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The association of genetic instrumental variables for lung function on coronary artery disease risk: A two-sample Mendelian randomization study

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Abstract

Background: Lung function, assessed by forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC), is inversely associated with coronary artery disease (CAD) but these associations could be due to confounding or reversed causality. We conducted a two-sample Mendelian randomization study, using publicly available data from relevant genome wide association studies (GWAS), to examine the role of FEV_1 or FVC on CAD.

Methods: We used the most recent GWAS on lung function to extract genetic instruments related to FEV_1 and FVC (n=92,749). Data on the association between genetic instruments and CAD were obtained from CARDIoGRAMplusC4D 1000 Genomes based GWAS (60,801 CAD cases and 123,504 controls). We used inverse variance weighting with a multiplicative random effect to estimate the genetic instrumented association of FEV_1 and FVC on CAD. Sensitivity analyses included weighted median and MR-Egger methods.

Results: Each standard deviation (SD) greater FEV_1 was associated with a lower risk of CAD (Odds ratio (OR): 0.78 per SD; 95% CI: 0.62 to 0.98) with a similar magnitude for FVC on CAD risk (OR: 0.82 per SD; 95% CI: 0.64 to 1.06). Estimates for FEV_1 were similar when using MR-Egger method (OR: 0.80 per SD; 95% CI: 0.33 to 1.94) although the magnitude was smaller for weighted median method (OR: 0.93 per SD; 95% CI: 0.75 to 1.17). Estimates for FVC in the sensitivity analyses were attenuated (median) or changed direction (MR-Egger).

Conclusion: Our study suggested an inverse relation between FEV_1 and CAD but for FVC, evidence is less clear.

Keywords: Coronary artery disease; lung function; Mendelian randomization

Introduction

Reduced lung function is consistently associated with higher risk of cardiovascular diseases (CVD), including coronary artery disease (CAD), in observational studies.[1-5] Possible mechanisms linking reduced lung function and cardiovascular diseases include increased inflammation as people with higher lung function tend to have lower levels of inflammatory markers such as C-reactive protein and fibrinogen,[6] inefficient cardio-toxic substance removal, or a mismatch of ventilation/perfusion ratio.[6] However, these inverse associations could be a result of residual confounding or reverse causality. Smoking and height, which are both strongly related to lung function and cardiovascular disease in directions that would result in the direction of association between these two are particularly important sources of confounding. [2, 7] Furthermore, early CAD may result in reduced perfusion and reduced lung function, i.e. reverse causation.[8] If improving lung function can reduce CAD risk this is a potentially modifiable risk factor that could have important public health benefit beyond that of improving lung function.

Mendelian randomization studies may provide more credible evidence concerning the causal role of lung function in CAD than that possible with conventional observational approaches. For example, previous studies have shown that Mendelian randomization studies generate comparable results with randomized controlled trials.[9] A Mendelian randomization study to test the effect of lung function on CAD would use genetic variants that are robustly associated with lung function as instrumental variables for its causal effect. As these variants are randomly allocated from parents to their offspring at conception they are less susceptible to reverse causation than observational studies.[10] Furthermore, the random allocation also implies that the genetic variants were less likely to be affected by the many factors that commonly confound observational studies, which is supported by empirical evidence.[10, 11] To

our knowledge, no previous Mendelian randomization studies have explored the effect of lung function on CAD. Therefore, we conducted a two-sample Mendelian randomization study to clarify the relation of lung function (forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC)) and CAD risk using summary statistics from large genome wide association studies (GWAS).[12, 13] We hypothesized higher lung function (higher FEV_1 and FVC) would be associated with lower risk of CAD. Further, as FVC is particularly related to height (more so than FEV_1), we hypothesized that inverse association between FVC and CAD would be weaker than those between FEV_1 and CAD in our Mendelian randomization study, if height is a key confounder of FVC with CAD.

Method

The data and methods are publicly available.

Summary of Study design

We used two-sample Mendelian randomization with summary data from publicly available GWAS databases.[12, 14] Firstly, we identified independent ($r^2<0.05$) genetic instruments (single nucleotide polymorphisms (SNP)) strongly associated with FEV_1 or FVC ($p < 5x10^{-8}$) from GWAS of lung function and obtained the estimates between each of these SNPs and lung function parameters (i.e. difference in mean FEV_1 or in FVC and their corresponding standard errors) – sample one.[15-17] Secondly, we obtained the corresponding genetic associations for each of the lung function SNPs with CAD risk from CARDIoGRAMplusC4D 1000 Genomes-based GWAS – sample two.[18] We then used these summary results to calculate the Mendelian randomization estimates of FEV_1 and FVC on CAD risk, using various statistical methods as described in the statistical analysis section.[19, 20]

GWAS of lung function (FEV_1 and FVC – Sample 1)

For this study we used data from the most recent GWAS (which included both FEV_1 and FVC as outcomes),[15] which built on previous GWAS,[16, 17] and had a combined discovery and replication sample size of 92,749 (discovery: 38,199; replication: 54,550). It was undertaken in European origin participants, and was imputed based on the 1000 Genomes Project Phase 1 reference panel.[15] Variants with imputation information <0.3, assessed using r2.hat (MACH and minimac) or .info (IMPUTE2), were excluded. The GWAS used linear regression adjusted for age, age^2, sex, height, and principal component for population structure, separately for never and ever smokers. The residuals from FEV_1 and FVC were converted to ranks and then transformed to normally distributed z scores, which were then used in an additive genetic model, separately for never and ever smokers, with the results from these two (never and ever smokers) strata then meta analyzed. In the GWAS, only SNPs previously not identified were genotyped and analyzed in both discovery and replication stages whereas previously known SNPs were only genotyped and analyzed in the discovery stage. As such, we extracted information for 17 SNPs related to FEV_1 (10 from both stages and 7 from stage 1) and 11 SNPs related to FVC (5 from both stages and 6 from stage 1).[15]

GWAS of CAD: CARDIoGRAMplusC4D 1000 Genomes (Outcome – Sample 2)

We obtained summary data on the estimates of each lung function GWAS SNP with CAD from CARDIoGRAMPLUSC4D (downloading these data from www.CARDIOGRAMPLUSC4D.ORG).[18] CARDIoGRAMplusC4D 1000 Genomes-based GWAS is a meta-analysis of GWAS of CAD case-control studies of people of mainly European (~91%), South Asian, and East Asian descent imputed using the 1000 Genomes phase 1 v3 training set with 38 million variants. The study interrogated 9.4 million variants and included 60,801 CAD cases and 123,504 controls.[18] Case status was defined as having

CAD if they had received a diagnosis of myocardial infarction, acute coronary syndrome, chronic stable angina, or coronary stenosis >50%. Diagnoses were ascertained in various ways in different studies; extracted from medical records, clinical diagnosis at the time of study, procedures (coronary angiography results or by-pass surgery), medications or symptoms that indicate angina, or self-report of a doctor diagnosis, as described elsewhere.[18] Covariate adjustment in the GWAS included study-specific covariates (e.g. age and sex) and genomic control. The GWAS reported the corresponding log odds and standard error on CAD for each SNP effect allele using an additive model.[18]

Harmonization of the two samples

To ensure correct allele harmonization, SNPs were orientated so that the effect allele was the same for both the exposure and the outcome datasets. Palindromic SNPs were checked and corrected using the effect allele frequency (EAF) reported in the exposure and outcome GWAS to identify corresponding strands between the two GWAS. If EAF is >0.42 and ≤0.50, a proxy non-palindromic SNP (R^2=1) would be used instead to avoid ambiguity in strand direction since alleles of palindromic SNPs are the same on both strands.[21] Linkage disequilibrium of the SNPs were checked via 1000 Genomes project to avoid inclusion of correlated SNPs. If two SNPs had an R^2 of >0.05, the SNP with the smaller p value was retained and the other SNP was discarded.

Statistical analysis

Main Mendelian randomization analysis

Inverse variance weighting (IVW) with multiplicative random effects was used to give our main Mendelian randomization effect estimate, which is a weighted regression of SNP-outcome association (log odds of CAD per effect allele) on SNP-exposure association (mean

difference in FEV_1 or FVC per effect allele), with the intercept constrained to zero.[22] One key assumption for this method to produce valid estimate is that there is no other way SNPs could affect the outcome (CAD here) than through the risk factor that the SNPs are instrumenting for (FEV_1 or FVC). Specifically with MR it assumes no horizontal pleiotropy.[12] We also calculated Wald ratio for each SNP ((i.e. SNP-outcome association divided by SNP-exposure association), with the standard error calculated by dividing the standard error of the SNP-outcome association by the effect size of the SNP-exposure association). We then meta-analysed these ratios using a fixed effect method and compared the results to the multiplicative random effects IVW. Both methods are supposed to estimate the same overall effect, but standard errors will be larger when using multiplicative random effects IVW in the presence of heterogeneity of effect across SNPs. We also estimated the I^2 statistics to explore whether there was heterogeneity in effects between SNPs, which could be suggestive that one or more SNPs are invalid instruments.

Sensitivity analyses

We undertook a number of sensitivity analyses to test the robustness of our main analyses. Since the IVW multiplicative random effect models can give biased estimates if one or more instruments are invalid due to horizontal pleiotropy,[20] we particularly focus on sensitivity analyses that allow the assumption of no horizontal pleiotropy to be relaxed. These analyses have different assumptions to the IVW (main analyses) method, and also to each other. In comparison to the IVW method they have less statistical efficiency; this is particularly the case with MR-Egger. Therefore, the aim of these sensitivity analyses was mostly to examine if the magnitude and direction of effect estimates were consistent across methods.

i. Leave one out analysis

The IVW estimates were recalculated removing one SNP at a time aiming to explore whether the overall estimate was primarily driven by any (outlying) SNP.

ii. MR-Egger method

Similar to the IVW, the MR-Egger method is a weighted regression of the SNP-outcome on the SNP-exposure association, but unlike the IVW method, the intercept is not constrained to be zero.[19] This means that if there is unbalanced pleiotropy the Mendelian randomization (slope of the regression line) effect estimate will differ from the IVW estimate, and the intercept of the regression line will be non-zero. The MR-Egger method slope will give unbiased estimate even if all instruments are invalid (i.e. due to the presence of horizontal pleiotropy).[19] However, it has an additional assumption known as the InSIDE (instrument strength independent of direct effect), which requires no correlation between the strength of the direct effect of instrument on outcome and the instrument strength (strength of association between genetic variants for lung function and lung function). One possible scenario through which the InSIDE assumption could be violated is if the pleiotropic effects of genetic instruments are mediated via the same exposure-outcome confounder.[20] The MR-Egger method is statistically inefficient and expected to have considerably wider confidence intervals than our main analyses or other sensitivity analyses. As such we focus on whether the odds ratio is consistent in terms of magnitude and direction with our main analysis results and also whether the intercept value suggests evidence of pleiotropy.

iii. Weighted median method

The weighted median method is a further method that allows some relaxation of the horizontal pleiotropy assumption. It weights each of the Wald ratio effects of each SNP by the corresponding precision and then takes the median weighted ratio as the causal effect.[20] It

will provide an unbiased estimate so long as more than 50% of the weights were derived from valid instruments.[20] This means that the estimate will be biased if one single SNP is pleiotropic and contributes more than 50% of the weight for the overall effect, or if a group of SNPs are pleiotropic and together they contribute more than 50% of the weight.

iv. Difference in the imputation reference panel for lung function GWAS

To examine whether the results were robust to the difference in the imputation reference panel, we also extracted genome wide significant SNPs for lung function from the most recent GWAS which used HapMap reference populations (CEU) and repeated the analyses.[16, 17] For FEV_1, the SNPs were extracted from a GWAS with a sample size of 94,612 (discovery: 48,201; replication: 46,411) of mainly European origin.[17] For FVC, the SNPs were extracted from a GWAS with a sample size of 85,170 (discovery: 52,253; replication: 32,917) of mainly European origins.[16] Similar adjustments, stratification by smoking and analytical methods, and imputation quality control threshold were used in these GWAS as in the one used in our main analyses with imputation using the 1000 Genome Project Phase 1 reference panel although the FVC GWAS used absolute FVC (mL) as the outcome.[16] To improve comparability with the other analyses, we rescaled the Mendelian randomization estimates of FVC (HapMap) into per standard deviation (SD). The SD was derived by pooling the SDs reported in the European cohorts in the FVC GWAS.

v. Exclusion of potential pleiotropic SNPs

Considering that height and smoking are key potential confounders, we checked whether any of our selected FEV_1 or FVC genetic instruments were related to height or smoking (ever smoking status and cigarettes smoked per day) using summary statistics from the relevant GWAS consortia (GIANT and TAG, respectively).[23, 24] Similarly, we explored whether any

of our FEV_1 or FVC genetic instruments were related to established cardiovascular risk factors by looking for their associations using data from relevant GWAS: triglycerides and LDL cholesterol (from GLGC);[25, 26] body mass index (GIANT);[27] and systolic blood pressure (UKB).[28, 29] We considered a SNP as potentially pleiotropic if there was evidence of it being associated with height, smoking or cardiovascular risk factors after applying a Bonferroni correction to account for multiple testing ($p<0.0004$),calculated by 0.05 (nominal p value) /126 (number of comparisons)). We repeated the main analysis (IVW estimate) after excluding the SNPs classified as pleiotropic.

vi. Multivariable Mendelian randomization analysis

Since some of the SNPs predicted both FEV_1 and FVC (i.e. with p value $<5 \times 10^{-8}$ for both parameters) based on the most recent GWAS,[15] we also conducted a multivariable Mendelian randomization analysis to adjust effects of FEV_1 by FVC, and vice-versa, in order to disentangle the effects of each exposure on CAD risk.[30]

Exploring instrument strength

Instrument strength was assessed by presenting the first stage (regression of FEV_1 and FVC on genetic instruments) F-statistics and R^2 values. For both of these statistics higher values related to stronger instruments.

We calculated the first stage F-statistics for the main analyses using the equation 1 in the supplementary material. The GWAS only reported R^2 of the instruments (known and novel) for the discovery stage.[15] We directly extracted the R^2 for known loci as reported in the GWAS discovery stage. However, the R^2 for novel loci in the discovery stage could be

susceptible to winner's curse.[31] As such, we calculated the R^2 for novel loci using equation 2 in the supplementary material using parameters from each novel loci based on the combined analyses (discovery + replication). Then, we summed up the R^2 and compute the F statistics using the sample size as stated in the GWAS.

All analyses were performed using R Version 3.3.2 (R Development Core Team, Vienna, Austria) with the R package (TwosampleMR). This study only used publicly available data and hence no ethical approval was required to conduct this study.

Results

Amongst 17 SNPs for FEV_1 (1000 Genomes imputation), rs1985524 was palindromic and was replaced by rs1989154 (R^2=1) to avoid ambiguity in the strand direction, rs11383346 was removed as this was an indel and was not available in CARDIoGRAMplusC4D, hence 16 SNPs were used. Amongst 11 SNPs for FEV_1 (HapMap imputation), rs17331332 and rs3995090 were in linkage disequilibrium with other SNPs and were removed, and hence 9 SNPs remained in these analyses. Amongst 11 SNPs for FVC (1000 Genomes imputation), rs1430193 was palindromic and was replaced by rs1430194 to avoid ambiguity in the strand direction; rs11383346 was removed as this was an indel and was not available in CARDIoGRAMplusC4D, thus 10 SNPs remained in these analyses. Amongst 6 SNPs for FVC (HapMap imputation), rs1430193 was palindromic and was replaced by rs1430194.

Supplemental Tables 1-2 summarize the genetic associations as reported in the relevant GWAS after allele harmonization. The first-stage F statistics and variance explained by the genetic instruments and were 49.5 and 0.8% for FEV_1 and 38.4 and 0.4% for FVC.

Supplemental Table 3 shows the associations of all SNPs that were used as instrumental variables with FEV_1 and FVC with smoking, height, BMI, LDLc, Triglycerides, and systolic blood pressure. After correcting for multiple testing, 5 of the SNPs we were using as genetic instruments were associated with height (rs1032296; rs134041; rs1430194; rs6923462; and rs7155279), one SNP (rs2036527) associated with number of cigarettes smoked per day, one with BMI (rs4237643) and 4 with systolic blood pressure (rs1989154; rs2571445; rs2863171; rs7068966); none of our genetic instruments were associated with ever smoking, LDLc or triglycerides. Based on the lung function GWAS, rs6681426, rs6441207, rs2274116, rs10850377 were associated with both FEV_1 and FVC.[15]

FEV_1 was inversely associated with CAD risk in the inverse variance weighting with multiplicative random effect model (Odds ratio: 0.78 per standard deviation (SD) increase, 95% Confidence Interval (CI): 0.62 to 0.98, I^2=62%)) (Table 1). Findings for the association of FEV1 with CAD risk were consistent across the range of sensitivity analyses. The leave one out plot suggested the overall estimate was not driven by any single outlying SNP (Figure 1). The estimate from MR-Egger, which is more robust to invalid variants was consistent with the main estimate (OR: 0.80, 95%CI: 0.33 to 1.94), although had wider confidence as expected because of the lower statistical power of this approach. In addition, there was no evidence for directional pleiotropy based on the MR-Egger intercept value (1000 Genomes imputation: -0.0009; p=0.96; HapMap imputation: -5.4×10^{-5}; p=0.998) (Table 1). The weighted median estimate was somewhat attenuated in comparison to the main inverse variance weighted analyses, but consistent with those and the MR-Egger results (OR: 0.93 per SD, 95% CI 0.75 to 1.17). Similar findings were observed when we used estimates from the GWAS imputed using HapMap reference panel (Table 1).

There was also an inverse association of genetic instruments for FVC with CAD risk in the inverse variance weighting with multiplicative random effect model using 1000 genome imputation (Odds ratio: 0.82 per SD increase, 95% CI: 0.64 to 1.06, I^2=35%) (Table 1). This point estimate is similar to that for the association of FEV_1 with CAD, but with wider confidence intervals. The estimate was weaker (closer to the null) using the weighted median method (OR: 0.92, 95%CI: 0.69 to 1.23) and the MR-Egger method yielded a directionally different estimate with wide confidence intervals (OR: 1.14, 95%CI: 0.36 to 3.61). There was no evidence for directional pleiotropy based on the MR-Egger intercept value (1000 Genomes imputation: -0.011, p=0.59; HapMap imputation: -0.022, p=0.48) (Table 1). The leave one out plot suggested the overall estimate was not driven by any single SNP, although it was somewhat attenuated when rs4237643 or rs6681426 were removed (Figure 2). When we used estimates from the GWAS with the HapMap imputation the main inverse variance weighed estimates were similar to the 1000 genome imputation (OR: 0.85, 95%CI: 0.49 to 1.48), as were the results for weighted median analyses (attenuating towards the null) and MR-Egger (showing a positive, rather than inverse association) (Table 1).

Repeating the analyses with the exclusion of SNPs related to height, smoking phenotypes, body mass index, and systolic blood pressure did not notably change the results (Supplemental Tables 4-5). The fixed effect Wald ratio meta-analysis gave similar results (Supplemental Table 6). The multivariable Mendelian randomization analysis produced directionally similar results compared to the MR-Egger regression for FEV_1 (OR: 0.82 per SD increase, 95% CI: 0.53 to 1.28) and FVC (OR: 1.84 per SD increase. 95% CI: 0.61 to 5.50).

Discussion

To our knowledge, this is the first Mendelian randomization study examining the role of lung function on CAD. Consistent with previous multivariable regression analyses, we found an inverse relation between FEV_1 and CAD.[1, 2] In our main analyses the association of FVC on CAD was similar to that of FEV_1. However, whilst the association of FEV_1 with CAD was robust to numerous sensitivity analyses, those for FVC were less so, with MR-Egger and the multivariable regression analyses (in which we adjusted for effect of SNPs on FEV_1) suggesting a possible positive association of FVC on CAD, albeit with wide confidence intervals including the null. Thus, our study does not corroborate previous multivariable regression studies of FVC, which suggested an inverse association of FVC with CAD risk.[6] Overall, our findings suggest that higher FEV_1 may reduce CAD risk, but that previous inverse associations of FVC with CAD from multivariable regression analyses are possibly explained by residual confounding, in particular due to height.

The inverse association of FEV_1 with CVD risk has been consistently seen in observational studies.[1-4] However, smoking is a strong determinant of FEV_1 and CAD, and it is difficult to fully adjust for it without very detailed information on life course frequency, amount and intensity of smoking. Therefore, residual confounding by smoking might have explained those previous results.[2, 7] Our Mendelian randomization study, which is less susceptible to such confounding,[10] suggests that greater FEV_1 may be related to reduced CAD risk. This conclusion is particularly supported by the consistency of results across several different Mendelian randomization approaches, including a meta-analysis of Wald ratio estimates for each SNP, MR-Egger and weighted median methods, as well as when results from FEV_1 GWAS with a different imputation reference panel used to identify genetic instruments, and when we removed SNPs with known associations with smoking, height and BMI (for which

we found some possible evidence of pleiotropy), and multivariable Mendelian randomization analysis. These different approaches each have differing underlying assumptions, and hence key sources of bias, and thus the triangulation of findings across them increases confidence that FEV_1 is causally related to CAD risk.[32] We did not find robust and consistent (across our sensitivity analyses) evidence of an association of FVC on CAD. It is possible that the null findings are due to lack of statistical power although some of these methods are less statistically efficient and with FEV_1 confidence intervals were wide and included the null is some sensitivity analyses. The difference between the sensitivity analyses of FEV_1 and CAD and of FVC and CAD is that the former were all directionally consistent and had broadly similar magnitudes, whereas with FVC two of the sensitivity analyses suggested positive, rather than inverse associations, which suggests less robust Mendelian randomization evidence for an inverse association.

That our study suggests possible differences in the association of FEV_1 and FVC on CAD is perhaps not surprising given they reflect very different aspects of lung function, as reflected by the differing genetic architecture observed in genome wide association studies.[16, 17] FEV_1 represents severity of airway obstruction,[33] whereas FVC is a measure overall lung capacity. These differences result in somewhat different confounding structures between the two measurements, with smoking being more strongly associated with FEV_1 and height with FVC. Thus, our results suggest that inability to fully account for confounding by height in previous multivariable regression analyses might have resulted in spurious inverse associations of it with CAD. Our results also suggest that airway obstruction, rather than lung size or capacity, may increase CAD risk.

Strengths and Limitations

Key strengths of this study are the large sample size, with over 60,000 CAD cases, and the use of Mendelian randomization to minimize the impact of confounding and reverse causation. The limitations of our study in inferring causal relation relate to the extent to which the key Mendelian randomization assumptions were satisfied – that the genetic instruments are (i) robustly associated with risk factor of interest (here FEV_1 and FVC), (ii) not associated with confounders of the risk factor-outcome association, and (iii) not related to the outcome via any path other than through the risk factor (i.e. that there is no horizontal pleiotropy). In two-sample Mendelian randomization as used here, weak instrument bias is expected to bias findings towards the null (rather than towards the confounded result as occurs in one-sample Mendelian randomization). However, this will depend on the extent of overlap between the two samples, with substantial overlap resulting in similar bias to that seen for one-sample Mendelian randomization.[12] Only one cohort (n=837, 0.5%) was used in both the lung function and CAD GWAS samples, and the high F-statistic and R^2 for the combined genetic instrumental variables, suggest that weak instrument bias is unlikely to have impacted our results. Horizontal pleiotropy is unlikely given the similarity of results across the different methods that we have used, and the intercept values from the MR-Egger being close to zero. One limitation of using summary GWAS data is that we are unable to explore the association of the genetic instruments with a broad range of observed potential confounders, but we did find similar results when we excluded SNPs that are known to be related to smoking and height, the two confounders we were mostly concerned about, one SNP associated with BMI, and 4 SNPs associated with systolic blood pressure. We did not find evidence for any of our genetic instrumental variables being robustly statistically associated with LDLc and triglycerides, which are important risk factors for CAD. In these analyses, the fact that the GWAS of both FEV_1 and FVC adjusted for height and smoking could introduce spurious associations through a phenomenon known as collider bias.

However, results were consistent after removing SNPs that had a residual association with height and smoking (Supplemental Table 4), suggesting that collider bias is unlikely to fully account for the inverse association between FEV_1 and CAD. Nevertheless, research using UK Biobank may help verify the findings since some of these biases can only be explored with individual data.[34] Lastly, we were unable to explore potential reverse causation using a bi-directional Mendelian randomization design because summary data across the whole genome for the lung function GWAS are currently not available. Whilst there may be a bidirectional association (i.e. a propensity to CAD causing reduced FEV_1 as well as vice versa), this would not negate the association of FEV_1 in CAD that we have identified here.

Conclusion

In this first Mendelian randomization study of the relation of FEV_1 and FVC with CAD risk that we are aware of, we find evidence for an inverse association of FEV_1 with CAD risk, but less robust evidence for an association of FVC with CAD risk. Whilst it has been suggested that increased systemic inflammation in those with poor lung function, inefficient cardio-toxic substance removal, or a mismatch of ventilation/perfusion ratio, are potential ways in which lower FEV_1 is related to increased CAD risk the exact mechanisms are unclear.[6] As our findings now provide more concrete evidence that there may be a causal effect that is not explained by confounding due to smoking or height, further research to explore potential mechanisms could identify novel targets for the prevention of CAD.

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Disclosures

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References

1. Sin DD, Wu L, Man SF. The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. *Chest.* 2005;127:1952-9.
2. Batty GD, Gunnell D, Langenberg C, Smith GD, Marmot MG, Shipley MJ. Adult height and lung function as markers of life course exposures: associations with risk factors and cause-specific mortality. *Eur J Epidemiol.* 2006;21:795-801.
3. Johnston AK, Mannino DM, Hagan GW, Davis KJ, Kiri VA. Relationship between lung function impairment and incidence or recurrence of cardiovascular events in a middle-aged cohort. *Thorax.* 2008;63:599-605.
4. Wannamethee SG, Shaper AG, Papacosta O, Lennon L, Welsh P, Whincup PH. Lung function and airway obstruction: associations with circulating markers of cardiac function and incident heart failure in older men-the British Regional Heart Study. *Thorax.* 2016;71:526-34.
5. Li J, Agarwal SK, Alonso A, Blecker S, Chamberlain AM, London SJ, et al. Airflow obstruction, lung function, and incidence of atrial fibrillation: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation.* 2014;129:971-80.
6. Schroeder EB, Welch VL, Couper D, Nieto FJ, Liao D, Rosamond WD, et al. Lung function and incident coronary heart disease: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol.* 2003;158:1171-81.
7. Anthonisen NR, Connett JE, Murray RP. Smoking and lung function of Lung Health Study participants after 11 years. *Am J Respir Crit Care Med.* 2002;166:675-9.
8. Dow L, Ebrahim S. Commentary: Lung function and risk of fatal and non-fatal stroke-The Copenhagen City Heart Study. *Int J Epidemiol.* 2001;30:152-3.
9. Ference BA, Julius S, Mahajan N, Levy PD, Williams KA, Sr., Flack JM. Clinical effect of naturally random allocation to lower systolic blood pressure beginning before the development of hypertension. *Hypertension.* 2014;63:1182-8.

10. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey-Smith G. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. *Stat Med.* 2008;27:1133-63.
11. Davey Smith G, Lawlor DA, Harbord R, Timpson N, Day I, Ebrahim S. Clustered environments and randomized genes: a fundamental distinction between conventional and genetic epidemiology. *PLoS Med.* 2007;4:e352.
12. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. *Int J Epidemiol.* 2016;45:908-15.
13. Borges MC, Lawlor DA, de Oliveira C, White J, Horta BL, Barros AJ. Role of Adiponectin in Coronary Heart Disease Risk: A Mendelian Randomization Study. *Circ Res.* 2016;119:491-9.
14. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, Consortium E-I. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol.* 2015;30:543-52.
15. Soler Artigas M, Wain LV, Miller S, Kheirallah AK, Huffman JE, Ntalla I, et al. Sixteen new lung function signals identified through 1000 Genomes Project reference panel imputation. *Nature communications.* 2015;6:8658.
16. Loth DW, Soler Artigas M, Gharib SA, Wain LV, Franceschini N, Koch B, et al. Genome-wide association analysis identifies six new loci associated with forced vital capacity. *Nature genetics.* 2014;46:669-77.
17. Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nature genetics.* 2011;43:1082-90.
18. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nature genetics.* 2015;47:1121-30.
19. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* 2015;44:512-25.
20. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol.* 2016;40:304-14.

21. Hartwig FP, Davies NM, Hemani G, Davey Smith G. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. *Int J Epidemiol.* 2016;45:1717-26.
22. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. *Stat Med.* 2017;36:1783-802.
23. Wood AR, Esko T, Yang J, Vedantam S, Pers TH, Gustafsson S, et al. Defining the role of common variation in the genomic and biological architecture of adult human height. *Nature genetics.* 2014;46:1173-86.
24. Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nature genetics.* 2010;42:441-7.
25. Liu DJ, Peloso GM, Yu H, Butterworth AS, Wang X, Mahajan A, et al. Exome-wide association study of plasma lipids in >300,000 individuals. *Nature genetics.* 2017;49:1758-66.
26. Global Lipids Genetics Consortium, Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, et al. Discovery and refinement of loci associated with lipid levels. *Nature genetics.* 2013;45:1274-83.
27. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature.* 2015;518:197-206.
28. Hemani G, Zheng J, Wade KH, Laurin C, Elsworth B, Burgess S, et al. MR-Base: a platform for systematic causal inference across the phenome using billions of genetic associations. *bioRxiv.* 2016
29. Churchhouse C, Neale B. Rapid GWAS of thousands of phenotypes for 337,000 samples in the UK Biobank 2017 [Available from: http://www.nealelab.is/blog/2017/7/19/rapid-gwas-of-thousands-of-phenotypes-for-337000-samples-in-the-uk-biobank.
30. Burgess S, Dudbridge F, Thompson SG. Re: "Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects". *Am J Epidemiol.* 2015;181:290-1.
31. Rosner B. Fundamentals of biostatistics. 4th ed. Belmont, Calif.: Duxbury Press 1995.
32. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *Int J Epidemiol.* 2016;45:1866-86.

33. Young RP, Hopkins R, Eaton TE. Forced expiratory volume in one second: not just a lung function test but a marker of premature death from all causes. *Eur Respir J.* 2007;30:616-22.

34. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* 2015;12:e1001779.

Table 1: Mendelian randomization estimates for lung function on coronary artery disease risk

		Inverse variance weighting with multiplicative random effects		MR-Egger			Weighted median	
*Lung function	Imputation	Odds ratio Per 1SD	95% CI	Odds ratio Per 1SD	95% CI	Intercept (p value)	Odds ratio Per 1SD	95% CI
FEV_1 (16 SNPs)	1000 Genomes	0.78	0.62 to 0.98	0.80	0.33 to 1.94	-0.0009 (0.96)	0.93	0.75 to 1.17
FEV_1 (9 SNPs)	HapMap	0.77	0.57 to 1.04	0.77	0.28 to 2.09	-5.4×10^{-5} (0.998)	0.80	0.62 to 1.04
FVC (10 SNPs)	1000 Genomes	0.82	0.64 to 1.06	1.14	0.36 to 3.61	-0.011 (0.59)	0.92	0.69 to 1.23
FVC (6 SNPs)	HapMap	0.85	0.49 to 1.48	2.37	0.17 to 33.5	-0.022 (0.48)	0.92	0.53 to 1.57

*FEV_1: Forced expiratory volume in 1 second; FVC: Forced vital capacity.

Figure legends

Figure 1: Leave one out plot for the association of forced expiratory volume in one second (FEV_1), per standard deviation, and coronary artery disease risk using Mendelian randomization (Single nucleotide polymorphisms with 1000 Genomes imputation)

Figure 2: Leave one out plot for the association of forced vital capacity (FVC), per standard deviation, and coronary artery disease risk using Mendelian randomization (Single nucleotide polymorphisms with 1000 Genomes imputation)