Chitotriosidase is a Biomarker for the Resistance to World Trade Center Lung Injury in New York City Firefighters

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Abstract

Purpose—World Trade Center (WTC) exposure caused airflow obstruction years after exposure. Chitinases and IgE are innate and humoral mediators of obstructive airway disease. We investigated if serum expression of chitinases and IgE early after WTC exposure predicts subsequent obstruction.

Methods—With a nested case-control design, 251 FDNY personnel had chitotriosidase, YKL-40 and IgE measured in serum drawn within months of 9/11/2001. The main outcome was subsequent Forced Expiratory Volume after one second/Forced Vital Capacity (FEV_1/FVC) less than the lower limit of normal (LLN). Cases (N=125) had abnormal FEV_1/FVC whereas controls had normal FEV_1/FVC (N=126). In a secondary analysis, resistant cases (N=66) had FEV_1 (107%) one standard deviation above the mean. Logistic regression adjusted for age, BMI, exposure intensity and post-exposure FEV_1/FVC modeled the association between early biomarkers and later lung function.

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Results—Cases and Controls initially lost lung function. Controls recovered to pre-9/11 FEV_1 and FVC while cases continue to decline. Cases expressed lower serum chitotriosidase and higher IgE levels. Increase in IgE increased the odds of airflow obstruction and decreased the odds of above average FEV_1. Alternately, increasing chitotriosidase decreased the odds of abnormal FEV_1/FVC and increased the odds of FEV_1 107%. Serum YKL-40 was not associated with FEV_1/FVC or FEV_1 in this cohort.

Conclusions—Increased serum chitotriosidase reduces the odds of developing obstruction after WTC-particulate matter exposure and is associated with recovery of lung function. Alternately, elevated IgE is a risk factor for airflow obstruction and progressive lung function decline.

Keywords

Chitotriosidase; Immunoglobulin E; WTC Particulate Matter; Pulmonary Function Testing

INTRODUCTION

World Trade Center (WTC)-particulate matter (PM) exposure from the 9/11 disaster resulted in significant airflow obstruction and reactive airway disease in Fire Department of New York (FDNY) rescue workers, other exposed workers, lower Manhattan residents and children (1–7). A recent longitudinal study by FDNY reported that exposure to WTC dust led to a dramatic decline in forced expiratory volume in one second (FEV_1) of FDNY rescue workers during the first year after exposure without subsequent recovery in a majority of those exposed (8). A minority of those exposed had accelerated decline of lung function suggesting greater than average susceptibility to lung injury (1, 8). Alternately, another subgroup recovered of FEV_1 suggesting greater than average resistance to the long-term effects of PM exposure (9). It is unclear why some individuals' lung function improved while others' continued to deteriorate. We therefore characterized biomarkers of resistance and susceptibility to WTC PM-related lung injury. We previously described a set of biomarkers of inflammation, metabolic syndrome and vascular injury in serum collected within six months of 9/11/2001 that predicted future decline or improvement in FEV_1 (9–11).

The ratio of FEV_1/Forced Vital Capacity (FVC) is another well-validated spirometric measure. Reduction of FEV_1/FVC indicates airflow obstruction that characterizes COPD and asthma. Genome wide association studies (GWAS) observed that a set of genetic variants are associated with only FEV_1, variation at other loci predict only FEV_1/FVC and variation at a third set of loci is associated with both FEV_1 and FEV_1/FVC (12, 13). This suggests independent but overlapping regulation of FEV_1 and FEV_1/FVC in health and disease. Biomarkers predicting abnormal FEV_1/FVC may therefore be distinct from FEV_1. Hence, we investigated if biomarkers expressed within 6 months of 9/11/2001 predicted future abnormal FEV_1/FVC in this WTC exposed cohort.

The glycosyl hydrolase 18 gene family contains true chitinases that bind and cleave chitin. Other chitinase-like proteins (CLP) bind but do not cleave the chitin polysaccharide. Among these chitinases/CLPs, chitotriosidase is the major enzymatically active chitinase in humans and the best characterized chitinase from a biologic and clinical perspective (14, 15).

Chitotriosidase is part of the innate host defense against bacterial and fungal infections since chitin is a major structural component in bacteria, fungi, insects and crustaceans, but not in mammals (16–20). YKL-40 is a CLP that is strongly associated with human diseases characterized by inflammation, remodeling and fibrosis (21–25). However, its biological function has not been clearly defined. Chitotriosidase is produced in mature monocyte-derived macrophages, lung macrophages and other specific subsets of tissue macrophages (26–29). Elevated chitotriosidase expression is associated with smoking induced and fibrotic lung disease (30, 31). The utility of chitotriosidase or YKL-40 as biomarkers of lung disease is under active investigation. However, their utility in particulate matter induced airway obstruction has not been elucidated.

Immunoglobulin E (IgE)-mediated humoral immunity is another important immune response mechanism in the respiratory tract (32). Elevated IgE is a key immune mediator in asthma. Children and adults with asthma have higher IgE than normal controls and anti-IgE antibody is an effective asthma treatment (33–36). Elevated serum IgE is strongly associated with low FEV_1/FVC in patients with chronic obstructive lung disease (37).

Our studies have focused on the well phenotyped WTC exposed FDNY firefighter cohort. The intense PM exposure at the WTC site overwhelmed the lung's normal protective barriers. We hypothesized that particulate matter exposure-induced lung injury is associated with immune mediators of asthma and COPD. We tested if serum chitinases and IgE, expressed soon after a massive PM exposure predict subsequent airway obstruction.

METHODS

Study design

We preformed a nested case-control study using a homogeneous previously healthy baseline cohort of 801 WTC exposed rescue and recovery workers. The study group did not contain individuals with lung disease or tobacco use to eliminate these confounders. The baseline cohort was derived from 1,720 exposed workers who needed subspecialty pulmonary evaluation (SPE) and treatment within 6.5 years of 9/11/2001. The study cohort was derived from three subgroups of the baseline cohort: 1) A randomly selected a subgroup to represent biomarker expression in the total cohort (171/801). 2) Those with the worst FEV_1 at SPE (100/801) to represent those susceptible to WTC induced lung injury. 3) Those with the best FEV_1 at SPE (100/801) to represent those resistant to lung injury (9). Serum was available in 124/171 of the representative subgroup, 66/100 of the resistant subgroup and 68/100 of the susceptible subgroup. Since there was overlap between subgroups, the study group was 251/801 of the baseline cohort. Cases were defined by FEV_1/FVC less than lower limit of normal (LLN, N=125) at SPE, whereas controls had FEV_1/FVC greater than LLN (N=126).

Demographics

Age, race and years of service at FDNY were obtained from the FDNY-WTC-monitoring database. BMIs were calculated from height and weight measured at the time of medical monitoring evaluation (MME) and SPE. Degree of exposure was self-reported at the first FDNY-WTC-monitoring and was categorized using the FDNY-WTC Exposure Intensity

Index (Arrival Time): i. Presented on the morning of 9/11/2001 ii. Arrived between afternoon on 9/11/2001 and 9/12/2001 (9–11). Those arriving after day three were excluded from analysis as a result of their low numbers in this sample.

Serum Sampling and Analysis

Blood drawn at the first post-9/11 FDNY-WTC monitoring exam was allowed to stand for one hour at room temperature before being centrifuged at 1,800g for ten minutes. Serum was stored at −80°C (Bio-Reference Laboratories, Inc. Elmwood Park, NJ). Serum was thawed once at four degrees and assayed using chitotriosidase (Quidel, USA), YKL-40 (MicroVue, USA) and IgE (R&D, Minnesota, USA) ELISA panels according to manufacturer's instructions. Data was analyzed using Graphpad Prism V (San Diego, USA).

Statistical Analysis

We tested normality using the Shapiro-Wilk test and Q-Q plots. We used unpaired Student's t-test or Wilcoxon rank-sum test for between group comparisons, as appropriate. Chi-squared test was used for inferences on proportions.

Given the dichotomous outcome of normal and abnormal FEV_1/FVC ratio, we tested if serum biomarkers predicted airflow obstruction using logistic regression. In addition to demographic variables, we tested if FEV_1/FVC ratio at the time serum was drawn altered the biomarker disease association. Equivalent analysis was done with the dichotomous outcome of FEV_1.

The Hosmer-Lemeshow goodness-of-fit test was used to assess calibration of the model. The model discrimination was evaluated through the receiver operating characteristic area under the curve (AUC). To test the robustness of the models, internal validation was performed using bootstrap procedures (10,000 bootstrap samples). Data are expressed as mean (standard deviation, SD), median (interquartile range, IQR) or Odds Ratio (95% confidence interval), unless otherwise stated. A two-sided P-value less than 0.05 was considered significant. All analyses were performed with STATA/SE version 12.1 (StataCorp LP, College Station, TX) and SPSS version 20 (IBM, USA).

Results

Demographics

Derivation of cases and controls is described in Figure 1. The demographics of baseline cohort, the study cohort, cases and controls are shown in Table 1. A study cohort of 251/801 had biomarkers measured in serum drawn soon after 9/11 during the first medical monitoring exam (MME). The median time to the serum draw at MME was 2.6 months post 9/11. The baseline cohort, study cohort, cases and controls had no significant difference in the time post 9/11 that serum biomarkers were measured.

Case status was defined by FEV_1/FVC ratio at subsequent subspecialty pulmonary evaluation (SPE). Cases of airflow obstruction (125/251) have had an abnormally low FEV_1/FVC ratio at SPE, while controls (126/251) have a normal ratio. The median time to SPE was 33.8 months post 9/11. The baseline cohort, study cohort, cases and controls had

no significant difference in the time post-9/11 that FEV_1/FVC was measured. Cases and controls had similar WTC exposure intensity, BMI and racial distribution. Cases were two years younger at 9/11 and had 2.2 less years of firefighting service than controls (P<0.01).

Pulmonary Function

By design, cases and controls were markedly different from one another in FEV_1/FVC ratio 34 months after 9/11/2001 on SPE spirometry (0.73 vs 0.82, Table 2). Cases and controls were also different from one another in pre-9/11 ratio (0.82 vs 0.87 P<0.001) and ratio at MME (0.81 vs 0.87 P<0.001). In a longitudinal analysis, the FEV_1/FVC ratio did not change in cases or controls from pre-9/11 spirometry to MME. In the interval from MME to SPE, the ratio declined in both cases (0.81 to 0.73 P<0.001) and controls (0.87 to 0.82 P<0.01) but cases had more deterioration than controls. To confirm that the median FEV_1/FVC in cases and controls represented individual deterioration, we used patients as their own control subjects. The mean individual reduction of the ratio from 9/11 to SPE was 9% (P=0.019) in cases and 4% in controls (P<0.001).

Similar to FEV_1/FVC ratio, FEV_1 % predicted was consistently lower in cases than controls at all time points. Surprisingly, in longitudinal analysis, cases lost 7% of their pre-9/11 FEV_1 while controls lost 11% at MME. Cases continued to lose FEV_1 from MME to SPE (86% to 81%), whereas FEV_1 in controls increased between two time points (97% to %108) returning to pre-9/11 values. Individual longitudinal lung function demonstrated the mean FEV_1 increased by 5% from 9/11 to SPE in cases (P=0.019) and decreased by 5% in controls (P=0.001). The mean individual FVC increased by 6% in cases (P<0.001) and 8% in controls (P<0.001).

At the time of SPE, cases had more air trapping and airway reactivity than controls. Cases had higher residual volume (RV) than controls (130% vs 111% P<0.001) without changes in functional residual capacity (FRC) or diffusing capacity for carbon monoxide (DLCO). Cases had more airway reactivity defined by FEV_1 increase in response to bronchodilator (9, IQR 4–16 in cases vs 4, IQR 0–8 in controls P<0.001) and FEV_1 decline in response to methacholine (MCT slope of cases 0.092, IQR 0.04–0.42 vs controls 0.038, IQR 0.02–0.07 P<0.001).

Serum Biomarkers

A total of 251 cases and controls had serum chitotriosidase, YKL-40 and IgE measured an average of 3 months after September 11, 2001. Compared to controls, cases had significant reduction of chitotriosidase (24.5ng/mL vs 32.2ng/mL, P<0.001) and significant elevation of IgE (50IU/mL vs 35.7IU/mL, P=0.01) (Table 3). There was no difference in YKL-40 between cases and controls (41.0ng/mL vs 44.0ng/mL, P=0.621). The range of chitotriosidase concentration was 1 to 174ng/mL and the range of IgE was 1.47 to 641IU/ml. Since the major source of chitotriosidase is phagocytes, we assessed if controls and cases had different leukocyte concentrations and differentials to rule out confounding effect. There was no significant difference in total leukocyte concentration, absolute neutrophil count and absolute lymphocyte count (P=0.549, 0.942 and 0.057 respectively) between cases and controls.

Logistic model predicting abnormal FEV_1/FVC

In an initial series of univariable analysis we observed significant association of abnormal FEV_1/FVC ratio at SPE with chitotriosidase, IgE, age and FEV_1/FVC ratio at the time serum was drawn. Increasing age, chitotriosidase and FEV_1/FVC ratio at MME reduced the odds of abnormal FEV_1/FVC ratio at SPE while increasing IgE increased the odds of airflow obstruction (Table 4). There was no association between YKL-40 and airflow obstruction (data not shown). In this small cohort, there was no significant association of WTC exposure intensity or BMI with abnormal FEV_1/FVC ratio at SPE (Table 4).

Multivariable logistic regression analysis showed that the biomarker-disease association remained after adjusting the model for potential confounders (Table 4). For each 10ng/mL increase of serum chitotriosidase the odds airflow obstruction at SPE declined by 18% (95%CI: 0.71, 0.95). We added the range of chitotriosidase to the adjusted logistic model and calculated the probability of abnormal FEV_1/FVC ratio (Figure 2). When holding all other variables in the model constant the probability of obstruction declined from 60.2% to 15.5% as the concentration of chitotriosidase increased from 1 to 174ng/mL (Figure 2 panel A). For each 100IU/mL increase of IgE, the odds of having a FEV_1/FVC less than LLN increased by 36% (95%CI: 1.09, 1.71). The probability of obstruction increased from 44.8% to 74.9% as the concentration of IgE increased from 1.47 to 641IU/mL (Figure 2 panel B). We then used receiver operating characteristic analysis to assess the sensitivity and specificity of the model for predicting airflow obstruction. The model had excellent performance with an AUC of 0.86 (Figure 2 panel C). The interaction term between chitotriosidase and IgE was not significant (data not shown).

FEV_1 as an outcome

FEV_1 is another well-validated spirometric measure to identify airway obstruction (9). We repeated the analysis with 66 individuals who had FEV_1 107% at SPE. These cases represent individuals with above average lung function who were resistant to the damaging effects of WTC exposure. We have previously reported the demographics and PFT of these resistant cases (9). Resistant cases had higher chitotriosidase than controls (26.2ng/mL vs 35.3ng/mL, P=0.007) and significant reduction of IgE (50.0IU/mL vs 35.7IU/mL, P=0.026). There was no difference in YKL-40 between cases and controls (43.2ng/mL vs 41.8ng/mL, P=0.621). For each 10ng/mL increase of serum chitotriosidase the odds of being in resistant group at SPE increased by 14% (95%CI: 1.012, 1.292). There was no association between YKL-40 and improvement in lung function after WTC exposure (95% CI: 0.991, 1.009). For each 100 IU increase in IgE the OR for resistance was 0.887 (95% CI: 0.71, 1.11). In addition, we tested if previously reported biomarkers of FEV_1 (9–11) were correlated with chitotriosidase or IgE. There was significant correlation between chitotriosidase and soluble vascular cell adhesion molecule (sVCAM) (R=0.197, P=0.002). Macrophage-derived chemokine (MDC), granulocyte-macrophage colony-stimulating factor (GM-CSF), leptin, amylin, ApoAII, C-reactive protein (CRP), mMacrophage inflammatory protein (MIP) were not associated with either chitotriosidase or IgE.

DISCUSSION

The WTC collapse produced a massive acute exposure to PM leading to a 439mL reduction of FEV_1 over the first six months in never smoking firefighters (8). FVC declined in parallel producing a pseudo-restrictive pattern on spirometry that was due to air trapping (38). Some individuals recovered while others had progressive lung function decline. To better understand intrinsic susceptibility and resistance to the damaging effects of WTC exposure we conducted a series of nested case-control studies (9–11). The study cohort was 801 never smokers with normal pre-9/11 lung function who sought subspecialty pulmonary evaluation (SPE) prior to March 2008. We have reported that levels of serum biomarkers of inflammation, metabolic syndrome and cardiovascular risk expressed within 6 months of September 11, 2011 were associated with increased risk of abnormal FEV_1 years later, with higher levels of some associated with increased risk and others associated with reduced risk. We now report early elevation of serum chitotriosidase reduces the risk of airway obstruction defined by abnormal FEV_1/FVC. It also predicts above average FEV_1 in the years following WTC exposure. Therefore chitotriosidase is a biomarker of resistance to lung injury. Alternately elevated IgE is a risk factor for abnormal FEV_1/FVC and reduces the odds of above average lung function post WTC exposure. Finally, there was no association of YKL-40 and any measure of lung function. This suggests that YKL-40 regulation in PM-induced asthma is significantly different than YKL-40 in allergic asthma (21–25).

The study cohort was nested within a larger, longitudinally followed population of symptomatic FDNY firefighters who were exposed to particulate matter from the WTC disaster. We narrowed 1720 subjects to a baseline cohort of 801 individuals. We chose to exclude active and prior smokers to eliminate a major confounder of abnormal lung function and chitotriosidase expression (30).

This investigation used FEV_1/FVC and FEV_1 as two separate outcomes in the analysis. We chose FEV_1/FVC below LLN to define lung injury since this cut point is derived from large cross sectional population studies. We chose FEV_1 107% to define resistance to lung injury since this value is one standard deviation above the mean at SPE in this WTC exposed cohort. Prior studies of this cohort used only FEV_1 as a measure of susceptibility or resistance to lung injury. Adding an explicit measure of airflow obstruction to the analysis enabled us to overcome the limitations of using of FEV_1 as a single measure of lung injury in this cohort. Using only FEV_1 can result in misclassification of disease since FEV_1 is reduced in both restrictive and obstructive lung disease. Furthermore patients who starting with pre-9/11 FEV_1 well above 107%, could suffer large, clinically significant decline in FEV_1 and remain above the cut point defining resistance to lung injury. Therefore FEV_1/FVC, when used in conjunction with FEV_1, provides a more complete measure of the resistance and susceptibility to the damaging effects of WTC exposure.

Both serum chitotriosidase and IgE expressed early after exposure are biomarkers for abnormal FEV_1/FVC and above average FEV_1 years later. The concept that FEV_1/FVC and FEV_1 may reflect distinct but overlapping pathophysiologic mechanisms of lung disease is supported by GWAS findings (12, 13). Some genetic polymorphisms are associated with

both FEV_1 and FEV_1/FVC, whereas other variants are associated with one or the other. To date chitotriosidase and IgE are the only biomarkers that predict susceptibility or resistance to FEV_1/FVC. Previously reported biomarkers of FEV_1(9–11); MDC, GM-CSF, leptin, amylin and apolipoproteins are not associated with airflow obstruction.

Patients with normal FEV_1/FVC and above average FEV_1 years after WTC exposure had higher serum chitotriosidase than controls. Multivariable logistic regression showed that patients with increased serum chitotriosidase soon after exposure had significantly reduced odds of subsequent airflow obstruction defined by abnormal FEV_1/FVC. They also had increased odds of above average FEV_1. In fact, these resistant individuals returned to their pre-9/11 FEV_1 and FVC on average 32 months post-9/11. Chitotriosidase is therefore a biomarker of recovery from lung injury. This suggests the group with high chitotriosidase levels had the capacity to repair irritant induced lung injury. We have previously observed sVCAM also predicts average FEV_1. Interestingly sVCAM expression and chitotriosidase expression are correlated raising the possibility that they are separate components of protective pathways.

Our nested case-control biomarker study has the advantage that serum biomarkers were measured on average 3 months after the acute exposure during the process of disease evolution. The outcome occurred on average 32 months after exposure. Because the biomarkers were measured prior to case definition it is unlikely that its expression is a consequence of good outcome. Chitotriosidase plays an important role in innate immunity and is associated with smoking, sarcoidosis, pulmonary infections, as well as interstitial lung disease (30, 31, 39–42). These cross sectional studies are unable to distinguish if elevated chitotriosidase cause disease, are a reparative response to disease or are a consequence of disease with no impact on lung function. The potential mechanism of chitotriosidase's role in protecting from WTC-PM induced lung injury is unknown. Our data are consistent with the hypothesis that chitotriosidase plays a beneficial role in injury repair after WTC-PM induced lung. Testing this hypothesis requires experiments in model systems in combination with larger longitudinal studies.

In contrast to chitotriosidase, IgE is critical for humoral immune function in the lung. IgE's association with airway obstruction and atopy is well known (32–37). As expected, increased IgE predicted subsequent airway obstruction in our WTC exposed cohort. We also observed that increasing IgE reduced the odds of above average FEV_1 post exposure. Importantly, no member of this cohort had atopic asthma prior to exposure since the presence of asthma precludes active firefighting duty. Also most of the population had IgE levels in the normal range. IgE's association with WTC-PM induced lung injury implies a complex pathophysiologic response to irritant induced lung injury reflected in the humoral immune system. Further investigation is required to assess if antigen specific antibodies are responsible for the observed phenotype or variation of IgE within the normal range reflects other attributes of the immune response to respiratory irritants that increases the risk for lung injury.

This study has several limitations. Our FDNY firefighters cohort is unique as they had massive acute exposure to WTC dusts. This limits the generalizability of these finding to

other study populations with lower level PM exposure produced by ambient air pollution. We did not have an unexposed control group to compare and therefore we could not determine the direct effect of WTC-PM exposure on chitotriosidase or IgE levels. Replication of these findings in other longitudinally followed populations with and without PM exposure will be important to demonstrate the generalizability of these findings.

In this study, increased serum chitotriosidase levels reduced the risk of developing airway obstruction after WTC-PM exposure and predicted above average FEV_1 post exposure. Alternately, elevated IgE was a risk factor for abnormal FEV_1/FVC and reduced the odds of above average FEV_1. These results suggest that both innate and humoral immune mediators are involved in the pathogenesis of WTC-PM mediated lung injury. Further investigation is required to define if chitotriosidase is a mediator of lung repair after PM exposure.

Acknowledgments

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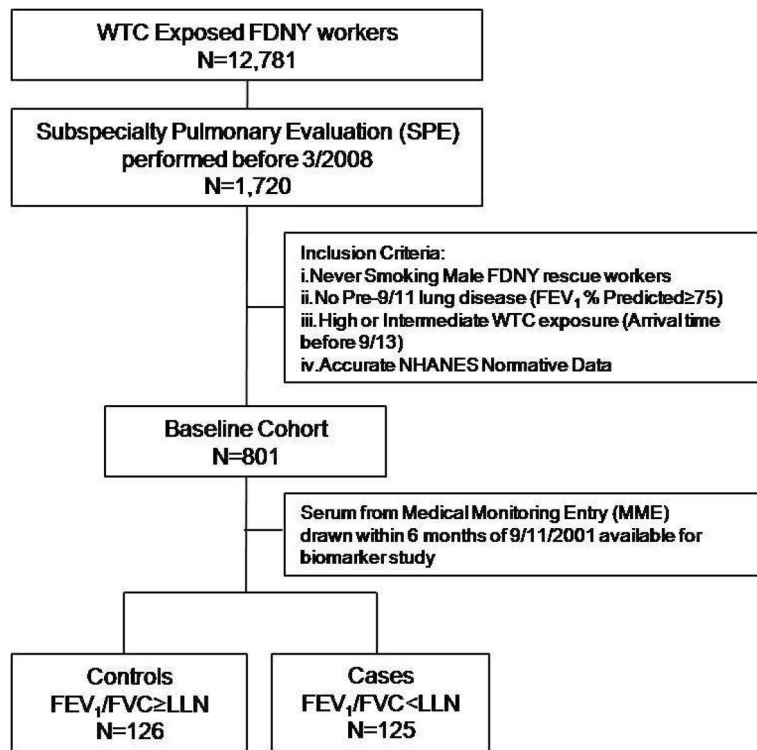
Figure 1.
Study design. WTC=World Trade Center; FDNY=Fire Department City of New York; NHANES=National Health and Nutrition Examination Survey; PFT=Pulmonary Function Test

Figure 2.
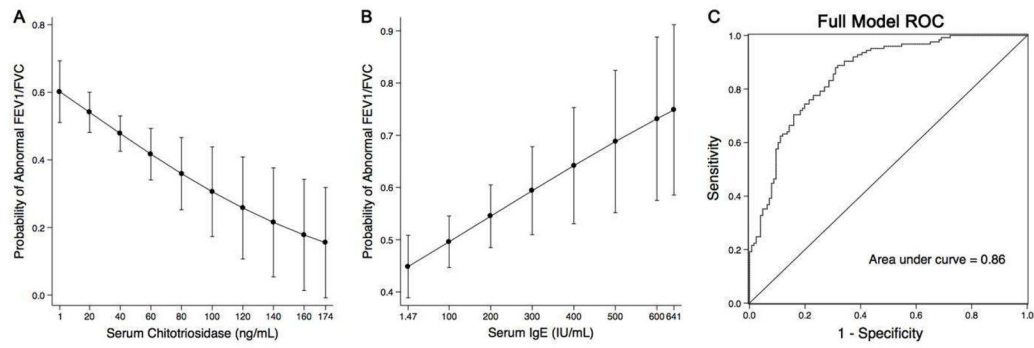Final logistic model prediction A. Calculated probability of $FEV_1/FVC<LLN$ with 95% CI as the concentration of chitotriosidase increased over the observed biomarker range with all other covariates held constant. B. Calculated probability of $FEV_1/FVC<LLN$ as IgE increases C. Receiver operating characteristic (ROC) of the final model to predict abnormal FEV_1/FVC. Area under the curve (AUC)=0.86.

Table 1

Demographics

	Baseline Cohort N=801	Study Group N=251	Controls N=126	Cases N=125
High WTC Exposure[*][‡]	197 (25)	52 (21)	30 (24)	22 (18)
Intermediate WTC Exposure[†][‡]	604 (75)	199 (79)	96 (76)	103 (82)
Months to MME[§]	2.7 (2–3.8)	2.6 (2.1–3.3)	2.6 (2.1–3.3)	2.7 (2.1–3.7)
Months to SPE[§]	33.8 (25–57)	33.6 (24–54)	32.0 (24–53)	35.5 (24–57)
BMI at MME, kg/m^2 [§]	28.0 (26–30)	27.9 (26–30)	27.6 (26–30)	28.1 (26–31)
BMI at SPE, kg/m^2 [§]	28.9 (27–31)	28.8 (27–31)	28.5 (26–31)	29.1 (27–31)
Age at hire, years[§]	27 (24–29)	27 (25–29)	27 (25–30)	27 (25–29)
Age at 9/11, years[§]	40 (36–45)	41 (37–46)	42 (38–46)	40 (36–45) [ll]
Years of Service at 9/11[§]	13.9 (7–19)	14.5 (8–18)	15.3 (10–20)	13.1 (7–17) [ll]
Race, Caucasian/African American[‡]	782 (98)/19 (2)	242 (96)/9 (4)	121 (96)/5 (4)	121 (97)/4 (3)

Definition of abbreviations: WTC = World Trade Center; MME = medical monitoring entry; SPE = subspecialty pulmonary evaluation; BMI = body mass index.

[*]Arrival at WTC 9/11 Morning,

[†]Arrival at WTC between noon of 9/11 and midnight of 9/12.

[‡]Expressed as N (%);

[§]Values Expressed as Medians (IQR);

[ll]p<0.01 by Wilcoxon rank-sum test between cases and controls.

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Table 2

Pulmonary Function Testing

		Study Group N=251	Controls N=126	Cases N=125	P-value*
Pre-9/11 Spirometry	FEV$_1$/FVC, %	84 (81–87)	87 (85–89)	82 (79–84)	<0.001
	FEV$_1$, L	4.3 (3.8–4.9)	4.5 (3.9–5.1)	4.1 (3.7–4.7)	<0.001
	FEV$_1$, % Predicted	104 (91–115)	109 (100–119)	93 (86–107)	<0.001
	FVC, L	5.0 (4.6–5.8)	5.1 (4.5–5.8)	4.9 (4.6–5.7)	0.564
	FVC, % Predicted	96 (87–108)	100 (91–110)	93 (85–106)	0.011
MME Spirometry	FEV$_1$/FVC, %	84 (80–87)	87 (84–89)	81 (77–84)	<0.001
	FEV$_1$, L	3.8 (3.3–4.3)	4.0 (3.6–4.5)	3.6 (3.2–4.2)	<0.001
	FEV$_1$, % Predicted	92 (81–103)	97 (89–107)	86 (77–97)	<0.001
	FVC, L	4.6 (4.1–5.2)	4.6 (4.1–5.2)	4.7 (4.1–5.1)	0.776
	FVC, % Predicted	88 (81–97)	90 (82–99)	86 (78–95)	0.034
SPE Spirometry	FEV$_1$/FVC, %	76 (73–80)	82 (79–84)	73 (69–76)	<0.001
	FEV$_1$, L	3.8 (3.2–4.5)	4.2 (4.1–5.2)	3.4 (3.0–4.0)	<0.001
	FEV$_1$, % Predicted	95 (76–109)	108 (94–114)	81 (73–95)	<0.001
	FVC, L	5.0 (4.3–5.7)	5.1 (4.3–5.8)	4.9 (4.3–5.5)	0.162
	FVC, % Predicted	96 (86–108)	101 (89–108)	91 (83–103)	0.003
SPE Plethysmograph and Diffusion	TLC% Predicted	103 (93–110)	102 (85–106)	104 (96–112)	0.031
	FRC% Predicted	95 (84–107)	86 (72–103)	100 (87–114)	0.212
	RV, % Predicted	123 (108–141)	114 (98–128)	132 (115–157)	<0.001
	DLCO, % Predicted	107 (15)	106 (21)	109 (15)	0.793
SPE Airway Reactivity	MCT Slope, % FEV$_1$/mg	0.054 (0.03–0.14)	0.038 (0.02–0.07)	0.092 (0.04–0.42)	<0.001
	BD Response, %	8 (3–15)	4 (2–8)	12 (5–18)	<0.001

Definition of abbreviations: MME = medical monitoring entry; SPE = subspecialty pulmonary evaluation. MCT = methacholine challenge test

All Values Expressed as Median (IQR) or Mean (SD);

*
Significance assessed by Wilcoxon rank-sum test or unpaired Student t-test between cases and controls. SPE Plethysmograph and Diffusion: Total N=121; Controls N=55; Cases N=66. BD Response: Total N=130; Controls N=56; Cases N=74. MCT Slope: Total N=182; Controls N=97; Cases N=85.

Table 3

Serum Levels of Analytes

Serum Analyte	Total	Controls	Cases	P-value[*]
Chitotriosidase (ng/mL)	29.9 (18–46)	32.2 (20–52)	24.5 (15–40)	<0.001
IgE (IU/mL)	44.6 (17–116)	35.7 (11–81)	50 (24–148)	0.010
YKL-40 (ng/mL)	42.1 (30–61)	41.0 (31–65)	44.0 (30–59)	0.621

All Values Expressed as Median (IQR);

[*] Significance assessed by Wilcoxon rank-sum test between cases and controls.

Table 4

Analysis Predicting $FEV_1/FVC<LLN$ at SPE

Model	Predictor	OR (95% CI)
Univariable	Age (yr)	0.94 (0.90, 0.98)
	BMI (kg/m^2)	1.02 (0.97, 1.08)
	Exposure High[*]	0.68 (0.40, 1.27)
	FEV$_1$/FVC at MME	0.78 (0.72, 0.83)
	Chitotriosidase[†]	0.84 (0.75, 0.94)
	IgE[‡]	1.23 (1.01, 1.50)
Multivariable[§]	Chitotriosidase[†]	0.82 (0.71, 0.95)
	IgE[‡]	1.36 (1.09, 1.71)

Definition of abbreviations: LLN = lower limit of normal; SPE = subspecialty pulmonary evaluation; OR = odds ratio; BMI = body mass index; MME = medical monitoring evaluation.

[*] Reference is intermediate exposure group.

[†] 10 ng/mL of Chitotriosidase

[‡] 100 IU/mL of IgE.

[§] Adjusted for age at 9/11/2001, BMI at SPE, exposure group and FEV$_1$/FVC at MME. χ^2 (6) = 49.465, P = <0.001. Hosmer and Lemeshow's goodness-of-fit test P=0.588. Area under ROC curve=0.86.