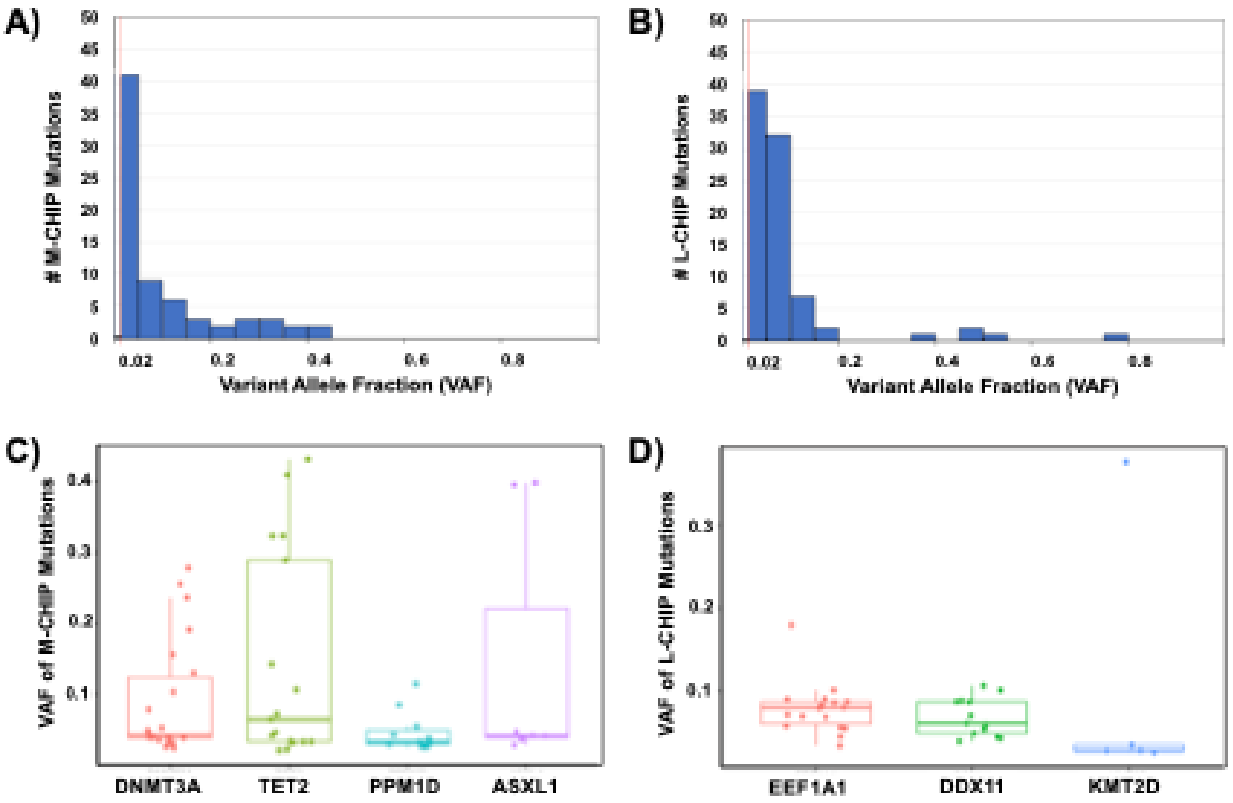

Figure 3. Prevalence and characteristics of M-CHIP and L-CHIP mutations in 345 WTC-debris-exposed first responders **A)** Number of M-CHIP mutations observed in each gene **B)** Number of L-CHIP mutations observed in each gene **C)** Variant allele fraction (VAF) distribution of mutations in the top M-CHIP genes **D)** VAF distribution of mutations in the top L-CHIP genes

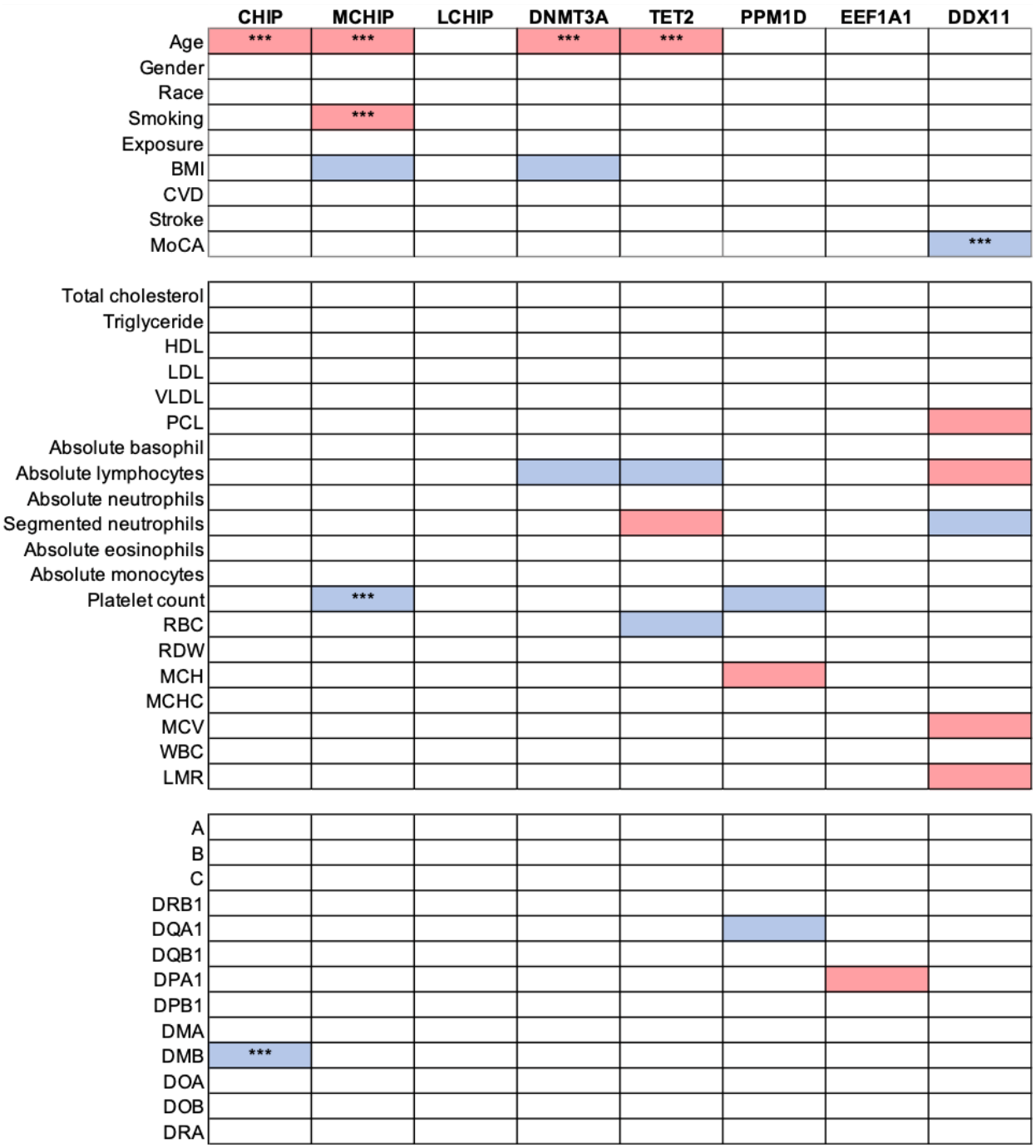

Figure 4: CHIP-Phenotype associations. Heatmaps showing associations between overall CHIP, M-CHIP, L-CHIP, M-CHIP driven by DNMT3A, TET2, PPM1D mutations and L-CHIP driven by EEF1A1, DDX11 mutations with clinical variables, blood count parameters and HLA zygosity. Blue and red correspond to significant negative and positive correlation ($p \leq 0.05$). White represents no correlation. Asterisk represents significant associations ($p \leq 0.05$) in the multivariate regression analysis.

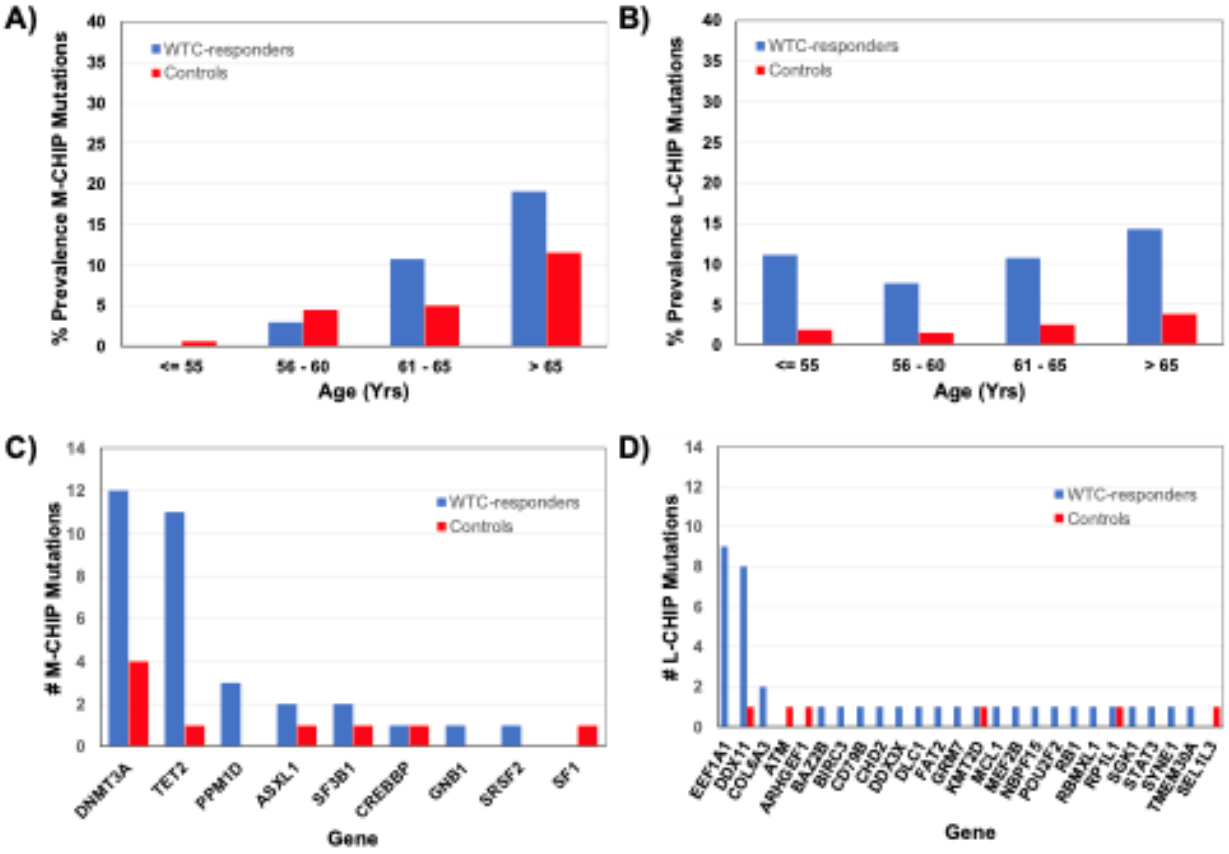

Figure 5. Prevalence and characteristics of M-CHIP and L-CHIP mutations in 345 WTC-exposed individuals (blue) and 293 unexposed controls (red) A) % Prevalence of M-CHIP mutations in the cohorts as a function of age B) % Prevalence of L-CHIP mutations in the cohorts as a function of age C) Genes identified with M-CHIP mutations D) Genes identified with L-CHIP mutations

Table 1. Characteristics of the World Trade Center Health Program (WTCHP) General Responders Cohort (GRC) members selected for WES in this study (the WTC cohort), which, after sample QC that removed duplicates, totaled 345 individuals.

Phenotype	Category	# Samples (%)
Sex (M/F =12.27)	Male (M)	319 (92.46%)
	Female (F)	26 (7.54%)
Smoking status	Current smoker	15 (4.35%)
	Previous smoker	154 (44.64%)
	Never smoker	176 (51.01%)
Race	White/ Caucasian	314 (91.01%)
	Black/ African-American	12 (3.48%)
	Other race	13 (3.77%)
	Unknown	6 (1.74%)
Age (yrs) Median= 59 SD= 5.90	≤ 55	27 (7.83%)
	56 - 60	171 (49.57%)
	61 - 65	84 (24.35%)
	66 - 70	32 (9.28%)
	> 70	31 (8.99%)
Exposure level	Very high	15 (4.35%)
	High	55 (15.94%)
	Intermediate	210 (60.87%)
	Low	50 (14.49%)
	Very low	6 (1.74%)
	Missing/ No record	9 (2.61%)

Table 2. Prevalence of M-CHIP and L-CHIP mutations

Age (Years)	# Samples	# Samples with M-CHIP mutation	# Samples with L-CHIP mutation
≤ 55	27	2 (7.41%)	6 (22.22%)
56-60	171	17 (9.94%)	33 (19.30%)
61-65	84	16 (19.05%)	23 (27.38%)
66-70	32	6 (18.75%)	7 (21.88%)
> 70	31	15 (48.39%)	5 (16.13%)

Table 3. Prevalence of M-CHIP and L-CHIP mutations in downsampled 345 WTC-exposed first responders and unexposed controls

Age (Years)	# Samples		#Sample w/ M-CHIP mutation		#Sample w/ L-CHIP mutation	
	WTC	Control	WTC	Control	WTC	Control
≤ 55	27	160	0 (0.00%)	1 (0.63%)	3 (11.11%)	3 (1.88%)
56-60	171	67	5 (2.92%)	3 (4.48%)	13 (7.60%)	1 (1.49%)
61-65	84	40	9 (10.71%)	2 (5.00%)	9 (10.71%)	1 (2.50%)
> 65	63	26	12 (19.05%)	3 (11.54%)	9 (14.29%)	1 (3.85%)

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Publications

Two scientific publications are in preparation, based on the results included in the Scientific Report.

Human Subjects:

Data and samples used in this research have been collected from WTC responders at Stony Brook Clinical Center of Excellence . Only de-identified data and samples have been used for this research. Approval for the research was obtained from Stony Brook IRB

Cumulative Inclusion Enrollment Table

Not applicable. Study subjects have been already included in the cohort of WTC rescue and recovery workers. The distribution of study subjects is shown in Table 1 of the Scientific Report.

Inclusion of Gender and Minority Study Subjects

WTC rescue and recovery workers are predominantly male and non-Hispanic White. No selection was performed for inclusion in this study based on gender or race/ethnicity, and that distribution of study subjects reflect that of the parent cohort.

Inclusion of Children

Not applicable

Materials Available for Other Investigators

Researchers requesting data from the Study must present a brief scientific proposal to the Contact PI of the Study, Dr. Boffetta, describing the aims of the intended research, the data requested, and the scientific approach including data analytic methods. This requirement applies to investigators from the same institutions as the Study investigators, and investigators from outside institutions. The researcher must also provide:

- name and title of the principal investigator,
- place of employment,

- academic affiliation,
- phone number and e-mail,
- name of project and web link if available,
- the name and contact information of the Institutional Review Board responsible for the study,
- the source and amount of budgetary support available for this project, if any,
- and descriptions of data security procedures that will insure the confidentiality of the study data

The group of investigators will review the proposal for scientific merit and burden to the current research staff and vote to approve, request modifications, or disapprove the proposal. Votes to approve must be unanimous. The Study Steering Committee may request that existing Study Investigators of their collaborators be included in the proposed project if they are interested.

No data will be released until all necessary IRB approvals have been obtained, including any amendments to existing IRB protocols, and any data sharing agreements with parties providing data are also approved and executed. Such agreements typically detail what the data can be used for, the time period identified for use of the data and the means for returning or destroying the data at the end of that time period. Also, any researcher requesting mortality data must submit a separate National Death Index application and receive approval before study mortality data will be provided.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

No

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Data or Databases	Once the manuscripts with the primary results of the research will be accepted for publication, de-identified data will be made available to external researchers upon request to the PI and the Stony Brook Clinical Center of Excellence.

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
PAOLO.BOFFETTA	Y	Boffetta, Paolo	MPH,MD	PD/PI	0.9	0.0	0.0			NA
	Y	Mascarenhas, John		PD,PI	7.5	0.0	0.0			NA
	Y	Gumus, Zeynep		Co-Investigator	0.8	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RS - Reentry Supplement
DS - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable

D.2.d New Other Significant Contributors

Not Applicable

D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

G. OVERALL SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Not Applicable

G.4.b Inclusion Enrollment Data

NOTHING TO REPORT

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

I. OVERALL OUTCOMES

I.1 What were the outcomes of the award?

The goal of this study is to determine whether WTC exposed subjects have an increased prevalence of clonal hematopoiesis of indetermined potential (CHIP), a condition associated with hematologic neoplasms and cardiovascular disease. Two types of CHIP mutations have been described, myeloid-CHIP (M-CHIP) and lymphoid-CHIP (L-CHIP). We analyzed blood samples from 357 WTC responders recruited at the Clinical Center of Excellence at Stony Brook University.

A total of 130 responders (36.4%) were positive for CHIP mutations, including 56 responders (15.7%) who were positive for M-CHIP mutations and 72 (20.2%) who were positive for L-CHIP mutations. The proportion of responders with M-CHIP mutation is higher than that found in unexposed populations, while limited data are available in the literature on the proportion of subjects with L-CHIP mutation.

The presence of M-CHIP mutation was associated with older age, smoking status, and overweight/obesity, whereas the presence of L-CHIP mutation was not associated with these or other factors under investigation. Some cardiovascular and metabolic biomarkers were higher in carriers of M-CHIP mutations, although the clinical significance of this finding is unclear.

Exposure to WTC, including being exposure to the dust cloud or being present on the day of the attack or the next day, was not associated with either M-CHIP or L-CHIP mutation status.