Spirometric traits show quantile-dependent heritability, which may contribute to their gene-environment interactions with smoking and pollution

Paul T. Williams

Molecular Biophysics & Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA, United States of America

ABSTRACT

Background. "Quantile-dependent expressivity" refers to a genetic effect that is dependent upon whether the phenotype (e.g., spirometric data) is high or low relative to its population distribution. Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and the FEV_1/FVC ratio are moderately heritable spirometric traits. The aim of the analyses is to test whether their heritability (h^2) is constant over all quantiles of their distribution.

Methods. Quantile regression was applied to the mean age, sex, height and smoking-adjusted spirometric data over multiple visits in 9,993 offspring-parent pairs and 1,930 sibships from the Framingham Heart Study to obtain robust estimates of offspring-parent (β_{OP}), offspring-midparent (β_{OM}), and full-sib regression slopes (β_{FS}). Non-parametric significance levels were obtained from 1,000 bootstrap samples. β_{OP}s were used as simple indicators of quantile-specific heritability (i.e., $h^2 = 2\beta_{OP}/(1+r_{spouse})$, where r_{spouse} was the correlation between spouses).

Results. $\beta_{OP} \pm$ standard error (SE) decreased by 0.0009 ± 0.0003 ($P = 0.003$) with every one-percent increment in the population distribution of FEV_1/FVC, i.e., $\beta_{OP} \pm$ SE were: 0.182 ± 0.031, 0.152 ± 0.015; 0.136 ± 0.011; 0.121 ± 0.013; and 0.099 ± 0.013 at the 10th, 25th, 50th, 75th, and 90th percentiles of the FEV_1/FVC distribution, respectively. These correspond to $h^2 \pm$ SEs of 0.350 ± 0.060 at the 10th, 0.292 ± 0.029 at the 25th, 0.262 ± 0.020 at the 50th, 0.234 ± 0.025 at the 75th, and 0.191 ± 0.025 at the 90th percentiles of the FEV_1/FVC ratio. Maximum mid-expiratory flow (MMEF) $h^2 \pm$ SEs increased 0.0025 ± 0.0007 ($P = 0.0004$) with every one-percent increment in its distribution, i.e.: 0.467 ± 0.046, 0.467 ± 0.033, 0.554 ± 0.038, 0.615 ± 0.042, and 0.675 ± 0.060 at the 10th, 25th, 50th, 75th, and 90th percentiles of its distribution. This was due to forced expiratory flow at 75% of FVC (FEF75%), whose quantile-specific h^2 increased an average of 0.0042 ± 0.0008 for every one-percent increment in its distribution. It is speculated that previously reported gene-environment interactions may be partially attributable to quantile-specific h^2, i.e., greater heritability in individuals with lower FEV_1/FVC due to smoking or airborne particles exposure vs. nonsmoking, unexposed individuals.

Conclusion. Heritabilities of FEV_1/FVC, MMEF, and FEF75% from quantile-regression of offspring-parent and sibling spirometric data suggest their quantile-dependent expressivity.

INTRODUCTION

Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and the FEV_1/FVC ratio are the most commonly measured spirometric traits (*Global initiative for Chronic Obstructive Lung Disease, 2020*; *Wood, Tan & Stockley, 2009*). FVC approximates lung volume and is a strong predictor of all-cause mortality (*Global initiative for Chronic Obstructive Lung Disease, 2020*; *Wood, Tan & Stockley, 2009*). Reduced FVC in the absence of reduced FEV_1/FVC indicates a tendency towards a restrictive ventilatory defect. Low FEV_1/FVC (e.g., < lower limit of normal or z-score < -1.64) identifies patients with airflow obstruction, and low FEV_1 (as a percentage of predicted values or as z-scores < -1.64) their obstruction severity. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines chronic obstructive pulmonary disease (COPD) as a post-bronchodilator FEV_1/FVC ratio < 0.70 and $FEV_1 \geq$ 80% of predicted (GOLD I), 50% $\leq FEV_1$ <80% (GOLD II), 30% $\leq FEV_1$ <50% (GOLD III), and FEV_1 <30% predicted (GOLD IV) (*Global initiative for Chronic Obstructive Lung Disease, 2020*). Maximum mid-expiratory flow (MMEF) and forced expiratory flows at X% of FVC (i.e., FEF25%, FEF50% and FEF75%) may assess small airway caliber especially in case of a normal FVC (*Wood, Tan & Stockley, 2009*), although its use in diagnosing small airway disease in individual patients is discouraged (*Quanjer et al., 2014*). Peak expiratory flow (PEF) is the maximum speed of expiration.

Heritability estimates range widely across family and twins studies, from 0.09 to 0.68 for FEV_1 (mean 0.39), 0.20 to 0.78 for FVC (mean 0.45), and 0.16 to 0.64 for the FEV_1/FVC ratio (mean 0.36) (*Devor & Crawford, 1984*; *Coultas et al., 1991*; *Klimentidis et al., 2013*; *Redline et al., 1989*; *Wilk et al., 2000*; *Tarnoki et al., 2013*; *Hukkinen et al., 2011*; *Hallberg et al., 2010*; *DeMeo et al., 2004*; *McClearn et al., 1994*; *Palmer et al., 2001*; *Astemborski, Beaty & Cohen, 1985*; *Beaty et al., 1987*; *Lewitter et al., 1984*; *Cotch, Beaty & Cohen, 1990*; *Chen et al., 1996*; *Chen et al., 1997*; *Givelber et al., 1998*; *Ingebrigtsen et al., 2011*; *Joost et al., 2002*; *Tian et al., 2017*; *Yamada et al., 2015*). Prior segregation analyses of pulmonary function generally favor its polygenic inheritance (*Chen et al., 1996*; *Chen et al., 1997*; *Givelber et al., 1998*). Forty-nine genetic loci have been significantly related to pulmonary function in meta-analyses of 38,199 individuals of European ancestry from 17 genome-wide association studies (GWAS) (*Loth et al., 2014*; *Soler Artigas et al., 2015*; *Hancock et al., 2010*; *Repapi et al., 2010*; *Soler Artigas et al., 2011*). As in other complex traits (*Manolio et al., 2009*), only a small proportion of the heritability is attributable to known single nucleotide polymorphisms (SNPs): 4.0% of the additive polygenic variance for FEV_1, 5.4% for FEV_1/FVC, and 3.2% for FVC (*Soler Artigas et al., 2015*). Estimates of heritability based on genome-wide SNPs are more consistent with pedigree-based estimates (*Klimentidis et al., 2013*; *Yamada et al., 2015*).

Smoking is the strongest environmental cause for reduced pulmonary function. The average decline of lung function with age is approximately 50% greater in smokers than

nonsmokers (*Gottlieb, 1999*). Gene-environment interactions with smoking and airborne particle exposure have been found in multiple reports (*Curjuric et al., 2012*; *Hallberg et al., 2010*; *He et al., 2004*; *Zhai et al., 2007*; *Mehta et al., 2014*; *Sigsgaard et al., 2000*; *Kim et al., 2015*) and used to improve power for identifying genetic variants associated with pulmonary function (*Hancock et al., 2012*). Having a first-degree relative with COPD is associated with a 13% mean reduction in FEV_1 in smokers but not in nonsmokers (*Aschard et al., 2017*). Moreover, COPD risk is two to four times greater in smokers having a first-degree relative with COPD than in smokers that do not (*Walter, Gottlieb & O'Connor, 2000*). Among persons with severe a-1 antitrypsin (AAT) deficiency, mortality occurs earlier and pulmonary function declines more rapidly with age in smokers than nonsmokers (*Walter, Gottlieb & O'Connor, 2000*). Reductions in FEV_1/FVC with smoking are reported to be greater among individuals who are genetically predisposed to lower FEV_1/FVC (*Aschard et al., 2017*). Gene-environment interactions have also been reported between smoking and chronic bronchitis (*Hallberg et al., 2008*), a condition indicating reduced FEV_1 (*Vestbo, Prescott & Lange, 1996*).

Elsewhere it has been shown that quantile-specific effects play a fundamental role in the genetics of body weight, lipoprotein concentrations, and coffee intake (quantile-dependent penetrance or expressivity) while not affecting other traits such as height (*Williams, 2012*; *Williams, 2020c*; *Williams, 2020b*; *Williams, 2020a*). It is not known whether quantile-specific genetic effects apply to pulmonary function. The aforementioned twin and family studies (*Devor & Crawford, 1984*; *Coultas et al., 1991*; *Klimentidis et al., 2013*; *Redline et al., 1989*; *Wilk et al., 2000*; *Tarnoki et al., 2013*; *Hukkinen et al., 2011*; *Hallberg et al., 2010*; *DeMeo et al., 2004*; *McClearn et al., 1994*; *Palmer et al., 2001*; *Astemborski, Beaty & Cohen, 1985*; *Beaty et al., 1987*; *Lewitter et al., 1984*; *Cotch, Beaty & Cohen, 1990*; *Chen et al., 1996*; *Chen et al., 1997*; *Givelber et al., 1998*; *Ingebrigtsen et al., 2011*; *Joost et al., 2002*; *Tian et al., 2017*; *Yamada et al., 2015*), segregation analyses (*Chen et al., 1996*; *Chen et al., 1997*; *Givelber et al., 1998*), and GWAS (*Loth et al., 2014*; *Soler Artigas et al., 2015*; *Hancock et al., 2010*; *Repapi et al., 2010*; *Soler Artigas et al., 2011*) are all based on the assumption that the genetic and other inherited effects are the same throughout the distributions of pulmonary function, i.e., the same whether pulmonary function is high or low relative to the population distribution. Quantile regression was therefore applied to spirometric data from the Framingham Study (*Dawber, Meadors & Moore, 1951*; *Kannel et al., 1979*; *Splansky et al., 2007*) to assess whether quantile-dependent expressivity affects its heritability.

POPULATION AND METHODS

The Framingham Study data were obtained from the National Institutes of Health FRAMCOHORT, GEN3, FRAMOFFSPRING Research Materials obtained from the NHLBI Biologic Specimen and Data Repository Information Coordinating Center. Approval for the analyses of these data were obtained from the Committee for the Protection of Human Subjects at Lawrence Berkeley National Laboratory, Department of Energy, Berkeley, California (Protocol Title: Gene-environment interaction vs. quantile-dependent penetrance of established SNPs. APPROVAL NUMBER: 107H021-13MR20).

The Original (generation 1) Framingham Cohort consisted of 5,209 men and women between the ages of 30 and 62 from the town of Framingham, Massachusetts who were recruited and examined between 1948 and 1953 and re-examined biannually thereafter (*Dawber, Meadors & Moore, 1951*). The Offspring Cohort (generation 2) consisted of 5,124 adult offspring of the original participants and their spouses who were first examined between 1971 and 1975, reexamined eight years later and then every three to four years thereafter (*Kannel et al., 1979*). Children of the Offspring Cohort were recruited to form the Third Generation Cohort, which was examined twice (*Splansky et al., 2007*). Participants used in the current analyses were at least 16 years of age and self-identified as white or non-Hispanic in the Offspring and Third Generation Cohorts (race and ethnicity were not requested in the Original Cohort, but reported to be overwhelmingly white). FVC and FEV_1 were measured at exams 5, 6, 16, 17 and 19 of the Original Cohort; exams 3 and 5-8 of the Offspring Cohort; and exams 1 and 2 of the Third Generation Cohort. MMEF, FEF25%, FEF50% and FEF75% were measured at exams 16, 17 and 19 of the Original; exams 3, 5-8 of the Offspring; and exams 1 and 2 of the Third Generation Cohort. PEF was measured at exam 19 of the Original Cohort, exams 3 and 5-8 of the Offspring Cohort, and exams 1 and 2 of Third Generation Cohort. There were no exclusions for chronic bronchitis, pulmonary disease, COPD, emphysema, or asthma.

Spirometry methodology and quality changed over the history of the Framingham Heart Study, particularly prior to 1980 (*Joost et al., 2002*; *Rice et al., 2013*; *Liao, Lin & Christiani, 2013*). Spirometry at examinations 5 (conducted between 1958-1959) and 6 (1960 and 1961) of the Original Cohort used a 13.5L Collins water-sealed bell spirometer. Each participant performed three FVC maneuvers while standing. FEV_1 and FVC were measured by hand from the chymograph tracings using the method of back extrapolation. Only the maneuver with the greatest FVC was recorded in the chart. Although these maneuvers were performed before the introduction of the American Thoracic Society guidelines for standardization of spirometry, a review of spirograms showed that 80% of the tracings met acceptability guidelines for smoothness of curve and forceful initial push. Raw values were multiplied by 1.10 to correct for body temperature, ambient pressure, saturated with water (BTPS), assuming a clinic temperature of 20°C.

Spirometry for later exams of the Original Cohort and examination 3 (1984–1987) of the Offspring Cohort used the 6L Collins water-sealed bell spirometer connected to a microprocessor that provided automatic correction for BTPS. At Offspring Cohort examination 5 (Cycle 5, 1992–1995), the spirometer was interfaced with lung function software that provided real-time quality assurance. Exams 6 and 7 of the Offspring Cohort used a Collins Survey II spirometer with pulmonary function data acquisition and quality control software (S&M Instruments, Doylestown, PA) that was calibrated daily. Spirometric data for the Third Generation Cohort were obtained on a Collins CPL system (Spire Health, Inc., Longmont, CO) that was calibrated daily. FVC maneuvers were performed standing while wearing nose clips and repeated until at least three acceptable spirograms were obtained, up to a maximum of eight spirograms, using American Thoracic Society standards to identify acceptable spirograms. The largest FEV_1 and the largest FVC

from among all acceptable maneuvers, and the FEV_1/FVC ratio from the maneuver with the largest sum of $FEV_1 + FVC$, were used in this analysis.

Applied definitions

Current cigarette smokers were defined as responding yes to the question "If ever smoked cigarettes regularly, have you smoked cigarettes regularly in the past year?" or reporting a positive number in response to the question "If you ever smoked cigarettes regularly, how many cigarettes do you smoke per day now?" Pulmonary function measures were not significantly affected by reported pipe or cigar smoking in these data and were therefore included in the never smoked cigarette group (*Givelber et al., 1998*). Cigarette smokers who reported not inhaling had about half the effect on lung function than those that inhaled, and were included in the cigarette smokers. Former smoker were defined as noncurrent smokers who were classified as a current smoker at any prior visit or who reported a history of having smoked.

Statistical analyses

Adjustment was performed separately in the Original, Offspring and Third Generation Cohorts which should partially correct for differences in methodology over time and differences between cohorts (e.g., Offspring Cohort being taller and heavier than members of the Original Cohort). All observations were adjusted for sex, age (age and age^2 separately in men and women), height (separately in men and women), and whether the participant currently smoked cigarettes or previously smoked cigarettes. Individual participant values were taken as the average of the residuals over all available exams (i.e., the average of up to five exams for the Original Cohort, up to five exams for the Offspring Cohort, and up to two exams for the Third Generation Cohort). Population-based studies suggest that FEV_1 and FVC are approximately normally distributed in the general population (*Palmer et al., 2001*; *Dockery et al., 1985*).

Offspring-parent correlations and regression slopes were computed using offspring and parents from: (1) Offspring and Original Cohorts, and (2) Third Generation and Offspring Cohorts. Offspring-parent correlations and regression slopes were computed by assigning a weight of one-half to the father-child and one-half to the mother-child pair (if both parents available), or assigning a weight of one to the parent–child pair if only one parent was available. Sibling correlations and full-sib regression slopes were obtained by forming all $k_i(k_i-1)$ sibpair combinations for the k_i siblings in sibship i and assigning equal weight to each individual (*Karlin, Cameron & Williams, 1981*). The number of degrees of freedom for the standard error was Σk_i-2 for offspring-parent regression slopes and correlations, and $\Sigma(k_i-1)$ for sibship correlations and regression slopes, where the summation is taken over all i, $i = 1,\ldots,$ N families. Heritability in the narrow sense (h^2) was estimated as $h^2 = 2\beta_{OP}/(1+r_{spouse})$ from the offspring-parent regression slope (β_{OP}), as $h^2 = \beta_{OM}$ from the offspring-midparental regression slope (β_{OM}), and as $h^2 = [(1 + 8r_{spouse}\beta_{FS})^{0.5} - 1]/(2r_{spouse})$ from full-sibs regression slopes (β_{FS}), where r_{spouse} is the spouse correlation (*Falconer & Mackay, 1996*). Means are presented with their associated standard deviations (SD), while slopes and h^2 are presented $\pm$ standard

error ($\pm$SE). "Quantile-specific heritability" refers to the heritability statistic (h^2), whereas "quantile-dependent expressivity" is the biological phenomenon of the trait expression being quantile-dependent.

Simultaneous quantile regression is a well-developed statistical method (*Koenker & Hallock, 2001*; *Gould, 1992*) that estimates the regression coefficients for multiple quantiles using linear programming to minimize the sum of asymmetrically weighted absolute residuals, and bootstrap resampling to estimate their corresponding variances and covariances. Simultaneous quantile regression was performed using the sqreg command of Stata (version. 11, StataCorp, College Station, TX) with one thousand bootstrap samples drawn to estimate the variance–covariance matrix for the 91 quantile regression coefficients between the 5th and 95th percentiles, and the post-estimation procedures (test and lincom) to test linear combinations of the slopes after estimation with Σk_i-2 degrees of freedom for offspring-parent regression slopes (β_{OP}) and $\Sigma(k_i$-1) degrees of freedom for full-sib regression slopes (β_{FS}). Quantile-dependent expressivity was assessed by: (1) estimating quantile-specific β-coefficient for the 5th, 6th,..., 95th percentiles of the sample distribution using simultaneous quantile regression (Fig. 1, the < 5th and > 95th percentiles ignored because they were thought to be less stable); (2) plotting the quantile-specific β coefficients vs. the quantile of the trait distribution; and (3) testing whether the resulting graph is constant, or changes as a linear, quadratic, or cubic function of the percentile of the trait distribution using orthogonal polynomials (*Winer, Brown & Michels, 1991*).

Data availability: The data that support the findings of this study are available from dbGaP (*dbGaP genotypes & phenotypes, 2020a*). Restrictions apply to the availability of these data, which were used under license for this study. Data are available with the permission of dbGaP with appropriate human use approval. The public summary-level phenotype data may be browsed at the dbGaP study home page (*dbGaP genotypes & phenotypes, 2020b*).

RESULTS

Table 1 presents the characteristics of the sample, Table 2 presents standard and quantile-regression analyses of spouses, offspring and parents, and among siblings.

Classical estimates of heritability

When adjusted for assortative mating, and ignoring shared environment and dominance in sibs, the classical estimates of heritability (h^2) from offspring-parent regression slopes and full-sib correlations respectively, were: (1) 0.49 and 0.61 for FVC; (2) 0.42 and 0.54 for FEV$_1$; (3) 0.26 and 0.53 for the FEV$_1$/FVC ratio; (4) 0.34 and 0.45 for PEF; (5) 0.55 and 0.59 for MMEF; and (6) 0.37 and 0.46 for FEF25%, 0.48 and 0.54 for FEF50%, and 0.49 and 0.54 for FEF75%.

FEV$_1$/FVC ratio

Figure 1A presents the offspring-parent regression slopes (β_{OP}) for selected quantiles of the offspring's FEV$_1$/FVC ratio. The slopes became progressively weaker (i.e., less steep) with increasing quantiles of the index ($P = 0.003$). These quantile-specific regression slopes were
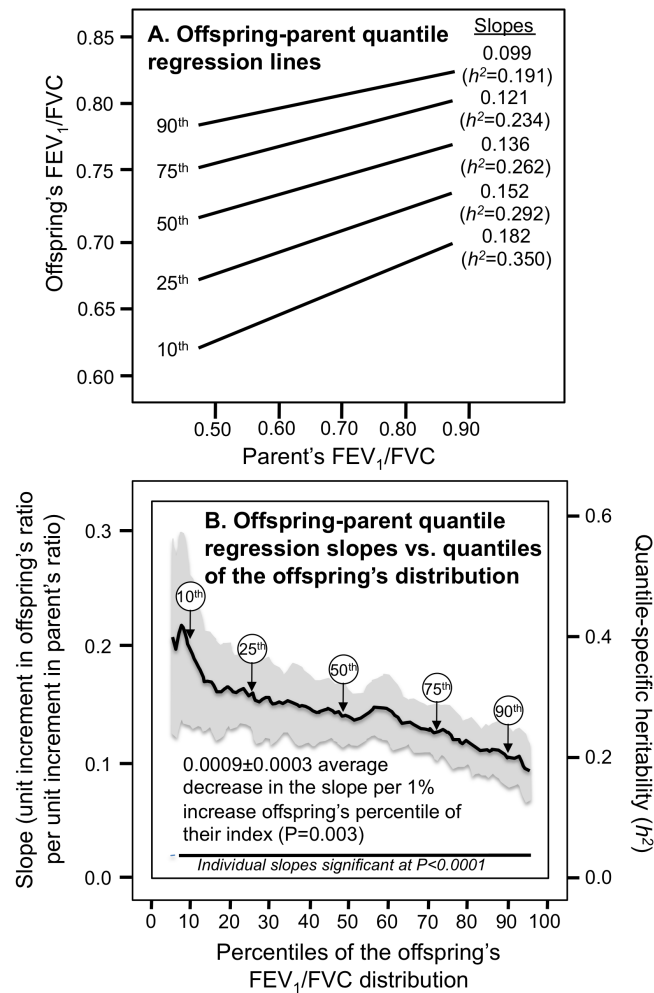
Figure 1 **Offspring-parent regression slopes (β_{OP}) for selected quantiles of the offsprings' FEV1/FVC ratio (10th, 25th, 50th, 75th, 90th).** (A) Offspring-parent regression slopes (β_{OP}) for selected quantiles of the offspring's FEV_1/FVC distribution (10th, 25th, 50th, 75th, 90th) for quantile regression analyses of 6,223 offspring, showing increasing regression slope with decreasing percentiles of the offspring's distribution. (B) The slopes from A were included with those of other quantiles to create the quantile-specific heritability plot. The shaded area presents the 95% confidence intervals for the individual slopes at each quantile. Quantile-specific heritability (h^2) was calculated as $2\beta_{OP}/(1+r_{spouse})$ where $r_{spouse} = 0.04$.

included with those of other quantiles to create the quantile-specific heritability function in Fig. 1B, i.e., where the offspring-parent slopes (Y-axis) are plotted as a function of the quantile of the offspring's sample distribution (X-axis). Specifically, the Y-axis represents the slope at the 5th quantile of the index, the 6th quantile of the index,..., and the 95th quantiles of the offspring's index. The shaded area presents the 95% confidence intervals for the individual slopes at each quantile. The figure shows that each unit increment in the parents' value was associated with an offsprings' increase of (slope ± SE) 0.182 ± 0.031 units at the 10th percentile of the offspring's distribution ($P = 6.4 \times 10^{-9}$), 0.152 ± 0.015 increase at the 25th percentile ($P < 10^{-15}$), 0.136 ± 0.011 increase at the 50th percentile

	Parents	Offspring
Sample	4601	6231
Male,%	47.3	47.0
Height, m	1.66 (0.09)	1.69 (0.09)
Ever smoked, %	68.4	51.4
Age, years	59.4 (8.2)	48.2 (11.4)
FVC, l	3.34 (0.95)	4.24 (1.06)
FEV_1, l	2.46 (0.73)	3.22 (0.86)
FEV_1/FVC	0.74 (0.09)	0.76 (0.08)
FEF25%, l/s	5.38 (2.09)	6.83 (2.03)
FEF50%, 1/s	2.91 (1.31)	3.88 (1.41)
FEF75%, l/s	0.74 (0.44)	1.21 (0.61)
MMEF, l/s	1.98 (0.92)	2.91 (1.12)
PEF, l/s	7.11 (2.32)	8.44 (2.17)

Table 1 Sample characteristics.

Notes.
  Means (standard deviations) are presented. Sample size is for FVC and varies between variables (see figure legends).

$(P < 10^{-15})$, 0.121 ± 0.013 increments at the 75th percentile $(P < 10^{-15})$, and 0.099 ± 0.013 increment at the 90th percentile $(P < 10^{-15})$. These correspond to $h^2 \pm$ SEs of 0.350 ± 0.060 at the 10th, 0.292 ± 0.029 at the 25th, 0.262 ± 0.020 at the 50th, 0.234 ± 0.026 at the 75th, and 0.191 ± 0.025 at the 90th percentiles of the FEV_1/FVC ratio. If the offspring-parent slope for the FEV_1/FVC ratio was the same for all offspring quantiles as traditionally assumed, then Fig. 1A would display parallel regression lines, and Fig. 1B would present a simple horizontal line. In fact, the graph shows that the slopes became progressively weaker with increasing quantiles of its offspring's distribution, such that on average each 1-percent increase in the offspring's distribution was associated with a 0.0018 unit linear decrease in h^2 ($P = 0.003$).

Heritability was nearly 50% lower at the 90th than at the 10th percentile of offspring's ratio. The h^2 difference between the most healthy and least healthy index (i.e., 90th −10th percentile): was -0.159 ± 0.062 ($P = 0.01$). Figure 2 shows that the quantile-specific heritability for the FEV_1/FVC ratio was also evident from the regression slopes between sibling. Specifically, the full-sib regression slope: (1) declined 0.0022 ± 0.0005 ($P = 2.1 \times 10^{-6}$) for each percentile increase in the distribution of the ratio, and (2) was 50% lower at the 90th (0.181 ± 0.021) than the 10th percentile (0.380 ± 0.037) of the sibs' distribution. The offspring-parent and full-sib slopes were statistically significant ($P < 10^{-6}$) at every percentile between the 5th and the 95th percentiles of the offspring's distribution.

FVC and FEV1

Consistent with the classical model, Table 2 and Figs. 3A and 3B show that the parent–offspring and full-sib regression slopes for FVC were significant and remained relatively constant for all quantiles between the 5th and 95th percentile of their distribution.

Table 2 Traditional (least squares) and quantile regression analyses of spirometric traits between related individuals.[a]

	Least-squares regression analysis			Quantile regression analysis				
	Correlation	Least-squares regression slope			Increase in slope per 1% increase in the offspring or sibling distribution		Difference in slope between the 90th and 10th percentiles	
		Slope ± SE	P^{b}		Slope ± SE	P^{c}	Difference ± SE	P^{d}
FEV$_1$/FVC								
Spouse	0.04	0.047 ± 0.018	0.007		−0.0005 ± 0.0005	0.37	−0.008 ± 0.077	0.91
Offspring-parent	0.17	0.134 ± 0.010	$<10^{-15}$		−0.0009 ± 0.0003	0.003	−0.083 ± 0.032	0.01
Offspring-midparent	0.24	0.269 ± 0.018	$<10^{-15}$		−0.0021 ± 0.0005	4.1×10^{-5}	−0.192 ± 0.059	0.001
Full-sibling	0.27	0.266 ± 0.017	$<10^{-15}$		−0.0022 ± 0.0005	2.1×10^{-6}	−0.200 ± 0.040	5.6×10^{-7}
FVC								
Spouse	0.08	0.109 ± 0.033	0.001		0.0008 ± 0.0009	0.41	0.049 ± 0.089	0.58
Offspring-parent	0.26	0.263 ± 0.013	$<10^{-15}$		−0.0002 ± 0.0003	0.43	−0.031 ± 0.027	0.24
Offspring-midparent	0.32	0.463 ± 0.022	$<10^{-15}$		−0.0007 ± 0.0006	0.23	−0.050 ± 0.059	0.4
Full-sibling	0.32	0.321 ± 0.017	$<10^{-15}$		0.0001 ± 0.0004	0.76	−0.001 ± 0.039	0.99
FEV$_1$								
Spouse	0.08	0.118 ± 0.034	0.0005		−0.0010 ± 0.0008	0.23	−0.073 ± 0.090	0.40
Offspring-parent	0.23	0.227 ± 0.012	$<10^{-15}$		−0.0003 ± 0.0003	0.29	−0.026 ± 0.034	0.44
Offspring-midparent	0.30	0.407 ± 0.022	$<10^{-15}$		−0.0007 ± 0.0006	0.22	−0.009 ± 0.058	0.88
Full-sibling	0.28	0.282 ± 0.017	$<10^{-15}$		−0.0006 ± 0.0004	0.17	−0.070 ± 0.045	0.12
PEF								
Spouse	0.05	0.078 ± 0.045	0.08		0.0001 ± 0.0012	0.92	−0.047 ± 0.111	0.67
Offspring-parent	0.19	0.180 ± 0.014	$<10^{-15}$		0.0003 ± 0.0003	0.32	0.017 ± 0.034	0.61
Offspring-midparent	0.25	0.336 ± 0.026	$<10^{-15}$		0.0006 ± 0.0006	0.34	0.052 ± 0.055	0.34
Full-sibling	0.23	0.228 ± 0.017	$<10^{-15}$		0.0003 ± 0.0004	0.46	0.063 ± 0.046	0.17
MMEF								
Spouse	0.04	0.049 ± 0.038	0.20		−0.0012 ± 0.0009	0.20	−0.106 ± 0.097	0.28
Offspring-parent	0.25	0.285 ± 0.015	$<10^{-15}$		0.0013 ± 0.0004	0.0004	0.108 ± 0.037	0.003
Offspring-midparent	0.35	0.550 ± 0.028	$<10^{-15}$		0.0014 ± 0.0008	0.06	0.137 ± 0.071	0.05
Full-sibling	0.30	0.296 ± 0.017	$<10^{-15}$		0.0009 ± 0.0005	0.04	0.049 ± 0.049	0.32
FEF25%								
Spouse	0.07	0.101 ± 0.043	0.02		−0.0007 ± 0.0011	0.52	−0.097 ± 0.105	0.36
Offspring-parent	0.21	0.200 ± 0.013	$<10^{-15}$		−0.0002 ± 0.0003	0.45	−0.024 ± 0.032	0.47
Offspring-midparent	0.28	0.367 ± 0.024	$<10^{-15}$		−0.0005 ± 0.0006	0.35	−0.092 ± 0.060	0.13
Full-sibling	0.24	0.241 ± 0.017	$<10^{-15}$		−0.0007 ± 0.0004	0.12	−0.032 ± 0.035	0.36
FEF50%								
Spouse	0.06	0.077 ± 0.037	0.04		−0.0007 ± 0.0009	0.41	−0.124 ± 0.080	0.12
Offspring-parent	0.25	0.253 ± 0.013	$<10^{-15}$		0.0011 ± 0.0003	0.002	0.079 ± 0.037	0.03
Offspring-midparent	0.37	0.514 ± 0.024	$<10^{-15}$		0.0010 ± 0.0006	0.10	0.060 ± 0.060	0.32
Full-sibling	0.28	0.284 ± 0.017	$<10^{-15}$		0.0008 ± 0.0004	0.05	0.055 ± 0.044	0.21

Table 2 (*continued*)

| | | Least-squares regression analysis | | Quantile regression analysis | | | |
| | Correlation | Least-squares regression slope | | Increase in slope per 1% increase in the offspring or sibling distribution | | Difference in slope between the 90th and 10th percentiles | |
		Slope ± SE	P^b	Slope ± SE	P^c	Difference ± SE	P^d
FEF75%							
Spouse	0.01	0.015 ± 0.038	0.70	-0.0012 ± 0.0011	0.27	-0.114 ± 0.097	0.24
Offspring-parent	0.20	0.249 ± 0.017	$<10^{-15}$	0.0021 ± 0.0004	1.8×10^{-6}	0.153 ± 0.048	0.002
Offspring-midparent	0.28	0.507 ± 0.033	$<10^{-15}$	0.0035 ± 0.0009	0.0001	0.228 ± 0.090	0.01
Full-sibling	0.27	0.269 ± 0.017	$<10^{-15}$	0.0019 ± 0.0005	0.0002	0.147 ± 0.052	0.005

Notes.
[a] See figure legends for sample sizes.
[b] Significance determined from standard least-squares regression giving each offspring equal weight.
[c] Significance determined from linear contrast from the variance–covariance matrix determined from 1000 bootstrap samples.
[d] Significance determined from difference in coefficients between the 10th and 90th quantiles and their associated standard errors (±SE) and covariance determined from 1,000 bootstrap samples. Significance levels are those reported by Stata, but it is important not to interpret significance levels much less than $P<0.001$ literally but rather as indicators of strong significance.
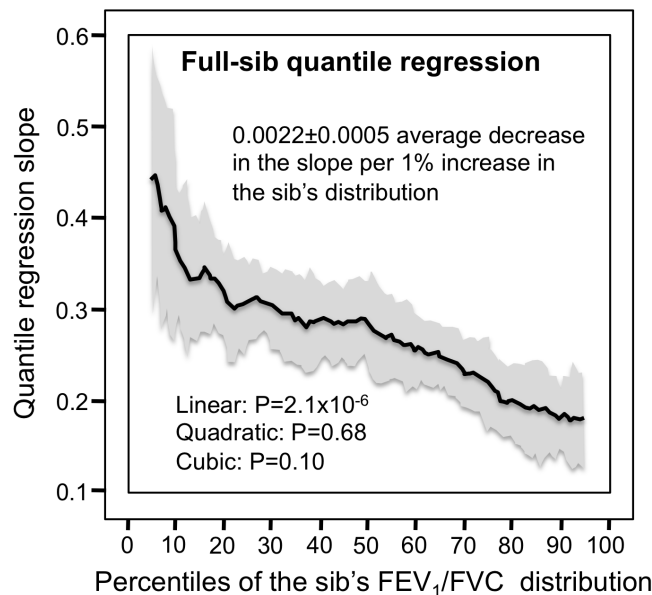

Figure 2 Full-sib regression slopes for FEV1/FVC ratio. Full-sib regression slopes (β_{FS}) for 5,122 sibling from 1,930 sibships showing higher full-sib regression slope for the lower percentiles of the siblings' FEV_1/FVC distribution. The shaded area presents the 95% confidence intervals for the individual slopes at each quantile from 1,000 bootstrapped samples. The significance levels of the linear, quadratic and cubic component of the quantile-specific slope function were computed from orthogonal contrasts.
Full-size 🖻 DOI: 10.7717/peerj.9145/fig-2

Figures 4A and 4B and Figs. 5A and 5B show that the slopes for FEV_1 and PEF were also generally constant, albeit FEV_1 exhibited some convexity.

MMEF and FEF

Offspring-parent and full-sib regression slopes for MMEF increased significantly with the percentiles of the trait distribution (Figs. 6A and 6B). At the 90th percentile, h^2 was 44%
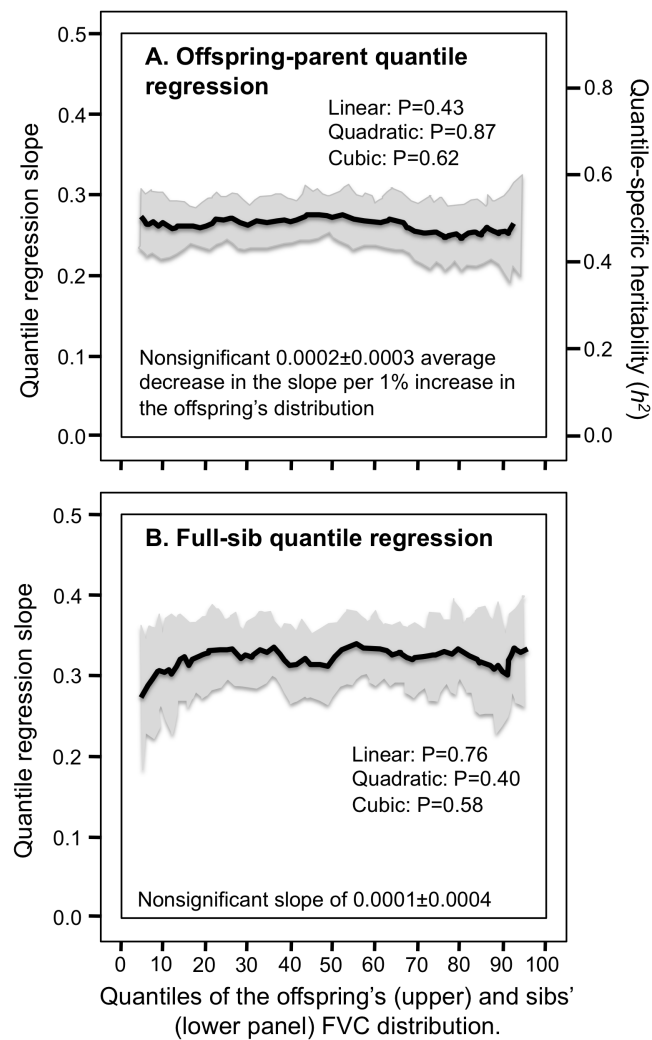
Figure 3 Offspring-parent and full-sib quantile regression slopes for forced vital capacity (FVC). (A) Offspring-parent ($N = 6{,}231$ offspring) and (B) full-sib quantile regression slopes (5,122 offspring in 1,930 sibships) for FVC. The shaded area presents the 95% confidence intervals for the slopes at each quantile from 1,000 bootstrapped samples. The nonsignificant linear, quadratic and cubic component of the quantile-specific heritability (A) and slope functions (B) were computed from orthogonal contrasts. Quantile-specific heritability (h^2) was calculated as $2\beta_{OP}/(1 + r_{spouse})$ where $r_{spouse} = 0.08$.

Full-size ▣ DOI: 10.7717/peerj.9145/fig-3

greater than at the 10th percentile (0.675 ± 0.060 vs. 0.468 ± 0.046). This was largely due to FEF75% (Figs. 7A and 7B). Specifically, those whose expiratory force was high relative to the population (90th percentile) were more strongly associated with their parents and siblings than those whose force was relatively low (10th percentile, i.e., regression slope 80% and 70% greater for parent offspring and sibs, respectively). In contrast, offspring-parent and full-sib regression slopes were relatively constant at FEF25%.
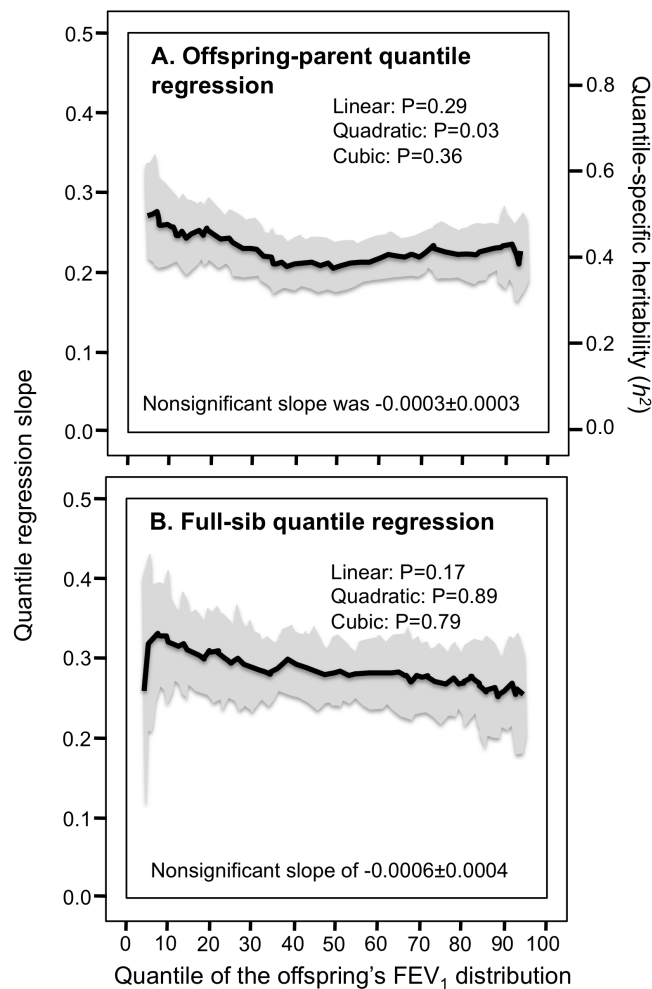
Figure 4 Offspring-parent and full-sib quantile regression for forced expiratory volume at 1 second (FEV_1). (A) Offspring-parent ($N = 6{,}223$ offspring) and (B) full-sib quantile regression slopes ($N = 5{,}122$ offspring in 1,930 sibships) for FEV_1. The shaded area presents the 95% confidence intervals for the slopes at each quantile from 1,000 bootstrapped samples. Significance for linear, quadratic and cubic component of the quantile-specific heritability and slope functions were computed from orthogonal contrasts. Quantile-specific heritability (h^2) was calculated as $2\beta_{OP}/(1 + r_{spouse})$ where $r_{spouse} = 0.08$.

Full-size 🖾 DOI: 10.7717/peerj.9145/fig-4

DISCUSSION

Prior studies tacitly assume that the genetic and non-genetic factors affecting pulmonary function in parents, offspring, and other related individuals apply to all quantiles of the phenotype. This assumption appears valid for FVC and PEF, and generally applies to FEV_1. It does not appear valid for the FEV_1/FVC ratio, whose heritability at the 90th percentile of the population distribution ($h^2 \pm$ SE: 0.191 ± 0.025) was about one-half of that at the 10th percentile ($h^2 \pm$ SE: 0.350 ± 0.060). Quantile-specific effects were also significant for full-sib association. These quantile-specific effects are a departure from the underlying statistical models used in the analyses of twin pairs, and pedigrees, segregation analyses, and GWAS.
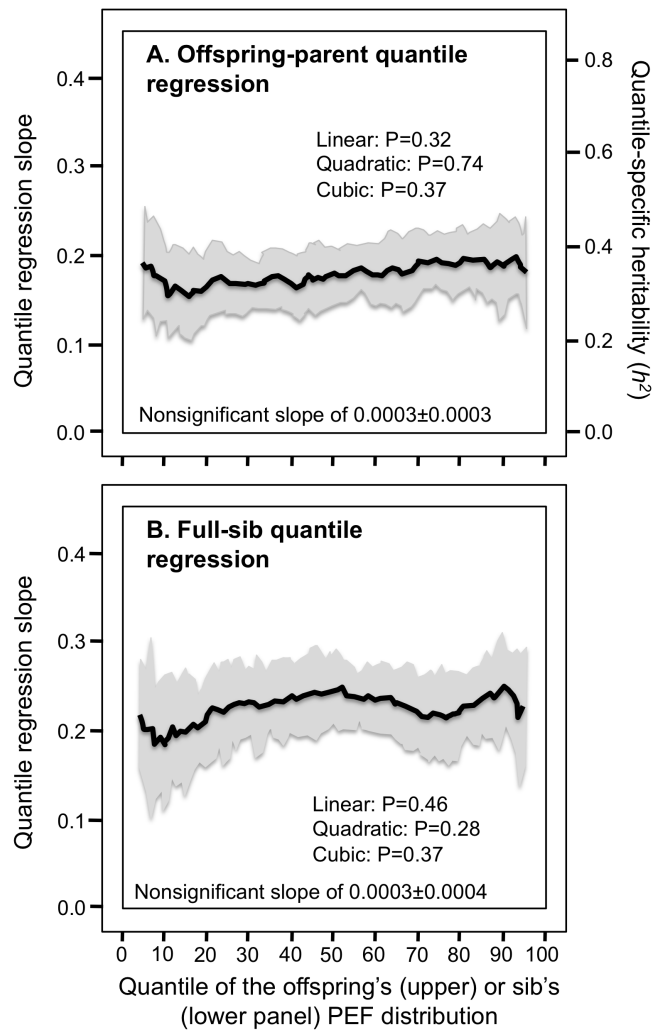
Figure 5 Offspring-parent and full-sib quantile regression slopes for peak expiratory flow (PEF). (A) Offspring-parent ($N = 4,686$ offspring) and (B) full-sib quantile regression slopes ($N = 5,122$ offspring in 1,930 sibships) for PEF. The shaded area presents the 95% confidence intervals for the slopes at each quantile from 1,000 bootstrapped samples. The nonsignificant linear, quadratic and cubic component of the quantile-specific heritability and slope function were computed from orthogonal contrasts. Quantile-specific heritability (h^2) was calculated as $2\beta_{OP}/(1 + r_{spouse})$ where $r_{spouse} = 0.05$.

Full-size 🖼 DOI: 10.7717/peerj.9145/fig-5

Implications regarding gene-environment interactions

Figures 1 and 2 suggest that estimates of genetic heritability, cultural transmission, or shared environmental are likely to increase with decreasing levels of the FEV_1/FVC ratio. Correspondingly, environmental factors that tend to distinguish normal vs. abnormal pulmonary function such as smoking and air pollution will likely manifest differences in genetics, cultural transmission, or shared environment in accordance with the Figures. In this regard, it is hypothesize that the environment (e.g., smoking) lowers the FEV_1/FVC ratio, which in turn increases the phenotypic expression of genetic traits (quantile-dependent expressivity: environment $\rightarrow$ phenotype $\rightarrow$ gene expression) rather than the
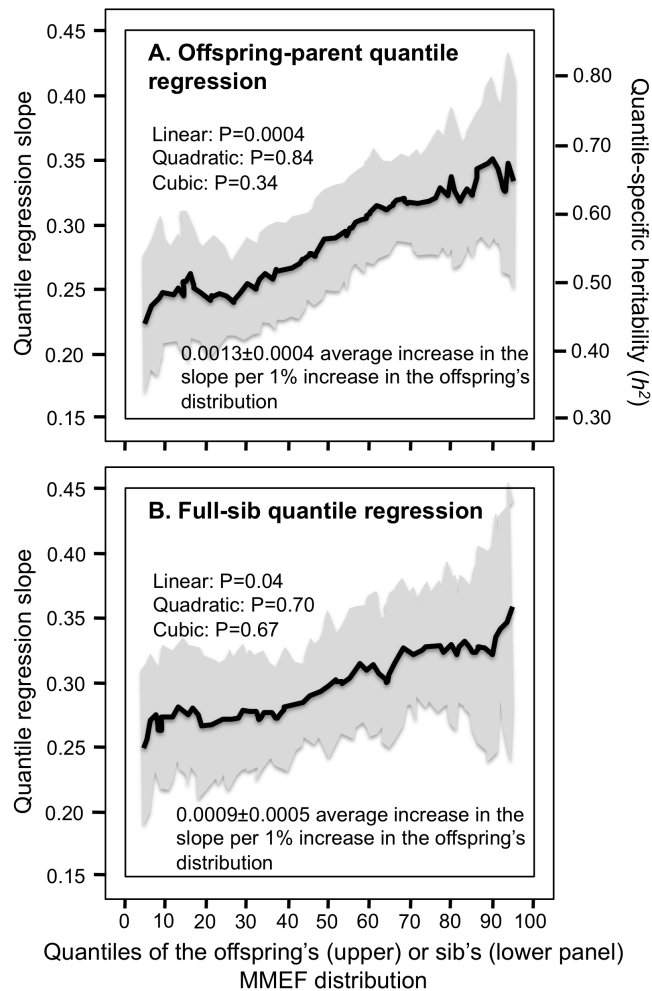
Figure 6 **Offspring-parent and full-sib quantile regression for maximum-mid expiratory flow (MMEF).** (A) Offspring-parent ($N = 5{,}497$ offspring) and (B) full-sib quantile regression slopes ($N = 5{,}122$ offspring in 1,930 sibships) for MMEF. The shaded area presents the 95% confidence intervals for the slopes at each quantile from 1,000 bootstrapped samples. The nonsignificant linear, quadratic and cubic component of the quantile-specific heritability function were computed from orthogonal contrasts. Quantile-specific heritability (h^2) was calculated as $2\beta_{OP}/(1+r_{spouse})$ where $r_{spouse} = 0.04$.

Full-size ◩ DOI: 10.7717/peerj.9145/fig-6

more traditional interpretation of gene-environment interaction (environment → gene expression → phenotype) (*Williams, 2012*). This scenario offers an alternative to the interpretation of several published reports purporting gene-environment interaction for pulmonary function.

Aschard et al. (2017) analyzed the association of a 26-SNP genetic risk with FEV_1/FVC by smoking status in 50,047 participants of European ancestry from 19 studies. They found that the difference in FEV_1/FVC between individuals who currently or previously smoked versus those who had never smoked was significantly greater if they had a high genetic risk score (GRS). They interpreted this association as a gene-environment interaction, with the susceptibility of the FEV_1/FVC ratio to smoking being significantly greater among
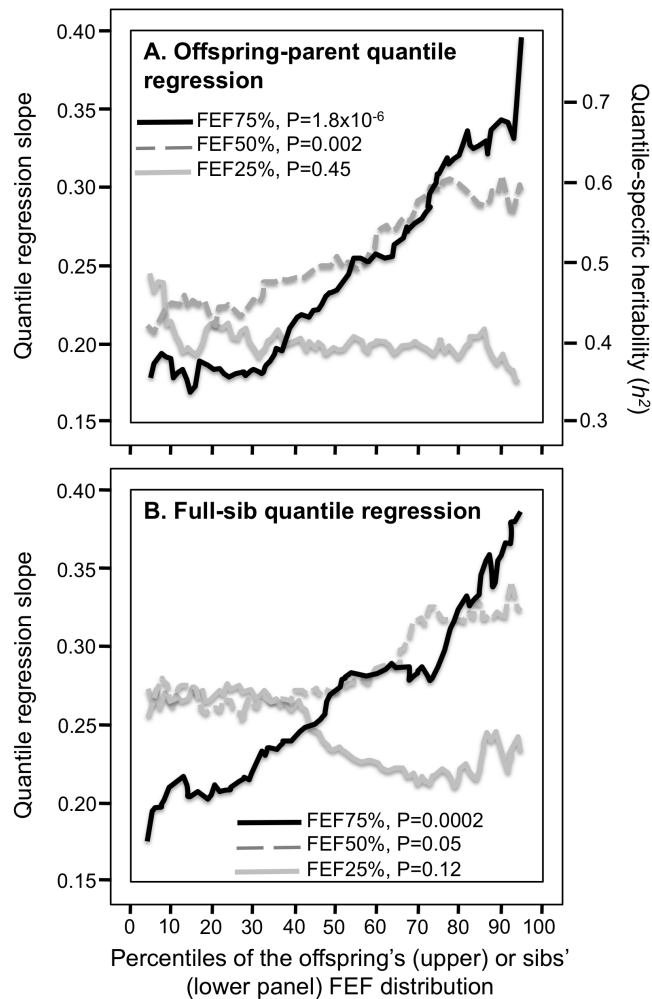
Figure 7 **Offspring-parent and full-sib quantile regression slopes for FEF25%, FEF50% and FEF75%.**
(A) Offspring-parent ($N = 5{,}497$ offspring) and (B) full-sib quantile regression slopes (5,122 offspring in 1,930 sibships) for FEF25%, FEF50% and FEF75%. The shaded area presents the 95% confidence intervals for the slopes at each quantile from 1,000 bootstrapped samples. The nonsignificant linear, quadratic and cubic component of the quantile-specific heritability function were computed from orthogonal contrasts. Quantile-specific heritability is for forces expired flow at 75% ($r_{spouse} = 0.01$), those for 50% ($r_{spouse} = 0.06$) and 25% ($r_{spouse} = 0.07$) would be slightly lower.

Full-size 🖼 DOI: 10.7717/peerj.9145/fig-7

individuals who are genetically predisposed to having a low ratio. They proposed that the GRS could be used "to identify subgroups in the population whose genetic background makes them more susceptible to the deleterious effects of smoking". Moreover, in the "public health setting, programmes targeting smoking cessation campaigns to individuals who are genetically predisposed to low pulmonary function may have a stronger impact in preventing COPD."

Aschard et al. (2017) interpreted their cross-sectional association as an effect of the GRS on the consequence of smoking. In their data, there was a significantly greater reduction in the FEV_1/FVC ratio per increment in the GRS in individuals who currently or previously

smoked ($\beta_{GRS} = -0.0462$) than in those who had never smoked ($\beta_{GRS} = -0.0363$, $P_{interaction} = 5.7 \times 10^{-4}$). Figure 1B shows that the heritability of the FEV$_1$/FVC ratio increases with decreasing percentiles of the FEV$_1$/FVC ratio in the population. On average, those who currently or previously smoked in the Framingham Cohorts had significantly lower FEV$_1$/FVC ratio than those who had never smoked. Therefore it is speculated that smoking lowers the FEV$_1$/FVC ratio, which in turn increases the expressivity of the GRS vis-à-vis its effect in those who have never smoked.

The best-documented inherited effect on pulmonary function is AAT deficiency (*Global initiative for Chronic Obstructive Lung Disease, 2020*). AAT is an anti-inflammatory protease inhibitor (Pi) of neutrophil elastase that is encoded by the SERPINA1 gene. Neutrophil elastase breaks down elastin in the lung parenchyma. Isoelectric focusing may be used to genotype the SERPINA1 gene, including the PiMM genotype having normal AAT concentrations, the PiMZ genotype with mild AAT deficiency, and the PiZZ genotype with severe AAT deficiency (*Global initiative for Chronic Obstructive Lung Disease, 2020*).

Mehta et al. (2014) examined the interaction between the SERPINA1 PiMZ and PiMM genotypes and concentrations of outdoor particulate matter of ≤ 10 mm (PM10) on changes in lung function over time. They reported that higher PM10 particle concentrations produced significantly greater declines in the ratio of FEV$_1$/FVC in PiMZ genotypes ($\beta = -0.003$) than PiMM genotypes ($\beta = 0.001$), representing a significant gene-environment interaction ($P = 0.03$). Alternatively, their results could also represent larger differences in the FEV$_1$/FVC ratio between the PiMZ and PiMM genotypes with increasing particle concentrations (i.e., decreasing FEV$_1$/FVC ratio), reflecting significant quantile-dependent expressivity.

Bronchial hyper-responsiveness predicts declining pulmonary function and is present in approximately two-thirds of patients with non-severe COPD (*Tashkin et al., 1996*). *Sigsgaard et al. (2000)* reported that bronchial hyper-responsiveness in farming students increased with the expected severity of AAT deficiency of their Pi genotypes, but not in rural controls. They attributed this difference to the faming students' exposure to inflammatory agents such as farm and swine confinement dust versus the rural controls' lack of exposure to these agents. The traditional gene-environment interpretation would suggest that the effects of farm dust on bronchial hyper-responsiveness was modified by the Pi-genotype, whereas quantile-dependent expressivity would suggest that farm dust reduced pulmonary function, which in turn accentuates the genetic effect of the Pi-genotypes on bronchial hyper-responsiveness.

Quantile-dependent expressivity may also contribute to the gene-smoking interactions of the SNPs rs9862443 and rs7941377 reported by *Hancock et al. (2012)*, and the increased effect of the FAM13A-gene haplotype on FEV$_1$/FVC from 0 ($\beta_{effect} = -0.51$), >0 to 15 ($\beta = -0.38$), >15 to 30 ($\beta = -0.61$), and >30 pack years ($\beta = -1.40$) in Koreans (*Kim et al., 2015*).

MMEF and FEF

FEF75% and MMEF both exhibited significant quantile-dependent expressivity of offspring-parent and sibling similarity. These measures may assess small airway

function (*Wood, Tan & Stockley, 2009*), although its use in diagnosing small airway disease in individual patients is discouraged (*Quanjer et al., 2014*). Lung elastic recoil and pleural pressure are lower and the location of the flow-limiting segment (choke point) is further upstream in airway segments towards the end of a forced expiratory maneuver then earlier. In addition, obstructions in small airways are hypothesized to have a greater effect on airflow at low than high lung volumes (*Wood, Tan & Stockley, 2009*).

Methodological issues

Offspring-parent and sibling regression coefficients are simple estimates of heritability relative to its estimation from twin studies, family studies and segregation analyses. As has been reported previously in this and other cohorts (*Devor & Crawford, 1984*; *Coultas et al., 1991*; *Klimentidis et al., 2013*; *Redline et al., 1989*; *Wilk et al., 2000*; *Tarnoki et al., 2013*; *Hukkinen et al., 2011*; *Hallberg et al., 2010*; *DeMeo et al., 2004*; *McClearn et al., 1994*; *Palmer et al., 2001*; *Astemborski, Beaty & Cohen, 1985*; *Beaty et al., 1987*; *Lewitter et al., 1984*; *Cotch, Beaty & Cohen, 1990*; *Chen et al., 1996*; *Chen et al., 1997*; *Givelber et al., 1998*; *Ingebrigtsen et al., 2011*; *Joost et al., 2002*; *Tian et al., 2017*; *Yamada et al., 2015*), these analyses showed moderate to strong heritabilities for pulmonary function. The generally greater heritabilities for the sib-based vs. offspring-parent-based estimates have been reported by others (*Givelber et al., 1998*) and suggest that shared environment or dominance effects might not be negligible. Table 2 presents the significance levels reported by Stata, but it is important not to interpret significance levels much less than $P < 0.001$ literally, but rather as a indicator of strong statistical significance. Although results for MMEF and FEF were statistically significant, these measurements are difficult to make and not encouraged for use clinically due to their variability. Their variability is partly technique related, which is particularly apropos given the changing Framingham methodology over the course of data collection. The calculations of offspring-parent and full-sib quantile regression presented here assigned equal weights to each offspring (*Karlin, Cameron & Williams, 1981*), but there may be additional clustering within family sets not addressed by these analyses.

CONCLUSIONS

These analyses suggest that the heritability of FEV_1/FVC ratio, MMEF, and FEF75% are strongly dependent upon whether the offspring or sib is high or low relative to its distribution in the population. Quantile-dependent expressivity may account for gene-environment interactions between pulmonary function and smoking or airborne PM10. Caution is warranted when assigning risk from smoking or PM10 concentration based on specific genotypes when in fact low FEV_1/FVC may be the cause for greater susceptibility.

Abbreviation Key

AAT	α-1 antitrypsin
β_{FS}	Full-sib regression slope
β_{OM}	Offspring mid-parental regression slope
β_{OP}	Offspring parent regression slope

COPD	Chronic obstructive pulmonary disease
FEF25%	Forced expiratory flows at 25% of FVC
FEF50%	Forced expiratory flows at 50% of FVC
FEF75%	Forced expiratory flows at 75% of FVC
FEV$_1$	Forced expiratory volume in 1 second
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GWAS	Genome-wide association studies
h^2	Heritability in the narrow sense
MMEF	Maximum mid-expiratory flow
PEF	Maximum speed of expiration
PM10	Particulate matter ≤ 10 mm
SD	Standard deviation
SE	Standard error
SNP	Single nucleotide polymorphisms

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The author declares there are no competing interests.

Author Contributions

- Paul T. Williams conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The Lawrence Berkeley National Laboratory Human Subjects Committee (HSC) protocol granted Ethical approval: "Gene-environment interaction vs quantile-dependent penetrance of established SNPs" (IRB Approval number: 107H021-13MR20). LBNL holds Office of Human Research Protections Federal wide Assurance number FWA 00006253.

Data Availability

The following information was supplied regarding data availability:

The raw data are available at the National Institutes of Health: https://biolincc.nhlbi.nih.gov/studies/framcohort/, https://biolincc.nhlbi.nih.gov/studies/gen3/, https://biolincc.nhlbi.nih.gov/studies/framoffspring/.

The code is available as a Supplemental File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.9145#supplemental-information.

REFERENCES

Aschard H, Tobin MD, Hancock DB, Skurnik D, Sood A, James A, Vernon Smith A, Manichaikul AW, Campbell A, Prins BP, Hayward C, Loth DW, Porteous DJ, Strachan DP, Zeggini E, O'Connor GT, Brusselle GG, Boezen HM, Schulz H, Deary IJ, Hall IP, Rudan I, Kaprio J, Wilson JF, Wilk JB, Huffman JE, Zhao JHua, Jong Kde, Lyytikäinen LP, Wain LV, Jarvelin MR, Kähönen M, Fornage M, Polasek O, Cassano PA, Barr RG, Rawal R, Harris SE, Gharib SA, Enroth S, Heckbert SR, Lehtimäki T, Gyllensten U, Society Scientific Group U, Jackson VE, Gudnason V, Tang W, Dupuis J, Soler Artigas M, Joshi AD, London SJ, Kraft P. 2017. Evidence for large-scale gene-by-smoking interaction effects on pulmonary function. *International Journal of Epidemiology* **46**:894–904.

Astemborski JA, Beaty TH, Cohen BH. 1985. Variance components analysis of forced expiration in families. *American Journal of Medical Genetics* **21**:741–753 DOI 10.1002/ajmg.1320210417.

Beaty TH, Liang KY, Seerey S, Cohen BH. 1987. Robust inference for variance components models in families ascertained through probands. II. Analysis of spirometric measures. *Genetic Epidemiology* **4**:211–221 DOI 10.1002/gepi.1370040306.

Chen Y, Horne SL, Rennie DC, Dosman JA. 1996. Segregation analysis of two lung function indices in a random sample of young families: the Humboldt family study. *Genetic Epidemiology* **13**:35–47 DOI 10.1002/(SICI)1098-2272(1996)13:1<35::AID-GEPI4>3.0.CO;2-5.

Chen Y, Rennie DC, Lockinger LA, Dosman JA. 1997. Major genetic effect on forced vital capacity: the Humboldt family study. *Genetic Epidemiology* **14**:63–76 DOI 10.1002/(SICI)1098-2272(1997)14:1<63::AID-GEPI5>3.0.CO;2-6.

Cotch MF, Beaty TH, Cohen BH. 1990. Path analysis of familial resemblance of pulmonary function and cigarette smoking. *American Review of Respiratory Disease* **142(6 Pt1)**:1337–1343 DOI 10.1164/ajrccm/142.6_Pt_1.1337.

Coultas DB, Hanis CL, Howard CA, Skipper BJ, Samet JM. 1991. Heritability of ventilatory function in smoking and non-smoking New Mexico Hispanics. *American Review of Respiratory Disease* **144**:770–775 DOI 10.1164/ajrccm/144.4.770.

Curjuric I, Imboden M, Nadif R, Kumar A, Schindler C, Haun M, Kronenberg F, Künzli N, Phuleria H, Postma DS, Russi EW, Rochat T, Demenais F, Probst-Hensch NM. 2012. Different genes interact with particulate matter and tobacco smoke exposure in affecting lung function decline in the general population. *PLOS ONE* **12**:e40175.

Dawber TR, Meadors GF, Moore FEJ. 1951. Epidemiological approaches to heart disease: the framingham study. *American Journal of Public Health* **41**:279–286 DOI 10.2105/AJPH.41.3.279.

DeMeo DL, Carey VJ, Chapman HA, Reilly JJ, Ginns LC, Speizer FE, Weiss ST, Silverman EK. 2004. Familial aggregation of FEF(25-75) and FEF(25-75)/FVC in families with severe, early onset COPD. *Thorax* **59**:396–400 DOI 10.1136/thx.2003.012856.

Devor EJ, Crawford MH. 1984. Family resemblance for normal pulmonary function. *Annals of Human Biology* **11**:439–448 DOI 10.1080/03014468400007331.

Dockery DW, Ware JH, Ferris Jr BG, Glicksberg DS, Fay ME, Spiro 3rd A, Speizer FE. 1985. Distribution of forced expiratory volume in one second and forced vital capacity in healthy, white, adult never-smokers in six U.S. cities. *American Review of Respiratory Disease* **131**:511–520 DOI 10.1164/arrd.1985.131.4.511.

Falconer DS, Mackay TFC. 1996. *Introduction to quantitative genetics.* Fourth edition. Harlow: Pearson Education Limited.

dbGaP genotypes phenotypes. 2020a. Authorized access portal. *Available at https://view.ncbi.nlm.nih.gov/dbgap-controlled* (accessed on 8 March 2020).

dbGaP genotypes phenotypes. 2020b. Framingham cohort. dbGaP Study Accession: phs000007.v30.p11. *Available at https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v30.p11* (accessed on 8 March 2020).

Givelber RJ, Couropmitree NN, Gottlieb DJ, Evans JC, Levy D, Myers RH, O'Connor GT. 1998. Segregation analysis of pulmonary function among families in the Framingham Study. *American Journal of Respiratory and Critical Care Medicine* **157**(5 Pt 1):1445–1451 DOI 10.1164/ajrccm.157.5.9704021.

Global initiative for Chronic Obstructive Lung Disease, Inc. 2020. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (2020 Report). *Available at https://goldcopd.org/wp-content/uploads/2019/12/GOLD-2020-FINAL-ver1.2-03Dec19_WMV.pdf* (accessed on 19 January 2020).

Gottlieb DJ. 1999. New understandings of the natural history and epidemiology of chronic obstructive lung disease. *Seminars in Respiratory and Critical Care Medicine* **20**:289–300 DOI 10.1055/s-2007-1021326.

Gould WW. 1992. Quantile regression with bootstrapped standard errors. *Stata Technical Bulletin* **9**:19–21.

Hallberg J, Dominicus A, Eriksson UK, Gerhardsson de Verdier M, Pedersen NL, Dahlbäck M, Nihlén U, Higenbottam T, Svartengren M. 2008. Interaction between smoking and genetic factors in the development of chronic bronchitis. *American Journal of Respiratory and Critical Care Medicine* **177**:486–490 DOI 10.1164/rccm.200704-565OC.

Hallberg J, Iliadou A, Anderson M, De Verdier MG, Nihlén U, Dahlbäck M, Pedersen NL, Higenbottam T, Svartengren M. 2010. Genetic and environmental influence on lung function impairment in Swedish twins. *Respiratory Research* **11**:92 DOI 10.1186/1465-9921-11-92.

Hancock DB, Soler Artigas M, Gharib SA, Henry A, Manichaikul A, Ramasamy A, Loth DW, Imboden M, Koch B, McArdle WL, Smith AV, Smolonska J, Sood A, Tang W, Wilk JB, Zhai G, Zhao JH, Aschard H, Burkart KM, Curjuric I, Eijgelsheim M, Elliott P, Gu X, Harris TB, Janson C, Homuth G, Hysi PG, Liu JZ, Loehr LR, Lohman K, Loos RJ, Manning AK, Marciante KD, Obeidat M, Postma DS, Aldrich MC, Brusselle GG, Chen TH, Eiriksdottir G, Franceschini N, Heinrich J, Rotter JI, Wijmenga C, Williams OD, Bentley AR, Hofman A, Laurie CC, Lumley T, Morrison AC, Joubert BR, Rivadeneira F, Couper DJ, Kritchevsky SB, Liu Y, Wjst M, Wain LV, Vonk JM, Uitterlinden AG, Rochat T, Rich SS, Psaty BM, O'Connor GT, North KE, Mirel DB, Meibohm B, Launer LJ, Khaw KT, Hartikainen AL, Hammond CJ, Gläser S, Marchini J, Kraft P, Wareham NJ, Völzke H, Stricker BH, Spector TD, Probst-Hensch NM, Jarvis D, Jarvelin MR, Heckbert SR, Gudnason V, Boezen HM, Barr RG, Cassano PA, Strachan DP, Fornage M, Hall IP, Dupuis J, Tobin MD, London SJ. 2012. Genome-wide joint meta-analysis of SNP and SNP-by-smoking interaction identifies novel loci for pulmonary function. *PLOS Genetics* **8**:e1003098 DOI 10.1371/journal.pgen.1003098.

Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciante KD, Franceschini N, Durme YMvan, Chen TH, Barr RG, Schabath MB, Couper DJ, Brusselle GG, Psaty BM, Van Duijn CM, Rotter JI, Uitterlinden AG, Hofman A, Punjabi NM, Rivadeneira F, Morrison AC, Enright PL, North KE, Heckbert SR, Lumley T, Stricker BH, O'Connor GT, London SJ. 2010. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nature Genetics* **42**:45–52 DOI 10.1038/ng.500.

He JQ, Connett JE, Anthonisen NR, Pare PD, Sandford AJ. 2004. Glutathione S-transferase variants and their interaction with smoking on lung function. *American Journal of Respiratory and Critical Care Medicine* **12**:388–394.

Hukkinen M, Kaprio J, Broms U, Viljanen A, Kotz D, Rantanen T, Korhonen T. 2011. Heritability of lung function: a twin study among never-smoking elderly women. *Twin Research and Human Genetics* **14**:401–407 DOI 10.1375/twin.14.5.401.

Ingebrigtsen TS, Thomsen SF, Van der Sluis S, Miller M, Christensen K, Sigsgaard T, Backer V. 2011. Genetic influences on pulmonary function: a large sample twin study. *Lung* **189**:323–330 DOI 10.1007/s00408-011-9306-3.

Joost O, Wilk JB, Cupples LA, Harmon M, Shearman AM, Baldwin CT, O'Connor GT, Myers RH, Gottlieb DJ. 2002. Genetic loci influencing lung function: a genome-wide scan in the Framingham Study. *American Journal of Respiratory and Critical Care Medicine* **165**:795–799 DOI 10.1164/ajrccm.165.6.2102057.

Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. 1979. An investigation of coronary heart disease in families. The Framingham offspring study. *American Journal of Epidemiology* **110**:281–290 DOI 10.1093/oxfordjournals.aje.a112813.

Karlin S, Cameron EC, Williams PT. 1981. Sibling and parent–offspring correlation estimation with variable family size. *Proceedings of the National Academy of Sciences of the United States of America* **78**:2664–2668 DOI 10.1073/pnas.78.5.2664.

Kim S, Kim H, Cho N, Lee SK, Han BG, Sull JW, Jee SH, Shin C. 2015. Identification of FAM13A gene associated with the ratio of FEV1 to FVC in Korean population by genome-wide association studies including gene-environment interactions. *Journal of Human Genetics* **60**:139–145 DOI 10.1038/jhg.2014.118.

Klimentidis YC, Vazquez AI, De Los Campos G, Allison DB, Dransfield MT, Thannickal VJ. 2013. Heritability of pulmonary function estimated from pedigree and whole-genome markers. *Frontiers Genetics* **4**:174.

Koenker R, Hallock KF. 2001. Quantile regression. *Journal of Economic Perspectives* **15**:143–156 DOI 10.1257/jep.15.4.143.

Lewitter FI, Tager IB, McGue M, Tishler PV, Speizer FE. 1984. Genetic and environmental determinants of level of pulmonary function. *American Journal of Epidemiology* **120**:518–530 DOI 10.1093/oxfordjournals.aje.a113912.

Liao SY, Lin X, Christiani DC. 2013. Gene-environment interaction effects on lung function- a genome-wide association study within the Framingham heart study. *Environ Health* **12**:101 DOI 10.1186/1476-069X-12-101.

Loth DW, Soler Artigas M, Gharib SA, Wain LV, Franceschini N, Koch B, Pottinger TD, Smith AV, Duan Q, Oldmeadow C, Lee MK, Strachan DP, James AL, Huffman JE, Vitart V, Ramasamy A, Wareham NJ, Kaprio J, Wang XQ, Trochet H, Kähönen M, Flexeder C, Albrecht E, Lopez LM, De Jong K, Thyagarajan B, Alves AC, Enroth S, Omenaas E, Joshi PK, Fall T, Viuela A, Launer LJ, Loehr LR, Fornage M, Li G, Wilk JB, Tang W, Manichaikul A, Lahousse L, Harris TB, North KE, Rudnicka AR, Hui J, Gu X, Lumley T, Wright AF, Hastie ND, Campbell S, Kumar R, Pin I, Scott RA, Pietiläinen KH, Surakka I, Liu Y, Holliday EG, Schulz H, Heinrich J, Davies G, Vonk JM, Wojczynski M, Pouta A, Johansson A, Wild SH, Ingelsson E, Rivadeneira F, Völzke H, Hysi PG, Eiriksdottir G, Morrison AC, Rotter JI, Gao W, Postma DS, White WB, Rich SS, Hofman A, Aspelund T, Couper D, Smith LJ, Psaty BM, Lohman K, Burchard EG, Uitterlinden AG, Garcia M, Joubert BR, McArdle WL, Musk AB, Hansel N, Heckbert SR, Zgaga L, Van Meurs JB, Navarro P, Rudan I, Oh YM, Redline S, Jarvis DL, Zhao JH, Rantanen T, O'Connor GT, Ripatti S, Scott RJ, Karrasch S, Grallert H, Gaddis NC, Starr JM, Wijmenga C, Minster RL, Lederer DJ, Pekkanen J, Gyllensten U, Campbell H, Morris AP, Gläser S, Hammond CJ, Burkart KM, Beilby J, Kritchevsky SB, Gudnason V, Hancock DB, Williams OD, Polasek O, Zemunik T, Kolcic I, Petrini MF, Wjst M, Kim WJ, Porteous DJ, Scotland G, Smith BH, Viljanen A, Heliövaara M, Attia JR, Sayers I, Hampel R, Gieger C, Deary IJ, Boezen HM, Newman A, Jarvelin MR, Wilson JF, Lind L, Stricker BH, Teumer A, Spector TD, Melén E, Peters MJ, Lange LA, Barr RG, Bracke KR, Verhamme FM, Sung J, Hiemstra PS, Cassano PA, Sood A, Hayward C, Dupuis J, Hall IP, Brusselle GG, Tobin MD, London SJ. 2014. Genome-wide association analysis identifies six new loci associated with forced vital

capacity. Genome-wide association analysis identifies six new loci associated with forced vital capacity. *Nature Genetics* **46**:669–677 DOI 10.1038/ng.3011.

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. 2009. Finding the missing heritability of complex diseases. *Nature* **461(7265)**:747–753 DOI 10.1038/nature08494.

McClearn GE, Svartengren M, Pedersen NL, Heller DA, Plomin R. 1994. Genetic and environmental influences on pulmonary function in aging Swedish twins. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **49**:264–268.

Mehta AJ, Thun GA, Imboden M, Ferrarotti I, Keidel D, Künzli N, Kromhout H, Miedinger D, Phuleria H, Rochat T, Russi EW, Schindler C, Schwartz J, Vermeulen R, Luisetti M, Probst-Hensch N, SAPALDIA team. 2014. Interactions between SERPINA1 PiMZ genotype occupational exposure and lung function decline. *Occupational and Environmental Medicine* **71**:234–240 DOI 10.1136/oemed-2013-101592.

Palmer LJ, Knuiman MW, Divitini ML, Burton PR, James AL, Bartholomew HC, Ryan G, Musk AW. 2001. Familial aggregation and heritability of adult lung function: results from the Busselton Health Study. *European Respiratory Journal* **17**:696–702 DOI 10.1183/09031936.01.17406960.

Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. 2014. Measurement of FEF25–75% and FEF75% does not contribute to clinical decision making. *European Respiratory Journal* **43**:1051–1058 DOI 10.1183/09031936.00128113.

Redline S, Tishler PV, Rosner B, Lewitter FI, Vandenburgh M, Weiss ST, Speizer FE. 1989. Genotypic and phenotypic similarities in pulmonary function among family members of adult monozygotic and dizygotic twins. *American Journal of Epidemiology* **129**:827–836 DOI 10.1093/oxfordjournals.aje.a115197.

Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, Zhao JH, Ramasamy A, Zhai G, Vitart V, Huffman JE, Igl W, Albrecht E, Deloukas P, Henderson J, Granell R, McArdle WL, Rudnicka AR, Wellcome Trust Case Control Consortium, Barroso I, Loos RJ, Wareham NJ, Mustelin L, Rantanen T, Surakka I, Imboden M, Wichmann HE, Grkovic I, Jankovic S, Zgaga L, Hartikainen AL, Peltonen L, Gyllensten U, Johansson A, Zaboli G, Campbell H, Wild SH, Wilson JF, Glser S, Homuth G, Vlzke H, Mangino M, Soranzo N, Spector TD, Polasek O, Rudan I, Wright AF, Helivaara M, Ripatti S, Pouta A, Naluai AT, Olin AC, Torn K, Cooper MN, James AL, Palmer LJ, Hingorani AD, Wannamethee SG, Whincup PH, Smith GD, Ebrahim S, McKeever TM, Pavord ID, MacLeod AK, Morris AD, Porteous DJ, Cooper C, Dennison E, Shaheen S, Karrasch S, Schnabel E, Schulz H, Grallert H, Bouatia-Naji N, Delplanque J, Froguel P, Blakey JD, NSHD Respiratory Study Team, Britton JR, Morris RW, Holloway JW, Lawlor DA, Hui J, Nyberg F, Jarvelin MR, Jackson C, Kohnen M, Kaprio J, Probst-Hensch NM, Koch B, Hayward C, Evans DM, Elliott P, Strachan DP, Hall IP, Tobin MD. 2010. Genome-wide

association study identifies five loci associated with lung function. *Nature Genetics* **42**:36–44 DOI 10.1038/ng.501.

Rice MB, Ljungman PL, Wilker EH, Gold DR, Schwartz JD, Koutrakis P, Washko GR, O'Connor GT, Mittleman MA. 2013. Short-term exposure to air pollution and lung function in the Framingham Heart Study. *American Journal of Respiratory and Critical Care Medicine* **188**:1351–1357 DOI 10.1164/rccm.201308-1414OC.

Sigsgaard T, Brandslund I, Omland O, Hjort C, Lund ED, Pedersen OF, Miller MR. 2000. S and Z alpha1-antitrypsin alleles are risk factors for bronchial hyperresponsiveness in young farmers: an example of gene-environment interaction. *European Respiratory Journal* **16**:50–55 DOI 10.1034/j.1399-3003.2000.16a09.x.

Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, Zhai G, Zhao JH, Smith AV, Huffman JE, Albrecht E, Jackson CM, Evans DM, Cadby G, Fornage M, Manichaikul A, Lopez LM, Johnson T, Aldrich MC, Aspelund T, Barroso I, Campbell H, Cassano PA, Couper DJ, Eiriksdottir G, Franceschini N, Garcia M, Gieger C, Gislason GK, Grkovic I, Hammond CJ, Hancock DB, Harris TB, Ramasamy A, Heckbert SR, Heliövaara M, Homuth G, Hysi PG, James AL, Jankovic S, Joubert BR, Karrasch S, Klopp N, Koch B, Kritchevsky SB, Launer LJ, Liu Y, Loehr LR, Lohman K, Loos RJ, Lumley T, Balushi KAAl, Ang WQ, Barr RG, Beilby J, Blakey JD, Boban M, Boraska V, Brisman J, Britton JR, Brusselle GG, Cooper C, Curjuric I, Dahgam S, Deary IJ, Ebrahim S, Eijgelsheim M, Francks C, Gaysina D, Granell R, Gu X, Hankinson JL, Hardy R, Harris SE, Henderson J, Henry A, Hingorani AD, Hofman A, Holt PG, Hui J, Hunter ML, Imboden M, Jameson KA, Kerr SM, Kolcic I, Kronenberg F, Liu JZ, Marchini J, McKeever T, Morris AD, Olin AC, Porteous DJ, Postma DS, Rich SS, Ring SM, Rivadeneira F, Rochat T, Sayer AA, Sayers I, Sly PD, Smith GD, Sood A, Starr JM, Uitterlinden AG, Vonk JM, Wannamethee SG, Whincup PH, Wijmenga C, Williams OD, Wong A, Mangino M, Marciante KD, McArdle WL, Meibohm B, Morrison AC, North KE, Omenaas E, Palmer LJ, Pietiläinen KH, Pin I, Pola Sbreve Ek O, Pouta A, Psaty BM, Hartikainen AL, Rantanen T, Ripatti S, Rotter JI, Rudan I, Rudnicka AR, Schulz H, Shin SY, Spector TD, Surakka I, Vitart V, Völzke H, Wareham NJ, Warrington NM, Wichmann HE, Wild SH, Wilk JB, Wjst M, Wright AF, Zgaga L, Zemunik T, Pennell CE, Nyberg F, Kuh D, Holloway JW, Boezen HM, Lawlor DA, Morris RW, Probst-Hensch N, International Lung Cancer Consortium, GIANT consortium, Kaprio J, Wilson JF, Hayward C, Kähönen M, Heinrich J, Musk AW, Jarvis DL, Gläser S, Järvelin MR, Stricker BHCh, Elliott P, O'Connor GT, Strachan DP, London SJ, Hall IP, Gudnason V, Tobin MD. 2011. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nature Genetics* **43**:1082–1090 DOI 10.1038/ng.941.

Soler Artigas M, Wain LV, Miller S, Kheirallah AK, Huffman JE, Ntalla I, Shrine N, Obeidat M, Trochet H, McArdle WL, Alves AC, Hui J, Zhao JH, Joshi PK, Teumer A, Albrecht E, Imboden M, Rawal R, Lopez LM, Marten J, Enroth S, Surakka I, Polasek O, Lyytikäinen LP, Granell R, Hysi PG, Flexeder C, Mahajan A, Beilby J, Bossé Y, Brandsma CA, Campbell H, Gieger C, Gläser S, González JR, Grallert H,

Hammond CJ, Harris SE, Hartikainen AL, Heliövaara M, Henderson J, Hocking L, Horikoshi M, Hutri-Kähönen N, Ingelsson E, Johansson , Kemp JP, Kolcic I, Kumar A, Lind L, Melén E, Musk AW, Navarro P, Nickle DC, Padmanabhan S, Raitakari OT, Ried JS, Ripatti S, Schulz H, Scott RA, Sin DD, Starr JM, UK BiLEVE, Viñuela A, Völzke H, Wild SH, Wright AF, Zemunik T, Jarvis DL, Spector TD, Evans DM, Lehtimäki T, Vitart V, Kähönen M, Gyllensten U, Rudan I, Deary IJ, Karrasch S, Probst-Hensch NM, Heinrich J, Stubbe B, Wilson JF, Wareham NJ, James AL, Morris AP, Jarvelin MR, Hayward C, Sayers I, Strachan DP, Hall IP, Tobin MD. 2015. Sixteen new lung function signals identified through 1000 Genomes Project reference panel imputation. *Nature Communications* **6**:8658 DOI 10.1038/ncomms9658.

Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino Sr RB, Fox CS, Larson MG, Murabito JM, O'Donnell CJ, Vasan RS, Wolf PA, Levy D. 2007. The third generation cohort of the national heart, lung, and blood institute's framingham heart study: design, recruitment, and initial examination. *American Journal of Epidemiology* **165**:1328–1335 DOI 10.1093/aje/kwm021.

Tarnoki DL, Tarnoki AD, Lazar Z, Medda E, Littvay L, Cotichini R, Fagnani C, Stazi MA, Nisticó L, Lucatelli P, Boatta E, Zini C, Fanelli F, Baracchini C, Meneghetti G, Jermendy G, Préda I, Kiss RG, Karlinger K, Lannert A, Schillaci G, Molnar AA, Garami Z, Berczi V, Horvath I. 2013. Genetic and environmental factors on the relation of lung function and arterial stiffness. *Respiratory Medicine* **107**:927–935 DOI 10.1016/j.rmed.2013.02.002.

Tashkin DP, Altose MD, Connett JE, Kanner RE, Lee WW, Wise RA. 1996. Methacholine reactivity predicts changes in lung function over time in smokers with early chronic obstructive pulmonary disease. The Lung Health Study Research Group. *American Journal of Respiratory and Critical Care Medicine* **153**(**6 Pt 1**):1802–1811 DOI 10.1164/ajrccm.153.6.8665038.

Tian X, Xu C, Wu Y, Sun J, Duan H, Zhang D, Jiang B, Pang Z, Li S, Tan Q. 2017. Genetic and environmental influences on pulmonary function and muscle strength: the chinese twin study of aging. *Twin Research and Human Genetics* **20**:53–59 DOI 10.1017/thg.2016.97.

Vestbo J, Prescott E, Lange P. 1996. The Copenhagen City heart study group. Association of chronic mucus hypersecretion with FEV1 decline and COPD morbidity. *American Journal of Respiratory and Critical Care Medicine* **153**:1530–1535 DOI 10.1164/ajrccm.153.5.8630597.

Walter R, Gottlieb DJ, O'Connor GT. 2000. Environmental and genetic risk factors and gene-environment interactions in the pathogenesis of chronic obstructive lung disease. *Environmental Health Perspectives* **108**:733–742.

Wilk JB, Djousse L, Arnett DK, Rich SS, Province MA, Hunt SC, Crapo RO, Higgins M, Myers RH. 2000. Evidence for major genes influencing pulmonary function in the NHLBI family heart study. *Genetic Epidemiology* **19**:81–94 DOI 10.1002/1098-2272(200007)19:1<81::AID-GEPI6>3.0.CO;2-8.

Williams PT. 2012. Quantile-specific penetrance of genes affecting lipoproteins, adiposity and height. *PLOS ONE* **7(1)**:e28764 DOI 10.1371/journal.pone.0028764.

Williams PT. 2020a. Gene-environment interactions due to quantile-specific heritability of triglyceride and VLDL concentrations. *Scientic Reports* **10**:4486 DOI 10.1038/s41598-020-60965-9.

Williams PT. 2020b. Quantile-dependent expressivity of postprandial lipemia. *PLOS ONE* **15**:e0229495 DOI 10.1371/journal.pone.0229495.

Williams PT. 2020c. Quantile-specific heritability may account for gene-environment interactions involving coffee consumption. *Behavior Genetics* **50**:119–126 DOI 10.1007/s10519-019-09989-0.

Winer BJ, Brown DR, Michels KM. 1991. *Statistical principles in experimental design.* Third edition. New York: McGraw-Hill New York.

Wood AM, Tan SL, Stockley RA. 2009. Chronic obstructive pulmonary disease: towards pharmacogenetics. *Genome Medicine* **1(11)**:112 DOI 10.1186/gm112.

Yamada H, Yatagai Y, Masuko H, Sakamoto T, Iijima H, Naito T, Noguchi E, Hirota T, Tamari M, Hizawa N. 2015. Heritability of pulmonary function estimated from genome-wide SNPs in healthy Japanese adults. *Respiratory Investigation* **53**:60–67 DOI 10.1016/j.resinv.2014.10.004.

Zhai G, Valdes AM, Cherkas L, Clement G, Strachan D, Spector TD. 2007. The interaction of genes and smoking on forced expiratory volume: a classic twin study. *Chest* **132**:1772–1777 DOI 10.1378/chest.07-1438.