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Genetic Influences on Pulmonary Function: A Large Sample Twin Study

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Abstract Heritability of forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and peak expiratory flow (PEF) has not been previously addressed in large twin studies. We evaluated the genetic contribution to individual differences observed in FEV_1, FVC, and PEF using data from the largest population-based twin study on spirometry. Specially trained lay interviewers with previous experience in spirometric measurements tested 4,314 Danish twins (individuals), 46–68 years of age, in their homes using a hand-held spirometer, and their flow-volume curves were evaluated. Modern variance component sex-limitation models were applied to evaluate possible genetic differences between the sexes for FEV_1, FVC, and PEF. Estimates were adjusted for age, height, and smoking. For FEV_1, additive genetic effects of 61% (95% CI 56–65) were observed. For FVC, the additive genetic contribution was 26% (3–49%) and the dominant genetic contribution was 29% (4–54%). For PEF, our models showed an additive genetic contribution of 43% (31–52%) for men, but genetic influences were not significant in women. We found no significant differences between dizygotic same-sex twins and dizygotic opposite-sex twins for FEV_1, FVC, and PEF, suggesting absence of qualitative genetic differences between the sexes. Sex-difference heritability for PEF suggested possible quantitative genetic differences between the sexes for this index. Genetic effects contributed significantly to individual differences observed in FEV_1, FVC, and PEF. Qualitative sex differences were absent for all spirometric measures, while quantitative sex differences were observed only for PEF, with heritability being substantial in men but negligible in women.

Keywords Twins · FEV_1 · FVC · PEF · Genetics · Sex differences

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Introduction

A person's expiratory capacity is an important indicator of respiratory health [1]. Knowledge of the factors underlying expiratory capacity is important for the understanding of the pathophysiology and prognosis of respiratory diseases such as asthma and chronic obstructive pulmonary disease. Twin studies can provide important clues about the proportional contribution of genetic and environmental factors to the variation in lung function. Twin studies on asthma have shown that relative to the general population, the risk of asthma in a co-twin of an affected monozygotic twin is increased five to ten times compared with two to four times in a co-twin of an affected dizygotic twin [2]. Asthma has been shown to be genetically related to airway

hyperresponsiveness, and asthma and rhinitis have been shown to be genetically similar but environmentally distinct [3].

Only a few twin studies have examined clinically relevant lung function indices like forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) [4], and these studies were generally based on small sample sizes [5–11]. A large sample size of twins substantially increases the reliability of the genetic estimates. A very large sample size also provides the possibility of studying quantitative and qualitative genetic differences between the sexes in addition to estimating the heritability. The previous small-sample twin studies on the heritability of pulmonary function have lacked the power to adjust for important covariates, and so a very large twin study, such as we present here, constitutes an important advantage to the estimation of reliable genetic contributions to spirometric indices.

Methods

Population

From the Danish twin cohorts [12] born between 1931 and 1952 (Middle Aged Danish Twins—MADT), a total of 5,280 twins were selected for a questionnaire study in 1998 that also included measurement of lung function [13]. We randomly chose 40 monozygotic (MZ), 40 dizygotic same-sex (DZ), and 40 dizygotic opposite-sex (DOS) twin pairs from each birth-year cohort, equally divided between males and females. This resulted in 120 male and 120 female individuals in each of the 22 birth-year cohorts. Of the 5,280 twins selected for the study, 4,314 chose to participate in the investigation (81.7% response rate). All included twins were white Caucasians and nearly all had Danish genetic background, i.e., all twins were born in Denmark with very little ethnic admixture. Participation was not restricted by any kind of exclusion criteria. Zygosity was determined using four standard questions of similarity and mistaken identity [14].

The spirometric measurements were performed by 105 lay interviewers who all had previous experience in recording spirometry in another study. They all underwent a specially designed training program for the present study. All spirometric measurements were performed in the twin's homes using a Micro DL system (MicroMedical Ltd, Chatham, UK). The Micro DL system is a handheld system that allows spirometric values (FEV_1, FVC, and PEF) to be saved along with respective flow–volume curves. The system saved the flow–volume curve corresponding to the highest measured value for each subject (highest being the value with the highest percentage of the predicted values with respect to age- and height-regressed predicted values in the Micro DL system). Each twin was asked to perform at least three expiratory maneuvers, but in some cases only one or two satisfactory values were obtained. For PEF, 3,527 twins had three successful readings, 193 had two, 211 had just one, and 383 did not achieve any PEF readings. For FEV_1, 3,502 twins had three readings, 207 had two, 211 had only one, and 394 did not have any FEV_1 successful recordings. For FVC, 3,500 had three readings, 205 had two, 179 had only one, and 430 did not register any FVC values.

Inspection Criteria

To assess the quality of the spirometric measurements, we composed a set of selection criteria which are shown in Table 1. They were used to determine which flow–volume curves must be inspected. Those selected for inspection were reviewed by a respiratory specialist who decided from the appearance of the flow–volume curves which curves were acceptable and which should be rejected. Reasons for rejection included evidence of coughing affecting FEV_1 or FVC, inhaling in the expiratory phase, lack of forced expiration giving a slow rise time or premature termination, or too short an exhalation period. Rejected blows were recorded as missing data. From all the acceptable data for each subject, the maximum values for FEV_1, FVC, and PEF were used in the subsequent analysis.

From the 4,314 individual twins who made spirometric measurements, 4,131 had at least one FEV_1 value from a satisfactory blow to give a maximum value for FEV_1, and of these, 211 had missing FEV_1 values, leaving acceptable FEV_1 spirometric data for 3,920 individual twins. Of these 3,920 twins, there were 1,562 complete twin pairs with valid FEV_1 values and complete data on covariates available. In the same manner, we identified 1,531 complete twin pairs with data on FVC and 1,570 complete twin pairs with data on PEF.

Statistical Analysis

For the descriptive procedures and the ANOVA analysis, we used SPSS ver. 16.0 (SPSS, Inc., Chicago, IL, USA) [15]. The selection process used scripts written in Microsoft Access 2003 (Microsoft Corp., Redmond, WA, USA) that were applied to the spirometry results which were stored in an Access database.

To estimate the genetic proportion of phenotypic variation in the three spirometric measures, i.e., the heritability analyses, variance components models were fit to the data. These models were designed to estimate how much of the observed variance was due to genetic effects and how much was due to environmental effects. Initial correlation

Table 1 Selection criteria

Criteria to select flow–volume curves for visual inspection[a]

1. All curves with observed FEV_1 values larger than 6 L and values lower than 0.5 L
2. All curves with observed FVC values larger than 8 L and values lower than 0.5 L
3. All curves with observed PEF values lower than 100 L/min and values higher than 800 L/min
4. All curves for twins with only one observed blow
5. All curves for twins with two or three observed blows and a difference larger than 25% between largest and second largest value
6. All curves with a calculated $maxFEV_1$/maxFVC ratio larger than 0.98
7. All curves with a calculated $maxFEV_1$/maxFVC ratio lower than 0.40

Further selection criteria

1. If no curves were available for inspection and one of the criteria for visual inspection was fulfilled, the values were converted to missing.
2. FEV_1, FVC, and PEF were evaluated separately for each twin so that if, for example, PEF values were approved after evaluation of the flow-volume curves, FEV_1 and FVC could be converted to missing values as a result of the evaluation and vice versa.
3. For calculations of correlations and variance components, the max value of the observed data for each twin was chosen, providing us with $maxFEV_1$, maxFVC, and maxPEF.

[a] Visual inspection of the flow–volume curves was done by the first author and a chief respiratory physician together

analyses provide an indication of how genetics could be involved by applying Falconer's rule: $H^2 = 2*[r(\mathrm{MZ}) - r(\mathrm{DZ})]$, i.e., heritability is approximately twice the difference between the monozygotic and dizygotic correlations. Genetic dominance effects were involved if the initial correlation analyses showed that monozygotic twin correlations were more than twice as high as the dizygotic twin correlations. In this study, variance components maximum likelihood models for estimating genetic and environmental effects were fitted to the observed data using the statistical package Mx ver. 1.66b [16], which has been used in family studies since the 1990s because it flexibly accommodates twin and family data structures.

Following standard practice, the total phenotypic variance of our spirometric measures in our variance components models was partitioned into four sources of genetic and environmental variance: additive genetic effects (A), dominant genetic effects (D), shared environmental effects (C), and unique (nonshared) environmental effects (E). Additive genetic effects assume that the effects of alleles at a locus can be described with a linear model, while dominant genetic effects are defined as the deviations from this linear model. Environmental influences are partitioned into shared environmental effects (C), i.e., those environmental effects that create similarities between members of the same family, and nonshared environmental variance (E), i.e., environmental effects that result in differences between members of the same family. The E term also includes any measurement error and can therefore never be zero [17].

In variance components models, the covariance, i.e. similarity, between MZ twins and the covariance between DZ twins are functions of A, D, and C. The covariance between the MZ twins, the covariance between the DZ twins, and the total phenotypic variance are estimated, but the models include a total of four parameters (A, C, D, and E), with D and C having opposite effects on the DZ correlation, i.e., D decreases this correlation while C increases it. This means that D and C cannot be modeled simultaneously unless information of additional family members (e.g., parents or sibs of the twins) is available [18]. If such additional information is not available, one can either fit an ACE model or an ADE model. If the MZ correlations are higher than two times the DZ correlations, dominant effects are assumed to be present and an ADE model should be fitted to the data. If the ratio of these correlations is less than two, then dominance effects are assumed to be absent and an ACE model is applied [17]. The significance of the contribution of the individual variance components to the total trait variance is tested with a likelihood ratio test which compares the fit of the full model, including all variance components (i.e., ACE or ADE), with the fit of the successively fitted submodels in which one or more variance components are fixed to zero. The difference in fit between the full model and a submodel is indicative of the significance of the variance component that is fixed to zero in the submodel. For example, comparing an AE model with an ADE model evaluates the significance of keeping the D parameter in the model. It is important to note that DE models, in which additive genetic effects are absent while a dominance effect is assumed to be present, are not considered biologically plausible and are thus not fitted, while CE models can be fitted [19].

If the correlation analyses show significant differences between monozygotic males and monozygotic females, one should estimate variance components separately for men and women. If the correlations between monozygotic and dizygotic male twins are significantly different from the

correlations between monozygotic and dizygotic female twins, quantitative genetic differences between the sexes are implied, i.e., the genes affecting the trait are the same in males and females but the effects of the genes differ between the sexes [17]. If the dizygotic opposite-sex twin (DOS twins) correlations are significantly lower than the dizygotic same-sex twin correlations, qualitative genetic differences between the sexes are implied, i.e., different genes are expressed in males and females [17].

Possible differences between the sexes were investigated using a sex-limitation model, where the means and the variance decomposition were allowed to depend on sex. Such models can study whether the genetic contribution is similar in males and females [16]. Within these sex-limitation models, parameters were adjusted for age, height, and smoking. Age was treated as a categorical variable (five equally sized groups to approximate the assumption of a normal distribution of the covariates in the mathematical models underlying the Mx software), height as a continuous variable (in meters), and smoking as a categorical variable (yes/no according to the questionnaires). The same covariates were included in the correlation analyses.

We did a sensitivity analysis among twin pairs concordant for smoking status, defining nonsmoking concordant pairs as complete twin pairs who had all smoked less than five pack-years, and defining smoking concordant pairs as complete twin pairs who had all smoked more than five pack-years. Following this definition, we distinguished 795 twin pairs concordant for nonsmoking, and 699 twin pairs concordant for smoking, and 936 twin pairs discordant for smoking-status.

Results

Table 2 gives average pulmonary function measures and smoking status, while Table 3 gives the pulmonary function measures for each expiratory maneuver, stratified according to zygosity. There were no significant differences between monozygotic and dizygotic twins with respect to the mean values for all variables ($p > 0.10$). Examiner effects were not significant for FEV_1 ($p = 0.14$) and FVC ($p = 0.56$), but a small significant difference was observed for PEF ($p = 0.03$) (SPSS v16.0, one-way ANOVA).

Table 2 Mean (range) spirometric data and demographics for the participating Middle Aged Danish Twins (MADT)

Zygosity	*N*	Age[a]	PEF[b]	FEV_1	FVC	Height[c]	Smoking[d]		
							Current (%)	Ex (%)	Never (%)
MZ									
Males	744	57.0 (50.6–63.6)	482 (358–606)	3.27 (2.59–3.95)	4.07 (3.29–4.86)	176 (170–183)	29.2	29.6	41.2
Females	715	56.9 (50.5–63.6)	325 (241–409)	2.35 (1.84–2.87)	2.93 (2.34–3.53)	164 (158–170)	44.9	15.8	39.3
DZ									
Males	726	56.8 (50.4–63.3)	487 (361–613)	3.31 (2.58–4.05)	4.14 (3.31–4.98)	177 (170–183)	26.3	33.6	40.1
Females	681	56.7 (50.4–63.0)	333 (249–417)	2.41 (1.89–2.93)	3.01 (2.36–3.66)	165 (159–171)	41.9	18.3	39.8
DOS									
Males	728	57.0 (50.8–63.3)	480 (352–607)	3.25 (2.49–4.00)	4.07 (3.18–4.95)	177 (170–184)	25.5	32.2	42.4
Females	720	56.9 (50.6–63.2)	330 (239–421)	2.41 (1.87–2.95)	3.03 (2.42–3.63)	165 (159–171)	41.6	22.5	35.8
Males	2,198	56.9 (50.6–63.2)	483 (357–609)	3.28 (2.55–4.00)	4.09 (3.26–4.93)	176 (170–183)			
Current smokers	880	56.9 (50.5–63.3)	452 (326–578)	3.10 (2.38–3.82)	3.99 (3.15–4.83)	176 (170–183)			
Ex-smokers	678	57.4 (50.9–64.0)	497 (369–626)	3.31 (2.57–4.04)	4.10 (3.25–4.95)	176 (169–183)			
Never smokers	576	56.1 (50.2–62.0)	518 (408–627)	3.52 (2.89–4.16)	4.26 (3.47–5.05)	176 (170–183)			
Females	2,116	56.8 (50.5–63.1)	329 (243–416)	2.39 (1.86–2.92)	2.99 (2.37–3.61)	165 (159–171)			
Current smokers	792	56.7 (50.4–63.0)	308 (219–397)	2.24 (1.71–2.77)	2.88 (2.26–3.51)	165 (158–171)			
Ex-smokers	391	57.8 (51.2–64.3)	340 (254–426)	2.38 (1.83–2.94)	2.97 (2.36–3.59)	165 (159–171)			
Never smokers	886	56.6 (50.4–62.8)	343 (264–422)	2.52 (2.05–2.99)	3.08 (2.49–3.67)	164 (159–170)			
Total	4,314	56.9 (50.6–63.2)	407 (275–540)	2.84 (2.07–3.62)	3.55 (2.63–4.47)	171 (162–179)	34.8	25.4	39.8

MZ monozygotic twins, *DZ* same-sex dizygotic twins, *DOS* opposite sex dizygotic twins

[a] Age is the mean age in years

[b] PEF, FEV_1, and FVC are calculated from the max value of the observed 1, 2, or 3 blows with PEF in L/min, FEV_1 and FVC in L

[c] Height is the mean height in cm

[d] Smoking: shows distribution in percent of current smokers, ex-smokers, and never smokers in the different zygosity groups

Table 3 Measurement description for the Middle Aged Danish Twins (MADT)

	N	Range	mean ± SD
MZ			
PEF			
1. Blow	1,291	55–840	365 ± 133
2. Blow	1,277	57–867	384 ± 133
3. Blow	1,244	64–930	387 ± 135
MaxPEF	1,342	64–930	404 ± 132
FEV1			
1. Blow	1,315	0.46–4.91	2.70 ± 0.76
2. Blow	1,266	0.49–5.16	2.74 ± 0.76
3. Blow	1,222	0.49–5.18	2.76 ± 0.77
MaxFEV1	1,341	0.51–5.18	2.81 ± 0.76
FVC			
1. Blow	1,311	0.70–6.43	3.35 ± 0.91
2. Blow	1,261	0.73–6.72	3.39 ± 0.90
3. Blow	1,220	0.73–6.35	3.41 ± 0.91
MaxFVC	1,329	0.80–6.72	3.50 ± 0.90
DZ			
PEF			
1. Blow	1,229	58–761	372 ± 131
2. Blow	1,189	76–772	392 ± 133
3. Blow	1,157	33–796	395 ± 135
MaxPEF	1,265	76–796	413 ± 132
FEV1			
1. Blow	1,232	0.42–5.08	2.76 ± 0.79
2. Blow	1,186	0.50–5.07	2.80 ± 0.78
3. Blow	1,145	0.46–5.15	2.80 ± 0.79
MaxFEV$_1$	1,261	0.50–5.15	2.88 ± 0.78
FVC			
1. Blow	1,225	0.74–6.37	3.45 ± 0.95
2. Blow	1,180	0.81–6.68	3.47 ± 0.93
3. Blow	1,145	0.47–6.92	3.47 ± 0.94
MaxFVC	1,250	0.83–6.92	3.60 ± 0.94
DOS			
PEF			
1. Blow	1,287	48–792	364 ± 132
2. Blow	1,268	65–817	384 ± 134
3. Blow	1,236	66–844	386 ± 137
MaxPEF	1,324	66–844	406 ± 134
FEV$_1$			
1. Blow	1,290	0.40–5.29	2.71 ± 0.79
2. Blow	1,256	0.43–5.28	2.75 ± 0.78
3. Blow	1,218	0.46–5.14	2.77 ± 0.78
MaxFEV$_1$	1,318	0.46–5.29	2.83 ± 0.78
FVC			
1. Blow	1,289	0.52–6.92	3.39 ± 0.93
2. blow	1,244	0.47–6.71	3.41 ± 0.92
3. Blow	1,214	0.51–6.63	3.45 ± 0.92
MaxFVC	1,305	0.52–6.92	3.55 ± 0.92

N number of twins, *PEF* values measured in L/min, *FEV$_1$* and *FVC* measured in L. Values are the range and mean ± SD for spirometric measurements of PEF, FEV$_1$, and FVC among monozygotic twins (MZ), dizygotic same-sex twins (DZ), and dizygotic opposite-sex twins (DOS)

Table 4 Twin correlation coefficients adjusted for age, sex, and smoking

	FEV$_1$ *r* (95% CI)	FVC *r* (95% CI)	PEF *r* (95% CI)
Men			
MZ	0.60 (0.50–0.67)	0.52 (0.42–0.60)	0.49 (0.40–0.57)
DZ	0.22 (0.11–0.33)	0.20 (0.09–0.32)	0.18 (0.06–0.29)
Women			
MZ	0.63 (0.55–0.69)	0.58 (0.50–0.65)	0.29 (0.17–0.40)
DZ	0.26 (0.14–0.36)	0.18 (0.07–0.28)	0.16 (0.03–0.28)
DOS	0.32 (0.24–0.39)	0.22 (0.13–0.29)	0.11 (0.03–0.19)

MZ monozygotic twins, *DZ* same-sex dizygotic twins, *DOS* opposite-sex dizygotic twins

Table 4 presents the estimated twin correlations. The male monozygotic correlations were significantly higher than the male dizygotic correlations for FEV$_1$, FVC, and PEF ($p < 0.001$). The female monozygotic correlations were significantly higher than the female dizygotic correlations for FEV$_1$ and FVC ($p < 0.001$), but the difference was not significant for PEF ($p > 0.10$). These results indicate a highly significant genetic contribution to all variables except for the female PEF observations.

The monozygotic male and monozygotic female correlations for FEV$_1$ and FVC were not significantly different ($p > 0.10$), but the difference was significant for PEF ($p < 0.01$). There were no significant differences between the dizygotic male and the dizygotic female correlations for any of the observed variables ($p > 0.10$). These results imply the presence of quantitative genetic differences between males and females only for PEF, not for FEV$_1$ or FVC.

For all of the spirometric indices, the dizygotic same-sex and the dizygotic opposite-sex twin correlations were similar ($p > 0.10$), suggesting the absence of qualitative genetic differences between males and females.

Table 5 presents the results of the variance components analyses. For FEV$_1$, the preferred model (most parsimonious model) was an AE model estimating an additive genetic contribution of 61% (95% CI 56–65) and a unique environmental contribution of 39% (35–44). For FVC, the

Table 5 Variance components estimates for the additive (A), dominant (D), common environmental (C), and unique environmental (E) genetic effects

Variance component	A[a]	C	D	E	χ^2 dif[b]	Df[c]
FEV_1						
ADE[d]	50 (27–65)	–	12 (0–35)	39 (34–44)		
AE	**61 (56–65)**	**–**	**–**	**39 (35–44)**	**1**	**1**
E	–	–	–	100	315	2
FVC						
ADE[d]	**26 (3–49)**	**–**	**29 (4–54)**	**45 (40–51)**		
AE	52 (46–57)	–	–	48 (43–54)	5	1
E	–	–		100	206	2
PEF						
ACEm[e]	**43 (31–52)**	**3 (0–10)**	**–**	**54 (46–62)**		
ACEf	0 (0–0)	25 (21–29)	–	75 (71–79)		
AEm	47 (40–55)	–	–	53 (46–61)	5	1
AEf	0 (0–0)	–	–	100	113	1
CEm	–	22 (18–27)	–	78 (73–82)	33	1
CEf[e]	**–**	**25 (21–29)**	**–**	**75 (71–79)**	**0**	**1**
Em	–	–	–	100	113	2
Ef	–	–	–	100	113	2

[a] A, C, D, and E = variance component estimates in percent with (95% CI). *p* values are provided in the Results section

[b] χ^2 dif is difference in χ^2 value of submodels (AE, CE, or E) compared to full model (ADE or ACE)

[c] Df = difference in degrees of freedom of submodel compared to full model

[d] ADE model was chosen because the correlation analyses showed that monozygotic correlations were significantly more than two times higher than dizygotic correlations (see Table 4)

[e] m = male and f = female. PEF variance components were estimated for males and females separately since the correlations showed significant differences between monozygotic males and monozygotic females for PEF (see Table 4)

The bold highlighted models are the best model fits to the data. If a submodel was not a significantly worse fit, than the full model, the submodel was the preferred model (most parsimonious)

ADE model was the preferred model, with an additive genetic contribution of 26% (3–49), a dominant genetic contribution of 29% (4–54), and a unique environmental contribution of 45% (40–51). For PEF, the best model fit for the females was a CE model that estimated a common environmental contribution of 25% (21–29) and a unique environmental contribution of 75% (71–79). For PEF in the males, the ACE model showed the best fit, with an additive genetic contribution of 43% (31–52), a common environmental contribution of 3% (0–10), and a unique environmental contribution of 54% (46–62).

A sensitivity analysis was conducted among twins concordant for smoking status to determine whether the heritability of the spirometric measures differed across smoking status. For FEV_1, this analysis showed a heritability of 66% (43–71) among twins concordant for smoking more than 5 pack-years and a heritability of 63% (54–69) among twins concordant for nonsmoking (<5 pack-year smoking exposure). For FVC, the results were 55% (46–63) for the smoking-concordant pairs and 58% (48–66) among twins concordant for nonsmoking. For PEF, we found an additive genetic contribution of 43% (23–59) among males concordant for smoking and a heritability of 44% (28–58) among males concordant for nonsmoking, while female genetic effects were not significant.

Discussion

We have shown that in the largest twin study ever that looked at the heritability of lung function, there is strong evidence of genetic influences on spirometric indices in middle-aged twins. Our analysis showed a heritability of 61% for FEV_1, 55% for FVC, and, for males, PEF showed a heritability of 43%. The results are in line with findings from previous genetic linkage data on the heritability of pulmonary function [20]. A family study has shown a strong correlation between level of pulmonary function and biological relation, indicating that 35% and 49% of the residual variance in level of pulmonary function for FEV_1 and FVC, respectively, may be explained by genetic factors [21]. Our twin study shows even higher genetic contributions to the observed variance. As the authors of the family study mention, many genes that influence lung function

might not have been detected due to lack of power in their study, so genes with a large contribution to the individual differences in pulmonary measures might not have been detected. Therefore, our study supports the further investigation of genetic mechanisms with respect to pulmonary function.

A major strength of our study is that our genetic analyses for FEV_1, FVC, and PEF were based on more than 1,500 twin pairs, with complete data on covariates, which means that our findings are reliable estimates of the genetic epidemiology of spirometric indices. Furthermore, the large size of our study sample made it possible to study sex differences with respect to the genetic effects. Previous twin studies on the heritability of spirometric indices [4] were based on much smaller sample sizes resulting in less reliable estimates of the genetic contribution and they lacked power to adjust for important covariates. The pioneer study by Redline [5] in 1987 assessed genetic influences from a total of 256 monozygotic and 158 dizygotic twins (individuals) corresponding to 128 monozygotic and 79 dizygotic pairs. This sample size is hardly sufficient for the modern variance component models we have applied. Results from a relatively small-sized twin study tried to address the question of sex differences in heritability of FEV_1 and FVC by using variance components analysis and found sex differences in the genetic contribution to FEV_1, with heritability being nonsignificant for males [22]. That study, however, consisted of only 176 complete twin pairs compared to the more than 1,500 complete pairs in our study, and the selection criteria of that study were based on the presence of respiratory symptoms [22] which differed from our study. Our study was free from ascertainment bias since the selection of twins was random.

However, one limitation of our study is that the spirometric measurements were not performed by professional lung function technicians but by lay interviewers; this may have introduced measurement errors. Although the interviewers went through a thorough training program and had previous experience with spirometry, the results might have been more reliable if they were recorded in a clinical setting by lung function technicians. Any measurement error would be included in the E parameter of our analysis, resulting in an overestimation of the E parameter with a concomitant underestimation of the genetic or common environmental contribution. Despite this possible limitation, we found highly significant heritability estimates and common environmental estimates which might be somewhat underestimated.

There were no significant differences between the dizygotic same-sex twin correlations and the dizygotic opposite-sex twin correlations for FEV_1, FVC, or PEF, indicating absence of qualitative sex differences, i.e., the genetic and environmental factors responsible for individual differences in spirometric measures were the same in men and women.

We are cautious about concluding that the difference between male and female with respect to the genetic decomposition of PEF values actually reflects a difference in expression of the same genes. PEF is not as reliable a lung function index as are FEV_1 and FVC and is very effort dependent. It is possible the sex difference with respect to PEF could reflect measurement uncertainty of PEF between the sexes: for PEF the ANOVA analysis showed a small but significant effect related to the test operator. If monozygotic and dizygotic twins had responded differently to the instructions of the test operators, then the heritability estimates in this study would be inaccurate; however, we have no reason to believe that such a differential response should be likely.

A study such as ours might be affected by interactions. Two recent studies report evidence of gene–environment interaction in the context of respiratory measures. One large twin study reported interaction between the genetic contribution to FEV_1 and smoking [23], and another study indicated interaction between smoking and chronic bronchitis [24]. These studies are mutually supportive since chronic mucus hypersecretion has been shown to be an indicator of decline in FEV_1 [25]. These interactions suggest that genes can influence the extent to which environmental factors such as smoking may cause individual differences in FEV_1, or vice versa; e.g., environmental factors may determine the extent to which genes are expressed. Another indication of the influence of smoking is a recent genome-wide association study of COPD, a disease characterized by impaired lung function and decreased values of FEV_1. This study identified SNPs at the nicotinic acetylcholine receptor locus that were related to lung function in a case-control study and two large replication cohort studies [26]. In our present study, a sensitivity analysis among twins concordant for smoking and nonsmoking indicated that the heritability of spirometric measures was not dependent on smoking status.

Normative equations of lung function are widely used in lung function laboratories to help determine if an individual's lung function is within normal limits. These equations are applied based on self-reported race or ethnic group and this may introduce some misclassification. As described previously, twin studies provide information about how much of the observed variation in spirometric measures is caused by differences between subjects on a genetic level. The impact of twin estimates in large studies with sufficient power, such as we present here, was demonstrated in a study that found that when ancestry was incorporated into predictive regression analyses of lung function, it was possible to categorize disease severity more accurately in asthma [27]. It is likely that this finding will be just as

relevant for spirometric verification of COPD. These authors also pointed out that by not including ancestry in lung function predictive equations, there will be inaccuracy not only in assessing individuals but also in assessing population-specific disease prevalence and severity. Our twin study has shown that the genetic contributions to differences in pulmonary functions measures are large and this supports the view of increased focus on ancestry in predictive regression analyses of lung function.

In conclusion, we have presented the results from the largest ever twin study that investigated the genetic contribution to individual differences in spirometric indices. The results showed that genetic contributions were highly significant for FEV_1 and FVC in both sexes and for PEF in men. Qualitative sex differences were absent for all studied spirometric measures and quantitative sex differences were observed only for PEF, with the genetic contribution being considerable in men but negligible in women.

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References

1. Celli BR (2000) The importance of spirometry in COPD and asthma: effect on approach to management. Chest 117:15–19
2. Los H, Postmus PE, Boomsma DI (2001) Asthma genetics and intermediate phenotypes: a review from twin studies. Twin Res 4:81–93
3. Thomsen SF, Ulrik CS, Kyvik KO et al (2006) Multivariate genetic analysis of atopy phenotypes in a selected sample of twins. Clin Exp Allergy 36:1382–1390
4. Chen Y (1999) Genetic epidemiology of pulmonary function. Thorax 54:818–824
5. Redline S, Tishler PV, Lewitter FI et al (1987) Assessment of genetic and nongenetic influences on pulmonary function. A twin study. Am Rev Respir Dis 135:217–222
6. Redline S, Tishler PV, Rosner B et al (1989) Genotypic and phenotypic similarities in pulmonary function among family members of adult monozygotic and dizygotic twins. Am J Epidemiol 129:827–836
7. McClearn GE, Svartengren M, Pedersen NL et al (1994) Genetic and environmental influences on pulmonary function in aging Swedish twins. J Gerontol 49:264–268
8. Hubert HB, Fabsitz RR, Feinleib M et al (1982) Genetic and environmental influences on pulmonary function in adult twins. Am Rev Respir Dis 125:409–415
9. Man SFP, Zamel N (1976) Genetic influence on normal variability of maximum expiratory flow–volume curves. J Appl Physiol 41:874–877
10. Ghio AJ, Crapo RO, Elliott CG (1989) Heritability estimates of pulmonary function. Chest 96:743–746
11. Whitfield KE, Grant J, Ravich-Scherbo I et al (1999) Genetic and environmental influences on forced expiratory volume in midlife: a cross-cultural replication. Exp Aging Res 25:255–265
12. Skytthe A, Kyvik K, Bathum L et al (2006) The Danish Twin Registry in the new millennium. Twin Res Hum Genet 9:763–771
13. Gaist D, Bathum L, Skytthe A et al (2000) Strength and anthropometric measures in identical and fraternal twins: no evidence of masculinization of females with male co-twins. Epidemiology 11:340–343
14. Christiansen L, Frederiksen H, Schousboe K et al (2003) Age- and sex-differences in the validity of questionnaire-based zygosity in twins. Twin Res 6:275–278
15. Field A (2005) Discovering statistics using SPSS, 2nd edn. SAGE Publications Ltd, London
16. Neale MC, Boker SM, Xie G, Maes HH (1999) Mx statistical modeling, 5th edn. Department of Psychiatry, Medical College of Virginia, Richmond, VA
17. Neale MC, Cardon LR (1992) Methodology for genetic studies of twins and families. Kluwer Academic Publishers, Dordrecht, The Netherlands
18. Kuijper EAM, Lambalk CB, Boomsma DI (2007) Heritability of reproductive hormones in adult male twins. Hum Reprod 22:2153–2159
19. Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Harlow Longman: Scientifical & Technical, London
20. Wilk JB, DeStefano AL, Arnett DK et al (2003) A genome-wide scan of pulmonary function measures in the national heart, lung, and blood institute family heart study. Am J Respir Crit Care Med 167:1528–1533
21. Joost O, Wilk JB, Cupples A et al (2002) Genetic loci influencing lung function. A genomewide scan in the Framingham study. Am J Respir Crit Care Med 165:795–799
22. Hallberg J, Iliadou A, Anderson M et al (2010) Genetic and environmental influence on lung function impairment in Swedish twins. Respir Res 11:92
23. Zhai G, Valdes AM, Cherkas L et al (2007) The interaction of genes and smoking on forced expiratory volume: a classic twin study. Chest 132:1772–1777
24. Hallberg J, Dominicus A, Eriksson UK et al (2008) Interaction between smoking and genetic factors in the development of chronic bronchitis. Am J Respir Crit Care Med 177:486–490
25. Vestbo J, Prescott E, Lange P, The Copenhagen City Heart Study Group (1996) Association of chronic mucus hypersecretion with FEV1 decline and COPD morbidity. Am J Respir Crit Care Med 153:1530–1535
26. Pillai SG, Ge D, Zhu G et al (2009) A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. PloS Genet 5:e1000421
27. Kumar R, Seibold MA, Aldrich MC et al (2010) Genetic ancestry in lung-function predictions. N Engl J Med 363:321–330