Club Cell Secretory Protein Deficiency Leads to Altered Lung Function

Jing Zhai, Michael Insel, Kenneth J. Addison, Debra A. Stern, William Pederson, Alane Dy, Joselyn Rojas-Quintero, Caroline A. Owen, Duane L Sherrill, Wayne Morgan, Anne L Wright, Marilyn Halonen, Fernando Martinez, Monica Kraft, Stefano Guerra, and Julie G. Ledford

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Fig. E1. Lung function by serum CC16 levels from age 11 to 32. FEV1, FVC, FEF$_{25-75}$, and FEV1/FVC ratio **A**) before (N subject = 706) and **B**) after (N subject = 689) bronchodilator from ge 11 to age 32 years by serum CC16 tertile categories. Data are shown separately for males and females. Error bars indicate the standard error.
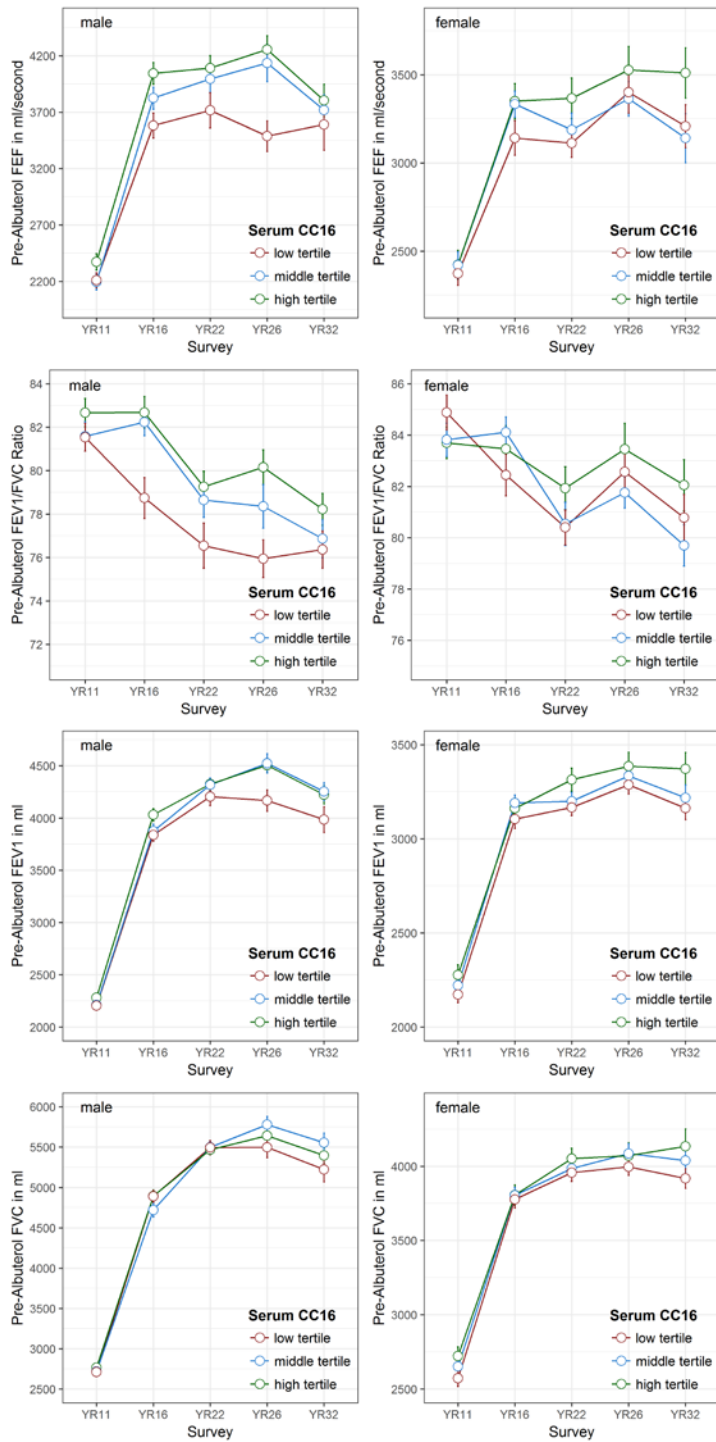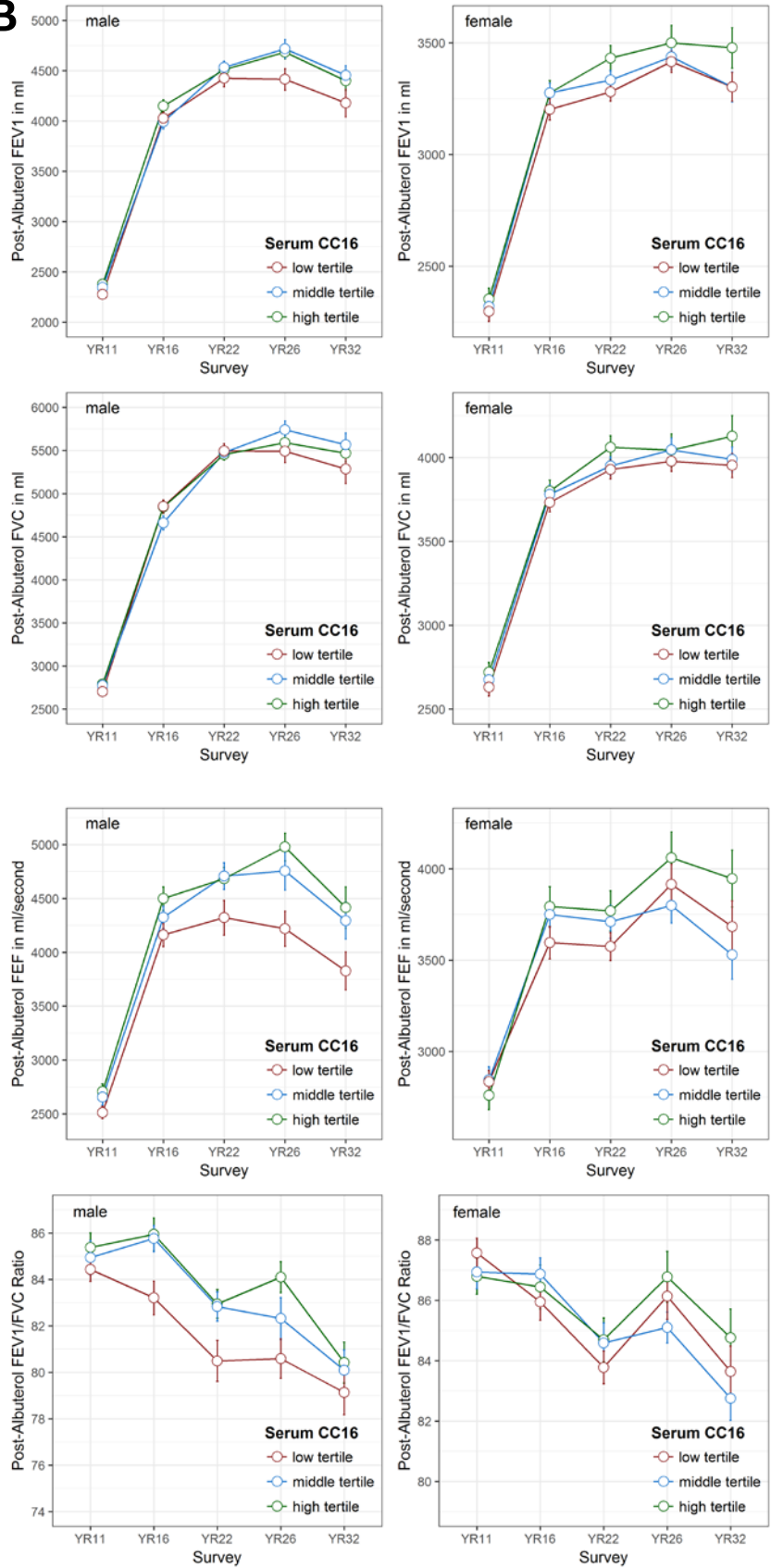
B

Fig. E2. Indices of acute inflammation are not present in CC16$^{-/-}$ naïve mice. After flexivent assessment, cells from the bronchoalveolar lavage were assessed in a subset of mice. There were no differences in **A**) total cells, **B**) macrophages and **C**) Neutrophils in WT (n=6) and CC16$^{-/-}$ (n=9) mice. **D**) TNF-α (n=6,6) and **E**) Muc5AC gene expression (n=6,6) and **F**) mucin production (n=12,12) as assessed by PAS score were not different in WT and CC16$^{-/-}$ mice. **G-H**) Airspace size was measured on formalin-fixed inflated lung sections. Representative pictures (10X) of alveolar areas of the lung from WT and CC16$^{-/-}$ naïve mice. The average alveolar chord length was ~20 um in both WT (n=9) and CC16-/- (n=6) mice at approximately 8-10 weeks of age.
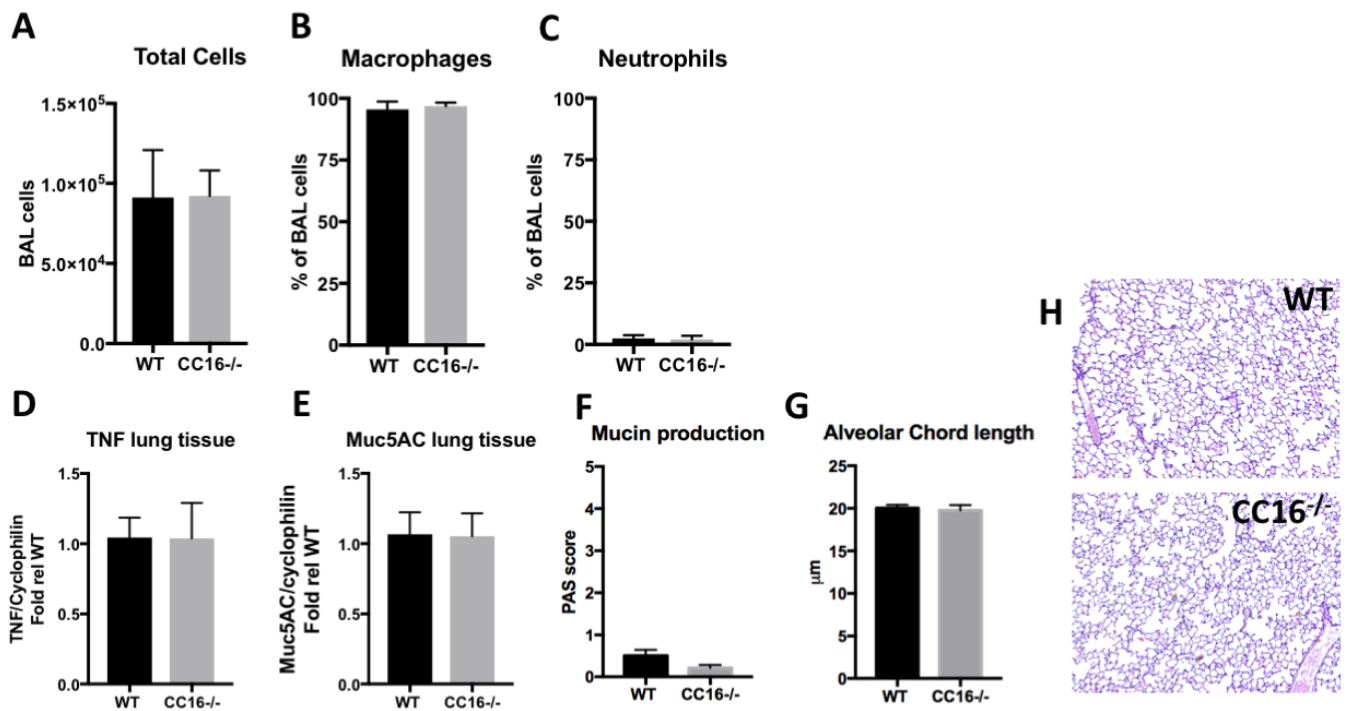
Table E1. Linear Regression Models for Lung Function* as Measured from Age 22 to Age 32 years <u>among Participants with No Asthma[‡]</u>.

		Pre-Albuterol							
Z-Scores CC16 [†]		**FEV1 (ml)** **N subjects = 427; N observations = 755**		**FVC (ml)** **N subjects = 427; N observations = 755**		**FEF (ml/second)** **N subjects = 427; N observations = 755**		**FEV1/FVC Ratio** **N subjects = 427; N observations = 755**	
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		65.3 (26.6, 104)	0.001	26.3 (-24.0, 76.6)	0.304	122 (43.6, 201)	0.002	0.802 (0.263, 1.34)	0.004
Category	High	(reference group)							
	Middle	-44.9 (-130, 40.1)	0.300	33.5 (-59.9, 127)	0.481	-179 (-352, -6.22)	0.042	-1.50 (-2.62, -0.376)	0.009
	Low	-142 (-237, -47.7)	0.003	-47.4 (-160, 65.6)	0.410	-293 (-483, -103)	0.003	-1.97 (-3.24, -0.703)	0.002
		Post-Albuterol							
Z-Scores CC16 [†]		**FEV1 (ml)** **N subjects = 412; N observations = 711**		**FVC (ml)** **N subjects = 412; N observations = 711**		**FEF (ml/second)** **N subjects = 411; N observations = 705**		**FEV1/FVC Ratio** **N subjects = 412; N observations = 711**	
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		55.7 (16.1, 95.3)	0.006	25.6 (-26.7, 77.8)	0.337	131 (52.9, 209)	0.001	0.608 (0.132, 1.08)	0.012
Category	High	(reference group)							
	Middle	-38.1 (-121, 45.1)	0.368	12.4 (-83.8, 109)	0.800	-209 (-392, -27.1)	0.024	-1.03 (-2.02, -0.039)	0.042
	Low	-122 (-216, -26.9)	0.012	-40.3 (-157, 76.3)	0.497	-348 (-547, -149)	0.001	-1.66 (-2.79, -0.520)	0.004

* Lung function was assessed pre- and post-albuterol.

† Circulating CC16 levels were included as z-scores in the model.

‡ Subject-clustered sandwich estimators of standard errors were used in linear regression models to adjust for within-subject correlation. All models were adjusted for sex, survey year, ethnicity, parental education, maternal smoking at year 6, and height (cm). The models are restricted to the non-asthmatics participants.

Table E2. Linear Regression Models for Lung Function* as Measured from Age 22 to Age 32 years <u>among Participants who did Not Smoke</u>‡.

		Pre-Albuterol							
Z-Scores CC16 †		**FEV1 (ml)** N subjects = 265; N observations = 499		**FVC (ml)** N subjects = 265; N observations = 499		**FEF (ml/second)** N subjects = 265; N observations = 499		**FEV1/FVC Ratio** N subjects = 265; N observations = 499	
		Coef (95% CI)	P	Coef (95% CI)	P	Coef (95% CI)	P	Coef (95% CI)	P
Continuous		70.9 (22.3, 119)	0.004	11.7 (-46.8, 70.1)	0.694	183 (81.3, 284)	<0.001	1.18 (0.444, 1.92)	0.002
Category	High	(reference group)							
	Middle	-72.5 (-172, 26.8)	0.152	13.4 (-101, 128)	0.818	-260 (-469, -50.8)	0.015	-1.77 (-3.19, -0.343)	0.015
	Low	-179 (-296, -62.1)	0.003	-54.4 (-195, 85.7)	0.445	-426 (-669, -183)	0.001	-2.67 (-4.34, -1.00)	0.002
		Post-Albuterol							
Z-Scores CC16 †		**FEV1 (ml)** N subjects = 260; N observations = 479		**FVC (ml)** N subjects = 260; N observations = 479		**FEF (ml/second)** N subjects = 260; N observations = 476		**FEV1/FVC Ratio** N subjects = 260; N observations = 479	
		Coef (95% CI)	P	Coef (95% CI)	P	Coef (95% CI)	P	Coef (95% CI)	P
Continuous		56.3 (7.42, 105)	0.024	18.6 (-40.1, 77.3)	0.533	155 (45.4, 264)	0.006	0.779 (0.090, 1.47)	0.027
Category	High	(reference group)							
	Middle	-35.5 (-134, 62.6)	0.477	-5.83 (-124, 113)	0.923	-191 (-414, 32.1)	0.093	-0.807 (-2.05, 0.440)	0.204
	Low	-139 (-255, -22.6)	0.019	-54.7 (-197, 87.9)	0.451	-386 (-645, -126)	0.004	-1.87 (-3.41, -0.332)	0.017

* Lung function was assessed pre- and post-albuterol.

† Circulating CC16 levels were included as z-scores in the model.

‡ Subject-clustered sandwich estimators of standard errors were used in linear regression models to adjust for within-subject correlation. All models were adjusted for sex, survey year, ethnicity, parental education, maternal smoking at year 6, and height (cm). All models were restricted to never-smokers, i.e. participants who never smoked or who smoked less than 100 cigarettes in their lifetime.

Table E3. Linear Regression Models for Lung Function* as Measured from Age 22 to Age 32 years including total IgE levels among covariates[‡].

		Pre-Albuterol							
Z-Scores CC16 [†]		**FEV1 (ml)** N subjects = 491; N observations = 906		**FVC (ml)** N subjects = 491; N observations = 906		**FEF (ml/second)** N subjects = 491; N observations = 906		**FEV1/FVC Ratio** N subjects = 491; N observations = 906	
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		49.6 (14.1, 85.0)	0.006	12.1 (-31.8, 56.0)	0.588	113 (37.3, 189)	0.004	0.747 (0.214, 1.28)	0.006
Category	High	(reference group)							
	Middle	-16.9 (-92.6, 58.9)	0.662	61.8 (-20.3, 144)	0.140	-138 (-299, 23.0)	0.093	-1.36 (-2.46, -0.257)	0.016
	Low	-114 (-199, -29.2)	0.009	-22.4 (-122, 77.0)	0.658	-273 (-447,-99.9)	0.002	-1.85 (-3.07, -0.632)	0.003
		Post-Albuterol							
Z-Scores CC16 [†]		**FEV1 (ml)** N subjects = 474; N observations = 855		**FVC (ml)** N subjects = 474; N observations = 855		**FEF (ml/second)** N subjects = 473; N observations = 849		**FEV1/FVC Ratio** N subjects = 474; N observations = 855	
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		35.8 (0.301, 71.3)	0.048	9.54 (-35.7, 54.8)	0.679	105 (29.3, 181)	0.007	0.526 (0.068, 0.984)	0.025
Category	High	(reference group)							
	Middle	5.18 (-68.6, 78.9)	0.890	45.6 (-40.4, 132)	0.298	-111 (-279, 57.8)	0.197	-0.750 (-1.71, 0.209)	0.125
	Low	-82.3 (-167, 2.28)	0.056	-13.1 (-115, 89.0)	0.801	-284 (-465, -104)	0.002	-1.42 (-2.50, -0.341)	0.010

* Lung function was assessed pre- and post-albuterol.
† Circulating CC16 levels were included as z-scores in the model.
‡ Subject-clustered sandwich estimators of standard errors were used in linear regression models to adjust for within-subject correlation. All models were adjusted for sex, survey year, ethnicity, parental education, maternal smoking at year 6, height (cm) and log10 transformed IgE (IU/ml).

Table E4. Linear Regression Models for Z-Scores of Lung Function* as Measured from Age 11 to Age 32 years‡.

Pre-Albuterol								
Z-Scores CC16 †		**Z-Scores FEV1** N subjects = 674; N observations = 1872		**Z-Scores FVC** N subjects = 674; N observations = 1872		**Z-Scores FEF** N subjects = 674; N observations = 1868		**Z-Scores FEV1/FVC Ratio** N subjects = 674; N observations = 1872
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		0.081 (0.045, 0.117)	<0.001	0.031 (-0.004, 0.065)	0.079	0.115 (0.058, 0.173)	<0.001	0.099 (0.037, 0.162)	0.002
Category	High	(reference group)							
	Middle	-0.057 (-0.143, 0.030)	0.198	0.0009 (-0.078, 0.080)	0.983	-0.138 (-0.259, -0.018)	0.025	-0.141 (-0.268, -0.014)	0.029
	Low	-0.173 (-0.265, -0.082)	<0.001	-0.054 (-0.142, 0.033)	0.224	-0.275 (-0.410, -0.139)	<0.001	-0.243 (-0.390, -0.097)	0.001
Post-Albuterol								
Z-Scores CC16 †		**Z-Scores FEV1** N subjects = 659; N observations = 1807		**Z-Scores FVC** N subjects = 659; N observations = 1806		**Z-Scores FEF** N subjects = 659; N observations = 1799		**Z-Scores FEV1/FVC Ratio** N subjects = 659; N observations = 1806
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		0.063 (0.027, 0.098)	0.001	0.029 (-0.007, 0.065)	0.110	0.094 (0.039, 0.149)	0.001	0.081 (0.021, 0.141)	0.008
Category	High	(reference group)							
	Middle	-0.032 (-0.115, 0.051)	0.446	-0.006 (-0.087, 0.075)	0.890	-0.076 (-0.194, 0.043)	0.212	-0.082 (-0.210, 0.046)	0.211
	Low	-0.137 (-0.223, -0.051)	0.002	-0.056 (-0.145, 0.032)	0.210	-0.240 (-0.370, -0.109)	<0.001	-0.207 (-0.352, -0.063)	0.005

* Lung function was assessed pre- and post-albuterol.

† Circulating CC16 levels were included as z-scores in the model.

‡ Subject-clustered sandwich estimators of standard errors were used in linear regression models to adjust for within-subject correlation. All models were adjusted for sex, survey year, ethnicity, parental education, maternal smoking at year 6, and height (cm).

Table E5. Linear Regression Models for Z-Scores of Lung Function* as Measured from Age 11 to Age 32 years <u>among Participants with No Asthma[‡]</u>.

Z-Scores CC16 [†]		Pre-Albuterol							
		Z-Scores FEV1 N subjects = 593; N observations = 1492		Z-Scores FVC N subjects = 593; N observations = 1492		Z-Scores FEF N subjects = 593; N observations = 1488		Z-Scores FEV1/FVC Ratio N subjects = 593; N observations = 1492	
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		0.087 (0.045, 0.128)	<0.001	0.039 (-0.0007, 0.078)	0.054	0.106 (0.044, 0.167)	0.001	0.088 (0.024, 0.152)	0.007
Category	High	(reference group)							
	Middle	-0.065 (-0.160, 0.030)	0.178	-0.003 (-0.091, 0.086)	0.955	-0.143 (-0.272, -0.015)	0.029	-0.148 (-0.280, -.017)	0.027
	Low	-0.177 (-0.277, -0.077)	0.001	-0.068 (-0.165, 0.029)	0.169	-0.247 (-0.388, -0.107)	0.001	-0.211 (-0.358, -0.064)	0.005
Z-Scores CC16 [†]		Post-Albuterol							
		Z-Scores FEV1 N subjects = 576; N observations = 1436		Z-Scores FVC N subjects = 576; N observations = 1436		Z-Scores FEF N subjects = 576; N observations = 1428		Z-Scores FEV1/FVC Ratio N subjects = 576; N observations = 1436	
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		0.077 (0.037, 0.116)	<0.001	0.039 (-0.001, 0.079)	0.057	0.098 (0.041, 0.156)	0.001	0.082 (0.020, 0.145)	0.010
Category	High	(reference group)							
	Middle	-0.059 (-0.151, 0.032)	0.200	-0.017 (-0.106, 0.073)	0.714	-0.107 (-0.235, 0.021)	0.102	-0.115 (-0.252, 0.021)	0.096
	Low	-0.155 (-0.251, -0.060)	0.001	-0.069 (-0.167, 0.029)	0.168	-0.240 (-0.375, -0.104)	0.001	-0.205 (-0.353, -0.058)	0.006

* Lung function was assessed pre- and post-albuterol.

[†] Circulating CC16 levels were included as z-scores in the model.

[‡] Subject-clustered sandwich estimators of standard errors were used in linear regression models to adjust for within-subject correlation. All models were adjusted for sex, survey year, ethnicity, parental education, maternal smoking at year 6, and height (cm). All models were restricted to participants with no asthma.

Table E6. Linear Regression Models for Z-Scores of Lung Function* as Measured from Age 11 to Age 32 years among Participants who did Not Smoke‡.

Z-Scores CC16 †		Z-Scores FEV1 N subjects = 572; N observations = 1255		Z-Scores FVC N subjects = 572; N observations = 1255		Z-Scores FEF N subjects = 572; N observations = 1252		Z-Scores FEV1/FVC Ratio N subjects = 572; N observations = 1255	
Pre-Albuterol									
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		0.083 (0.042, 0.125)	<0.001	0.023 (-0.019, 0.065)	0.279	0.136 (0.074, 0.199)	<0.001	0.122 (0.052, 0.192)	0.001
Category	High	(reference group)							
	Middle	-0.072 (-0.169, 0.024)	0.142	-0.016 (-0.113, 0.080)	0.741	-0.163 (-0.293, -0.034)	0.013	-0.137 (-0.275, 0.0004)	0.051
	Low	-0.200 (-0.304, -0.095)	<0.001	-0.061 (-0.168, 0.046)	0.263	-0.328 (-0.485, -0.171)	<0.001	-0.290 (-0.461, -0.120)	0.001

Z-Scores CC16 †		Z-Scores FEV1 N subjects = 568; N observations = 1232		Z-Scores FVC N subjects = 568; N observations = 1231		Z-Scores FEF N subjects = 568; N observations = 1227		Z-Scores FEV1/FVC Ratio N subjects = 568; N observations = 1231	
Post-Albuterol									
		Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*	Coef (95% CI)	*P*
Continuous		0.060 (0.017, 0.103)	0.006	0.023 (-0.021, 0.066)	0.306	0.099 (0.038, 0.160)	0.002	0.093 (0.024, 0.162)	0.009
Category	High	(reference group)							
	Middle	-0.042 (-0.138, 0.054)	0.389	-0.027 (-0.124, 0.071)	0.595	-0.069 (-.198, 0.060)	0.295	-0.054 (-0.191, 0.084)	0.442
	Low	-0.154 (-0.255, -0.053)	0.003	-0.063 (-0.170, 0.044)	0.250	-0.259 (-0.407, -0.112)	0.001	-0.236 (-0.404, -0.069)	0.006

* Lung function was assessed pre- and post-albuterol.

† Circulating CC16 levels were included as z-scores in the model.

‡ Subject-clustered sandwich estimators of standard errors were used in linear regression models to adjust for within-subject correlation. All models were adjusted for sex, survey year, ethnicity, parental education, maternal smoking at year 6, and height (cm). All models were restricted to never-smokers, i.e. participants who never smoked or who smoked less than 100 cigarettes in their lifetime.

Table E7. Multinomial Logistic Regression Models for Airway Hyper-responsiveness as Measured from Age 11 to Age 26 years including total IgE levels among covariates[†].

Airway Hyper-Responsiveness			All participant (N subjects = 552; N observations = 1198)		No asthma (N subjects = 490; N observations = 1007)		Non-smoker (N subjects = 483; N observations = 875)	
			adjRRR (95% CI)	*P*	adjRRR (95% CI)	*P*	adjRRR (95% CI)	*P*
No drop			(base outcome)					
Mild	z-score CC16 * continuous		0.943 (0.790, 1.13)	0.515	0.918 (0.753, 1.12)	0.395	0.890 (0.714, 1.11)	0.300
Moderate			0.761 (0.636, 0.910)	0.003	0.768 (0.627, 0.941)	0.011	0.652 (0.515, 0.826)	<0.001
Severe			0.678 (0.554, 0.831)	<0.001	0.637 (0.499, 0.813)	<0.001	0.601 (0.470, 0.768)	<0.001
No drop			(base outcome)					
Mild	z-score CC16* category	High	(reference group)					
		Middle	1.22 (0.831, 1.80)	0.308	1.28 (0.851, 1.92)	0.238	1.43 (0.925, 2.22)	0.107
		Low	1.18 (0.760, 1.85)	0.456	1.20 (0.750, 1.90)	0.453	1.39 (0.821, 2.36)	0.219
Moderate		High	(reference group)					
		Middle	1.26 (0.839, 1.90)	0.264	1.28 (0.814, 2.01)	0.287	1.47 (0.898, 2.42)	0.125
		Low	1.84 (1.19, 2.84)	0.006	1.86 (1.16, 2.98)	0.010	2.52 (1.50, 4.25)	0.001
Severe		High	(reference group)					
		Middle	1.40 (0.905, 2.16)	0.131	1.75 (1.06, 2.89)	0.029	1.44 (0.894, 2.31)	0.135
		Low	2.16 (1.34, 3.47)	0.001	2.51 (1.43, 4.40)	0.001	2.83 (1.63, 4.89)	<0.001

* Circulating CC16 levels were included as z-scores in the model.

† Subject-clustered sandwich estimators of standard errors were used in multinomial logistic regression models to adjust for within-subject correlation. All models were adjusted for sex, survey year, ethnicity, parental education, maternal smoking at year 6 and log10 transformed IgE (IU/ml).

AHR severity (mild, moderate, severe) was defined based on PD20 tertiles generated at each survey (see Methods section for more information)