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Lung function & cardiovascular disease. A Two Sample Mendelian Randomization Study

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Short title: MR study of lung function effects on risk of cardiovascular disease

1 **Abstract**

2 Background

3 Observational studies suggest an association between reduced lung function and risk of
4 coronary artery disease and ischaemic stroke, independent of shared cardiovascular risk
5 factors such as cigarette smoking. We use the latest genetic epidemiological methods to
6 determine if impaired lung function is causally associated with an increased risk of
7 cardiovascular disease.

8 Methods and Findings

9 Mendelian Randomization uses genetic variants as instrumental variables to investigate
10 causation. Preliminary analysis used two sample Mendelian Randomization with lung
11 function single nucleotide polymorphisms ~~shown to confer a high risk of COPD~~. To avoid
12 collider bias the main analysis used single nucleotide polymorphisms for lung function
13 identified from UKBiobank in a Multivariable Mendelian Randomization model conditioning
14 for height, body mass index and smoking.

15 Multivariable Mendelian Randomization shows strong evidence that reduced FVC causes
16 increased risk of coronary artery disease, Odds Ratio:1.32 (1.19-1.46) per Standard
17 Deviation. Reduced FEV₁ is unlikely to be cause increased risk of coronary artery disease as
18 evidence of its effect becomes weak after conditioning for height 1.08 (0.89, 1.30). There is
19 weak evidence that reduced lung function increases risk of ischaemic stroke.

20 Conclusion

21 There is strong evidence that reduced FVC is independently and causally associated with
22 coronary artery disease. Although the mechanism remains unclear, FVC could be taken into
23 consideration when assessing cardiovascular risk and considered a potential target for
24 reducing cardiovascular events. FEV₁ and airflow obstruction do not appear to cause
25 increased cardiovascular events, confounding and collider bias may explain previous findings
26 of a causal association.

27 Word Count abstract: 245

28

29 **Introduction**

30

31 Multi-morbidity, the co-existence of multiple diseases in an individual, is associated with
32 poor quality of life, mortality and polypharmacy.[1] Impaired lung function measures such as
33 Forced Expiratory Volume in one second (FEV₁) and Forced Expiratory Volume (FVC) have
34 been found to be strongly associated with multi-morbidity and are reported as independent
35 predictors of cardiovascular disease.[2] Although research has often focused on the
36 contribution of FEV₁ and obstructive airways disease to cardiovascular risk, FVC has been
37 shown to be a stronger predictor of survival, and appears to add value to the Framingham
38 Risk Score for prediction of mortality.[3, 4] However, it is unclear if there is a causal link
39 between lung function and multi-morbidity or if the association is due to confounding factors
40 such as cigarette smoking.

41

42 Observational studies have reported that Chronic Obstructive Pulmonary Disease (COPD),
43 decreased FEV₁, FVC and FEV₁/FVC ratio are all associated with an increased the risk of
44 coronary artery disease.[5, 6] However results are inconsistent, with some studies reporting no
45 association,[7] or that the association is limited to those with abnormally high blood
46 pressure.[8] There is also evidence suggesting that COPD and impaired lung function are
47 associated with an increased risk of stroke.[9]

48

49 Impaired lung function and associated lung diseases could have a direct detrimental effect on
50 cardiovascular health via a number of biological pathways including systemic inflammation
51 or oxidative stress.[10, 11] However the mechanisms may vary between different lung
52 function traits.[12]

53

54 Mendelian Randomization (MR) is a method which can overcome problems of unmeasured
55 confounding and reverse causation typical of conventional observational epidemiology.[13]
56 MR allows causal inference through the use of genetic variants as proxies for modifiable risk
57 factors or health outcomes.[14] MR uses genetic data, e.g. single nucleotide polymorphisms
58 (SNPs) that are associated with an exposure (in this case lung function), as instrumental
59 variables (IV) to assess the causal effect of the exposure on the outcome of interest (in this
60 case cardiovascular disease).[15]

61

62 MR has multiple advantages, it uses genetic variants which are randomly allocated at
63 conception so they can be exploited to simulate randomisation.[15] Genetic variants are not
64 influenced by behavioural or environmental factors and are far less susceptible to bias from
65 reverse causation. Additionally, the effects are equivalent to lifetime differences, reducing
66 issues relating to transient fluctuations in exposures.[16] Multivariable MR (MVMR) has
67 further advantages, it includes multiple exposures in the model allowing estimation of the
68 direct causal effect of each exposure on the outcome. Each exposure SNP has its effect on all
69 exposures e.g. lung function (LF) trait and height included in the MR model allowing for
70 conditioning. MVMR is a robust method when using two exposures that could act as a
71 confounder, mediator or collider of the exposure-outcome relationship.[17, 18] Our objective
72 was to determine if impaired lung function causally increases the risk of cardiovascular
73 disease.

74 **Methods**

75

76 Exposures – Shrine et al preliminary analysis [19]

77

78 We used data from the largest currently available lung function GWAS, by Shrine et al to
79 undertake a preliminary 2 sample Mendelian Randomization analysis. The Shrine et al

80 GWAS reported 279 genome wide significant SNPs ($p < 5 \times 10^{-9}$) in European ancestry
81 population and was adjusted for age, age², height, smoking status. Full details are provided
82 elsewhere.[19]

83

84 Given that the Shrine et al GWAS adjusted for covariates of lung function and cardiovascular
85 disease e.g. height and smoking, this can lead to collider bias as SNPs can be related to both
86 the covariates e.g. height and to other adverse risk factors.[16] This can lead to false positive
87 SNP discoveries and bias (towards null effect) in MR studies.[20]

88 Exposures – Main analysis MVMR

89 To avoid the collider bias we used exposure SNPs discovered in GWAS that had not been
90 adjusted for covariates in an MVMR model. To find suitable exposure SNPs we used the
91 UKBiobank, of 502,543 individuals aged between 40 and 69 at recruitment across the UK.^[21]
92 Participants completed detailed health questionnaires and blood samples were taken for
93 genotyping. Of these 353,315 participants have “best measures” of pre-bronchodilator FEV₁
94 and FVC, measured as absolute values in litres. We performed a GWAS on these individuals
95 (adjusting for sex). We also performed a GWAS based on 55,907 cases of airflow obstruction
96 (defined as FEV₁/FVC <0.70) and 297,408 controls (FEV₁/FVC ≥0.70). The SNPs discovered
97 in this unadjusted GWAS were then used in a two-sample MVMR model conditioning with
98 SNPs for covariates of exposure and outcome: standing height, body mass index (BMI) and
99 current smoking. SNPs for these covariates were identified in pre-existing GWAS performed
100 in the UKBiobank.[22] See online supplement for details. NB. Genetic variants function is
101 independent of age and adjusting for it in a two sample MR model is not necessary or possible
102 (as age is not genetically determined). All exposure SNPs were discovered in only European
103 ancestry populations.

104

105 Outcomes

106 We used CARDIOGRAMplusC4D GWAS based on 60,901 cases of coronary artery disease
107 and 123,504 controls, 77% of whom were of European ancestry.[23] Coronary artery disease
108 was defined by myocardial infarction, acute coronary syndrome, chronic stable angina or
109 coronary stenosis of >50%.

110 For stroke we used MEGASTROKE GWAS based on 34,217 cases of acute ischaemic stroke
111 and 406,111 controls, all of European ancestry.[24] There was no overlap between our
112 exposure and outcome population samples.

113 Statistical Analysis

114

115 Statistical analysis was done using R Studio version 3.6.1 with MRCIEU/TwoSampleMR and
116 MRInstruments packages.[17, 25]

117 F-statistics were calculated to assess exposure instruments strength.[26] Linkage
118 disequilibrium clumping (LD-clumping) and Steiger filtering were performed.[25] Duplicate
119 SNPs and palindromic SNPs were removed, and all SNPs were harmonised. Proxies were
120 identified when CAD was the outcome. See appendix 3 for more details.

121

122 Main Mendelian Randomization Analysis

123

124 Inverse Variance Weighting (IVW) was used for main effect estimate for both MVMR and
125 2S-MR analyses. This IVW is a weighted regression of SNP-outcome on SNP-exposure
126 associations combined.

127

128 **Results**

129 Shrine et al preliminary analysis

130 Due to collider bias, results from this analysis should be interpreted with caution. When
131 adjusting for a covariate the effect estimate of the SNP with lung function will be biased
132 by the correlation between the covariate and lung function multiplied by their
133 association with covariate. For example, if a SNP has a strong positive effect on height it
134 would reduce the observed effect on lung function. Adjusting for a covariate in a GWAS
135 could induce an association between SNPs associated with the covariate and the
136 adjusted trait that is inverse to the true association between each SNP and the
137 covariate.[20] This bias in the SNP-exposure association will feed through to any MR
138 estimates obtained using it and could lead to bias in the MR estimates obtained, either
139 towards or away from the null. The implications for MR estimates from covariate
140 adjusted GWAS are explained in detail elsewhere. [27]. [Please see appendix 8 for](#)
141 [directed acyclic graph and further detail.](#)

142 All analysis showed weak evidence of an effect, variable direction of effect and wide
143 confidence intervals. These results are reported in further detail in the supplementary
144 information. We proceeded with MVMR as our main analysis as a more robust method able
145 to account for collider bias.

146 MVMR

147 Using a threshold of $p < 5 \times 10^{-8}$, after quality control and LD-clumping the unadjusted GWAS
148 of lung function in UKBiobank produced 360 SNPs for FEV₁, 464 SNPs for FVC and 154
149 SNPs for FEV₁/FVC <0.70 explaining 3.6%, 4.8% and 0.9% of variance respectively. F-
150 statistic for FEV₁ = 38, FVC = 40 and Ratio <0.7 = 36. For covariates, F-statistic for standing
151 height, BMI and current smoking were 50, 39 and 32 respectively.

152

153

154 MVMR analysis – FEV₁ and FVC as exposure, CAD as outcome

155 Results are presented as per SD decrease in lung function trait. Analysis showed strong
156 evidence of an increased risk of CAD per SD decrease in FVC (OR:1.32 per SD; 95% CI:
157 1.19-1.46) as shown in **Table 1**. This effect did not attenuate after conditioning for BMI
158 (1.41; 1.25-1.59) or current smoking (1.32; 1.19-1.47) but was weaker after conditioning for
159 height (OR: 1.22; 1.03-1.44).

160

161 Table 1. Multivariable MR results of FEV₁ and FVC on Coronary Artery Disease and
162 Ischaemic Stroke using UKBiobank lung function GWAS

163

Lung function trait	Condition	No. SNPs (LF/condition)	OR (95% CI)* for Coronary Artery Disease	No. SNPs (LF/condition)	OR (95% CI)* for Ischaemic Stroke
FEV ₁	Nil	300/Nil	1.27 (1.12, 1.44)	291/Nil	1.11 (0.97-1.26)
FEV ₁	Height	194/744	1.08 (0.89, 1.30)	193/741	1.01 (0.83, 1.22)
FEV ₁	BMI	179/645	1.26 (1.08, 1.47)	185/660	1.03 (0.88, 1.20)
FEV ₁	Smoking	274/15	1.26 (1.10, 1.44)	273/12	1.11 (0.95, 1.29)
FVC	Nil	391/Nil	1.32 (1.19-1.46)	384/Nil	1.12 (1.01-1.24)
FVC	Height	272/726	1.22 (1.03, 1.44)	273/728	1.04 (0.88, 1.24)
FVC	BMI	227/599	1.41 (1.25, 1.59)	227/607	1.05 (0.93, 1.19)
FVC	Smoking	359/15	1.32 (1.19, 1.47)	368/11	1.11 (1.00, 1.23)

164 *per SD decrease in lung function trait

165 OR – Odds Ratio. 95% CI – 95% Confidence Interval. LF – Lung Function

166

167 ~~There is strong~~ Prior to any conditioning, there was evidence that reduced FEV₁ increases risk
168 of CAD (OR: 1.27 per SD; 95% CI: 1.12-1.44). However, when conditioning for height the
169 effect size decreases with widening of the confidence interval which cross 1.0 (1.08; 0.89-

170 1·30) **Table 1.** This is probably due to the pleiotropy in the MR analysis as the unadjusted
171 GWAS would have discovered SNPs that affected LF via height. Therefore, there is limited
172 evidence of a direct effect of FEV₁ on cardiovascular risk. Conditioning for BMI (1·26; 1·08-
173 1·47) and current smoking (1·26; 1·10-1·44) made minimal difference to the estimated effect.

174

175 MVMR analysis – FEV₁ and FVC as exposure, ischaemic stroke as outcome

176 There is little evidence to suggest that reduced FEV₁ increases the risk of ischaemic stroke
177 (OR: 1·11 per SD; 95% CI: 0·97-1·26) **Table 1.** The magnitude decreased further when
178 conditioning for both height and BMI, although the direction remained consistent. There is
179 evidence that a decrease in FVC increases risk of ischaemic stroke (1·23; 1·01-1·24) but the
180 effect size and strength of evidence attenuates after conditioning for height or BMI (1·16;
181 0·98-1·38 and 1·05; 0·93-1·19 respectively). Results for effects of FEV₁ and FVC on CAD
182 and ischaemic stroke after conditioning for all covariates together are in supplementary
183 information appendix 4.

184

185 MVMR analysis – FEV₁/FVC ratio <0·7 as exposure, CAD and ischaemic stroke as outcomes

186 Steiger filtering removed 87 SNPs for FEV₁/FVC ratio <0·7 with CAD as the outcome and 96
187 SNPs with ischaemic stroke as the outcome. We found very little evidence of an effect of
188 liability to airflow obstruction on CVD as can be seen in **Table 2.** ~~These results may be due to~~
189 ~~weak instruments, or they could be supporting the evidence that reduced FVC has more of an~~
190 ~~effect on CVD than obstructive ratio or low FEV₁.~~

191

192 Table 2. Multivariable MR results of and FEV₁/FVC <0.7 on Coronary Artery Disease and
 193 Ischaemic Stroke using UKBiobank lung function GWAS

Trait	Condition upon	No SNPs (LF/condition)	OR (95% CI)* for Coronary Artery Disease	No. SNPs (LF/condition)	OR (95% CI)* for Ischaemic Stroke
FEV ₁ /FVC <0.7	Nil	50/Nil	1.00 (0.60, 1.67)	39/Nil	0.96 (0.52, 1.79)
FEV ₁ /FVC <0.7	Smoking	49/17	1.00 (0.83, 1.21)	38/13	0.98 (0.82, 1.16)

194 *per SD increase in liability to ratio <0.7

195

196

197 **Discussion**

198

199 This MVMR study provides evidence that a one standard deviation reduction in FVC *causes*
 200 approximately a 20% increased risk of CAD. This finding confirms causality of previous
 201 observational associations.[5, 6] These results are unlikely to be affected by reverse causation
 202 or confounding factors due to the use of SNPs as instrumental variables. This effect was not
 203 seen in the preliminary non-MVMR analysis because of collider bias introduced to the model
 204 by covariate adjustment in the Shrine et al discovery GWAS. Our main analysis used MVMR
 205 which is a robust tool when a secondary exposure acts as a confounder, a mediator, a
 206 pleiotropic pathway and a collider.[28]

207 Although historically, most observational studies of cardiovascular morbidity have focused on
 208 FEV₁ and COPD, we found little evidence of a causal association between FEV₁ and liability
 209 to obstructive ratio on CVD risk. These results mirror findings that FVC is stronger predictor
 210 of overall survival than FEV₁. [3] Our findings suggests that the observed association between
 211 low FEV₁, obstruction and increased risk of CVD is unlikely to be causal. In healthy
 212 individuals, FEV₁ and FVC are highly correlated. Therefore, we hypothesise that the

213 unknown underlying biological mechanism linking lung function and cardiovascular disease
214 may be specific to FVC reduction.

215 Finding modifiable risk factors for CAD is important, however the majority of therapies
216 designed to improve lung function (such as inhaled bronchodilators) have a temporary and
217 limited impact on FVC and so are unlikely to be sufficient to modify cardiovascular risk.
218 Available treatments which do target decline in FVC are for specific and rare lung disease
219 such as pulmonary fibrosis.[29]

220 There are a number of strengths to our study, first it utilises large numbers of instrumental
221 variables, far more than were available in previous MR studies.[30] Second we used [a huge](#)
222 [exposure](#) sample populations and multiple robust methods and adhered to rigorous proposed
223 STROBE guidelines for MR papers.[31]. By using MR we accounted for unmeasured
224 confounding and reverse causation, problems typical of conventional observational
225 epidemiology and establish causality by the use of randomly assigned genetic instrumental
226 variables.[13, 32, 33] In addition, our study benefited from using MVMR to condition for
227 these covariates avoiding collider bias that could have contributed to the weak evidence found
228 in our preliminary analysis using the Shrine et al GWAS.[19] MVMR estimates the direct,
229 rather than total effect of an exposure allowing us to show that much of the effect of FEV₁ on
230 CAD risk was due to pleiotropic SNPs affecting FEV₁ via height (an established determinant
231 of cardiovascular risk). Finally, this is the first study to use SNPs for FEV₁/FVC <0.7 ratio.
232 MR has assumptions and is vulnerable to certain biases if not used properly. The sensitivity
233 analysis using plots, MR Egger, weighted median and mode did not indicate any violation of
234 assumptions. The use of Steiger filtering reduces the risk of reverse causality.

235 Limitations

236 Our exposure GWAS and the MEGASTROKE used only those of European heritage. The
237 CARDIOGRAMplusC4D GWAS was 23% non-European heritage. LF SNPs discovered in

238 European ancestral populations in the Shrine GWAS have been shown to have a smaller
239 effect in non-European populations.[19] As our own UKBiobank GWASs used a high
240 proportion of the same sample examining similar traits, it is likely that in a non-European
241 population the effects would be smaller. We did not have access to another sample population
242 to estimate the effects of SNPs discovered in our GWAS. As our SNPs were discovered and
243 effects estimated in the same population, the effects could have been over estimated due to
244 “Winner’s Curse” phenomena.[34] There was a reduction in number of instruments available
245 for analysis following LD-clumping, removal of duplicates, and extraction from exposure and
246 outcome GWAS. This reduces the strength of the instruments which may have reduced the
247 power to show an effect of FEV₁ or FEV₁/FVC <0.7 ratio. In our MVMR analysis we used
248 FEV₁/FVC <0.7 ratio as an exposure because this is a commonly used, threshold of
249 obstructive lung function. Using FEV₁/FVC ratio as a continuous trait has inherent issues in
250 MR analysis. High FEV₁/FVC ratio is a sign of restriction and low FEV₁/FVC ratio defines
251 airflow obstruction, both of which are pathological states that could affect cardiovascular
252 disease, making interpretation of the continuous variable challenging. Most MR analysis
253 assumes a linear effect, which would be violated when using FEV₁/FVC as a continuous trait.
254 Dichotomization of continuous traits in MR studies can make interpretation of the causal
255 estimate less reliable, but MR can still be a valid test of the causal null hypothesis for a binary
256 exposure.[35] An assumption of MR is that SNPs only affect the outcome via the exposure.
257 To ensure that our SNPs were not affecting our outcomes via amount smoked we checked to
258 see if any of our lung function SNPs are found in the 15q25 locus.[36] In the MVMR analysis
259 for FEV₁ only one SNP (rs72736802) is from the locus, none from the FVC analysis.
260 Therefore, we do not think this will affect our results. Lung function is a complex trait and
261 SNPs affect LF via differing pathological processes.[19] The differing processes may vary in
262 their impact on the risk of co-morbidities, perhaps reflected in the assessments of

263 heterogeneity. It is possible our study was limited by the number of ischaemic stroke cases in
264 the outcome population. If there is a causal effect of lung function on ischaemic stroke, it is
265 likely to only occur with large changes in lung function as seen with CAD.

266 Implications

267 There are several important implications of our findings, first is that it is Forced Vital
268 Capacity not obstructive lung function that is causally associated with coronary artery disease.
269 This suggests that we should focus our attention on understanding the mechanisms by which
270 FVC causes CAD. Second given, there are limited FVC specific therapies, it is most likely
271 that future interventions to improve CAD outcomes through modifying FVC are most likely
272 to be achieved through environmental/ behavioural public health interventions designed to
273 achieve optimal lung development and preventing lung function decline. Third, FVC is a
274 widely and routinely collected clinical measure (spirometry), this study supports the call for
275 FVC measurements to be evaluated as part of cardiovascular prognostication / secondary
276 prevention risk assessments.

277 It remains uncertain if lung function has a causal effect on the risk of ischaemic stroke.

278 ~~Although~~ Our MVMR models show very little weak evidence that reduced lung function
279 increases the risk of ischaemic stroke, ~~the evidence is weak~~. Larger outcome sample sizes ~~and~~
280 ~~more SNPs~~ may become available as genetic consortia grow which could provide more
281 conclusive results. Future studies are needed to determine the mechanism by which FVC
282 causes increased coronary artery disease.

283

284 Conclusions

285 There is strong evidence that reduced Forced Vital Capacity (FVC) is independently and
286 causally associated with Coronary Artery Disease. Although the mechanism remains unclear,
287 FVC may play an important contribution to the assessment of cardiovascular risk. Further

288 studies are needed to test whether interventions to improve or maintain FVC may also modify
289 cardiovascular risk. FEV₁ and Obstructive lung function do not appear to cause increased
290 cardiovascular events, confounding and collider bias may explain previous observational and
291 MR findings of a causal association.

292

293

294 **Acknowledgement**

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297

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