Evidence for Large-Scale Gene-by-Smoking Interaction Effects on Pulmonary Function

Hugues Aschard, Martin D. Tobin, Dana B. Hancock, David Skurnik, Akshay Sood, Alan James, Albert Vernon Smith, Ani W. Manichaikul, Archie Campbell, Bram P. Prins, Caroline Hayward, Daan W Loth, David J. Porteous, David P Strachan, Eleftheria Zeggini, George O'Connor, Guy G. Brusselle, H. Marike Boezen, Holger Schulz, Ian J. Deary, Ian P. Hall, Igor Rudan, Jaakko Kaprio, James F. Wilson, Jemma B. Wilk, Jennifer E. Huffman, Jing Hua Zhao, Kim de Jong, Leo-Pekka Lyytikäinen, Louise V. Wain, Marjo-Riitta Jarvelin, Mika Kähönen, Myriam Fornage, Ozren Polasek, Patricia A. Cassano, R. Graham Barr, Rajesh Rawal, Sarah E. Harris, Sina A. Gharib, Stefan Enroth, Susan R. Heckbert, Terho Lehtimäki, Ulf Gyllensten, Understanding Society Scientific Group, Victoria E. Jackson, Vilmundur Gudnason, Wenbo Tang, Josée Dupuis, María Soler Artigas, Amit D. Joshi, Stephanie J. London, Peter Kraft

Online Data Supplement

Single SNP-by-smoking interaction

Assuming that $E = (E_1, .. E_3)$ is a vector of smoking exposures including smoking status (ever/never), current smoking, and pack-years, E_k is the exposure tested for interaction (either smoking status or pack-years), and Y is the outcome (either FEV_1 or FEV_1/FVC), the parent study by Hancock et al.[9] they uses a model that saturate the main effect of smoking but only included a single interaction term:

$$Y \sim \beta_0 + \beta_G G + \beta_{GE_k} G E_k + \sum_{l=1...3} \beta_{E_l} E_l$$

where β_G and β_{E_l} are the main effect of G and exposure E_l, β_{GE_k} is the interaction effect between G and exposure E_k, and β_0 the intercept. For each single nucleotide polymorphisms (SNP), each outcome, and each interacting exposure E_k, Hancock et al.[1] used $\hat{\beta}_G$ and $\hat{\beta}_{GE_k}$, their variance $\hat{\sigma}_{\beta_G}$ and $\hat{\sigma}_{\beta_{GE_k}}$, and their covariance from *Equation 1* estimated within each study, and derived a meta-analysis joint test of β_G and β_{GE_k}. In this study, we used only the estimate of the interaction effect and its standard deviation ($\hat{\beta}_{GE_k}$ and $\hat{\sigma}_{\beta_{GE_k}}$), derived across all studies as part of the aforementioned meta-analysis to perform multivariate tests of interaction effects across multiple genetic variants. Finally, for clarity, main genetic effect refers to the estimated effect of genetic variants among never smokers, derived from a model with the interaction term. In contrast, marginal genetic effect refers to the estimated average genetic effect across all smoking categories, derived from a model without the interaction term.

Model characteristics

The main effect of smoking variables in the interaction model $\beta_{E_l}, l = (1, \dots 3)$ in *Equation 1* were not available in the summary statistics data. However their marginal effects, derived in a multivariate model similar to the one used in the genome-wide association (GWAS) but without the genetic component (no SNP main effect or SNP-by-smoking interaction effect), were available for each of the 19 studies.[1] For each of the three smoking exposures E, we first derived γ_{mE}, their marginal effect over all studies, using a standard inverse variance-weighted meta-analysis of study-specific estimates.

Using γ_{mE}, we then estimated $\gamma_{E_l}, l = (1, \dots 3)$, the main effect of smoking exposure from the genetic risk score (GRS)-by-smoking interaction model (*Equation 2*). For exposures $E_{l \neq k}$ not modeled to interact with the SNPs, the main effect was assumed to be equal to the marginal effect ($\gamma_{E_{l \neq k}} = \gamma_{mE_{l \neq k}}$). For E_k, the interacting exposure, the main effect estimate was approximated using the relationship defined in [2]:

$$\gamma_{E_k} = \gamma_{mE_k} - \gamma_{INT} \times \mu_{GRS}$$

where γ_{INT} is the interaction effect between the GRS and E_k, and μ_{GRS} is the mean of the GRS. The validity of this approximation mostly relies on independence between the GRS and E_k, but remains valid for low to moderate correlation (e.g., <0.1).

We then derived the mean and variance of each exposure across all studies using the sample size-weighted average. Study-specific descriptive statistics were available for all studies for ever/never smoking and pack-years, and for the largest studies for current smoking (Framingham Heart Study, Cardiovascular Health Study, Atherosclerosis Risk in Communities, LifeLines, European Prospective Investigation into Cancer and Nutrition, and British 1958 Birth Cohort). The means were used to approximate γ_0, the intercept of the interaction models with the GRS (*Equation 2*). Because both outcomes were standardized to have mean 0, γ_0 equals the opposite of the average effect of all predictors:

$$\gamma_0 = -\gamma_{GRS} \times \mu_{GRS} - \gamma_{INT} \times \mu_{GRS \times E_k} - \sum\nolimits_{l=1 \dots 3} (\gamma_{E_l} \times \mu_{E_l})$$

where $\mu_{.}$ are the mean of the predictors considered.

Derivation of Relative risk in ever smokers against never-smokers

We aimed at estimating the joint probability of having both FEV_1/FVC in the interval $[-\infty, \mathrm{FEV}_1/\mathrm{FVC}_{up}]$ and the GRS in the interval $[GRS_{low}, GRS_{up}]$, which can be expressed as the following integral:

$$\int_{-\infty}^{\mathrm{FEV}_1/\mathrm{FVC}_{up}} \int_{GRS_{low}}^{GRS_{up}} f_1(y|g) \times f_2(g)\, dy\, dg$$

In practice, we derived the bivariate cumulative distribution function of the GRS and FEV_1/FVC independently for ever smokers and never smokers using the R function *pmvnorm* from R package *mvtnorm* and the estimated effects from the interaction model. We assumed a normal conditional distribution of $\frac{\mathrm{FEV_1}}{FVC}$, which was standardized in the original analysis (i.e., $\sigma^2_{\frac{\mathrm{FEV_1}}{FVC}} = 1$), so that $\frac{\mathrm{FEV_1}}{FVC} \sim \mathcal{N}(\gamma_0 + \gamma_{GRS} \times \mu_{GRS}, 1)$ in never smokers and $\frac{\mathrm{FEV_1}}{FVC} \sim \mathcal{N}(\gamma_0 + (\gamma_{GRS} + \gamma_{INT}) \times \mu_{GRS} + \gamma_{E_k} + \sum_l (\gamma_{E_l} \times \mu_{E_l} | E_k = 1), 1)$ in ever smokers, where E_k is the ever-never smoking variable, γ_{E_k} its effect as defined in *Equation 2,* and $\mu_{.}$ are the mean of the predictors considered. We assumed the GRS was independent of the smoking variable, so that its distribution simply equals $\mathcal{N}(\mu_{GRS}, \sigma_{GRS})$. The covariance term of the bivariate distributions was defined as the GRS effect specific to each group times the standard deviation of the GRS (i.e., $cov\left(GRS, \frac{\mathrm{FEV_1}}{FVC} \middle| non-smokers\right) = \gamma_{GRS} \times \sigma_{GRS}$, and $cov\left(GRS, \frac{\mathrm{FEV_1}}{FVC} \middle| ever-smokers\right) = (\gamma_{GRS} + \gamma_{INT}) \times \sigma_{GRS}$).

Replication study

Two replication datasets were used. The first dataset included 8,859 unrelated individuals recruited as part of three studies: Lothian Birth Cohort 1936 (LBC1936, n = 991), United Kingdom Household Longitudinal Study (UKHLS, n = 7,449), and Young Finish Study (YFS, n = 419). The second dataset of 9,457 family-based samples included the following: CROATIA-Split (n = 493); GS:SFHS (n = 8,093); and NSPHS (Northern Sweden Population Health Study, n = 871). All datasets already had GWAS results available for marginal genetic effects stratified by ever-never smoking status as part of a recent meta-analysis of FEV_1 and FEV_1/FVC.[3] Detailed description of individual studies can be found in Soler Artigas et al.[3] except for UKLHS, which is described in the next section of this supplement.

Assuming the following stratified models for each SNP G_i, where G_i is coded additively (0, 1, or 2 corresponding to the number of coded allele): $Y_N \sim \gamma_0 + \gamma_{G_{i.never}} \times G_{i.never} + \boldsymbol{\gamma_C} \times \mathbf{C}$ in never smokers, and $Y_S \sim \gamma_0 + \gamma_{G_{i.ever}} \times G_{i.ever} + \boldsymbol{\gamma_C} \times \mathbf{C}$ in ever smokers, where γ_0 is the intercept, $\gamma_{G_{i.never}}$ is the marginal genetic effect in never smokers and $\gamma_{G_{i.ever}}$ is the marginal genetic effect in ever smokers, and $\boldsymbol{\gamma_C}$ is the effect of the covariates $\mathbf{C}$. Single-SNP interaction effect estimates ($\hat{\beta}_{INT_i}$) and standard error ($\hat{\sigma}_{\beta_{INT_i}}$) were approximated using the following equations:

$$\hat{\beta}_{INT_i} = \hat{\gamma}_{G_{i.ever}} - \hat{\gamma}_{G_{i.never}}$$

$$\hat{\sigma}_{\beta_{INT_i}} = \sqrt{\hat{\sigma}^2_{\gamma_{G_{i.ever}}} + \hat{\sigma}^2_{\gamma_{G_{i.never}}} - 2\,\rho\,\hat{\sigma}_{\gamma_{G_{i.ever}}}\hat{\sigma}_{\gamma_{G_{i.never}}}}$$

Where ρ is the Spearman rank correlation estimates between $\hat{\gamma}_{G_{i.ever}}$ and $\hat{\gamma}_{G_{i.never}}$ derived across all SNPs from the GWAS. However, for cohorts of unrelated individuals, we assumed $\rho = 0$, so that $\hat{\sigma}_{\beta_{INT_i}}$ simplifies to:

$$\hat{\sigma}_{\beta_{INT_i}} = \sqrt{\hat{\sigma}^2_{\gamma_{G_{i.ever}}} + \hat{\sigma}^2_{\gamma_{G_{i.never}}}}$$

We then performed a meta-analysis of each SNP G_i across K studies using standard inverse-variance formula, that is:

$$\hat{\beta}_{INT_i.META} = \frac{\sum_K \frac{\hat{\beta}_{INT_i}}{\hat{\sigma}^2_{\beta_{INT_i}}}}{\sum_K \frac{1}{\hat{\sigma}^2_{\beta_{INT_i}}}}$$

$$\hat{\sigma}^2_{INT_i.META} = \frac{1}{\sum_K \frac{1}{\hat{\sigma}^2_{\beta_{INT_i}}}}$$

GRS-by-ever smoking interaction was then derived using the approach described in the Method section.

Note that this approach has limitations and might lead to biased interaction effect estimates in the presence of covariates associated with both the exposure and the outcome. For illustration purposes we generated series of 1,000 simulations each including 10,000 individuals. For each series three outcomes were simulated as a function of a genotype G with minor allele frequency 0.3, a binary exposure E_1 with frequency 0.5, and two normally distributed continuous exposures $E_2$and E_3present in either unexposed only or exposed only (i.e., had value of zero in either exposure strata).

$$Y_1 = \beta_0 + \beta_G G + \beta_{E_1} E_1 + \beta_{GE_1} GE_1 + \varepsilon$$

$$Y_2 = \beta_0 + \beta_G G + \beta_{E_1} E_1 + \beta_{E_2} E_2 + \beta_{GE_1} GE_1 + \varepsilon$$

$$Y_3 = \beta_0 + \beta_G G + \beta_{E_1} E_1 + \beta_{E_3} E_3 + \beta_{GE_1} GE_1 + \varepsilon$$

We plotted in **Supplementary Figure S6** the point estimates and power for the standard interaction model applied to Y_1, and the stratified approach described above and applied to Y_2 and Y_3. Both of the later analyses display biased estimates with magnitude increasing with β_{E_2} and β_{E_3}, respectively.

UKHLS

The UKHLS, also known as Understanding Society (https://www.understandingsociety.ac.uk), is a longitudinal panel survey of 40,000 households (England, Scotland, Wales, and Northern Ireland) that are representative of the UK population. Beginning in 2009, participants are surveyed annually and contribute information relating to their socioeconomic circumstances, attitudes, and behaviors via a computer-assisted interview. The study includes phenotypic data for a representative sample of participants for a wide range of social and economic indicators and a biological sample collection encompassing biometric, physiological, biochemical, and hematological measurements and self-reported medical history and medication use. The UKHLS has been approved by the University of Essex Ethics Committee, and informed consent was obtained from every participant.

For a subset of individuals who took part in a nurse health assessment, blood samples were taken and genomic DNA extracted. Of these, 10,484 were genotyped at the Wellcome Trust Sanger Institute using the Illumina Infinium HumanCoreExome-12 v1.0BeadChip.

Lung function measures in samples from England and Wales were conducted with the NDD Easy On-PC spirometer (NDD Medical Technologies, Zurich, Switzerland). Participants were excluded in the following cases: pregnancy, having had abdominal or chest surgery in the past 3 weeks, admitted to the hospital with a heart complaint in the past 6 weeks, having had eye surgery in the past 4 weeks, or having a tracheostomy. Subjects were asked to perform up to eight blows that ideally lasted at least 6 seconds, uninterrupted by coughing, glottis closure, laughing, or leakage of air. Upon completion, the measurements were rated either acceptable or unacceptable by the NDD Easy On-PC software.

The study included 3,293 males (44.2%) and 4,509 (60.5%) ever smokers. Average age was 53.10 (SD=15.94), average FEV_1 (in liter) was 2.89 (SD=0.90), and average FEV_1/FVC was 0.753 (SD=0.09).

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Supplementary Table 1. Effect estimates from SNPs associated with cross-sectional FEV_1 or FEV_1/FVC measures.

chr	Gene	SNP	MAF*	A1	*FEV_1*			*FEV_1/FVC*			
					beta	*sd*	p	beta	*sd*	P	N
1	MFAP2	rs2284746	0.499	G	0.008	*0.007*	0.278	-0.042	*0.007*	2.47×10^{-9}	45944
1	TGFB2	rs993925	0.305	T	0.025	*0.007*	0.00151	0.04	*0.007*	2.54×10^{-7}	42402
2	HDAC4	rs12477314	0.201	T	0.032	*0.008*	0.000277	0.052	*0.008*	4.48×10^{-9}	45585
2	TNS1	rs2571445	0.396	G	0.047	*0.007*	9.83×10^{-11}	0.033	*0.007*	4.46×10^{-6}	45839
3	RARB	rs1529672	0.160	C	-0.037	*0.009*	0.000178	-0.06	*0.009*	7.75×10^{-10}	40624
3	MECOM	rs1344555	0.203	T	-0.042	*0.008*	1.91×10^{-6}	-0.019	*0.008*	0.0261	46067
4	FAM13A	rs2045517	0.400	T	-0.012	*0.007*	0.0893	-0.047	*0.007*	2×10^{-11}	47675
4	GSTCD-NPNT	rs10516526	0.066	G	0.108	*0.014*	4.75×10^{-14}	0.039	*0.014*	0.00617	47970
4	HHIP	rs11100860[a]	0.441	G (T)	0.047	*0.007*	4.27×10^{-9}	0.064	*0.007*	6.81×10^{-20}	47876
5	SPATA9	rs153916	0.454	T	-0.001	*0.007*	0.891	-0.033	*0.007*	2.06×10^{-6}	47530
5	ADAM19	rs11134779	0.359	G	-0.027	*0.007*	0.00024	-0.042	*0.007*	6.01×10^{-9}	48075
5	HTR4	rs11168048[b]	0.402	T (G)	-0.048	*0.007*	2.43×10^{-10}	-0.047	*0.007*	5.97×10^{-11}	44976
6	ZKSCAN3	rs6903823	0.206	G	-0.046	*0.008*	2×10^{-7}	-0.027	*0.008*	0.00228	47057
6	NCR3	rs2857595	0.160	G	0.04	*0.009*	1.46×10^{-5}	0.049	*0.009*	7.86×10^{-8}	45540
6	ARMC2	rs2798641	0.179	T	-0.046	*0.009*	5.39×10^{-7}	-0.047	*0.009*	2.81×10^{-7}	46369
6	AGER	rs2070600	0.050	T	0.025	*0.016*	0.127	0.126	*0.016*	9.07×10^{-15}	46314
6	LOC153910[c]	rs262129	0.294	G	0.031	*0.008*	5.44×10^{-5}	0.056	*0.008*	2.91×10^{-13}	47014
9	PTCH1	rs16909859	0.090	G	-0.014	*0.013*	0.293	0.08	*0.013*	7.45×10^{-10}	43353
10	CDC123	rs7068966	0.492	T	0.04	*0.007*	1.19×10^{-8}	0.045	*0.007*	1.28×10^{-10}	47085
10	C10orf11	rs11001819	0.470	G	-0.041	*0.007*	1.42×10^{-8}	-0.019	*0.007*	0.0065	45546
12	LRP1	rs11172113	0.396	T	-0.021	*0.007*	0.00355	-0.035	*0.007*	1.36×10^{-6}	45387
12	CCDC38	rs1036429	0.186	T	0.01	*0.008*	0.267	0.049	*0.008*	1.24×10^{-8}	47814
15	THSD4	rs8033889	0.202	T	-0.044	*0.009*	3.01×10^{-7}	-0.072	*0.008*	2.03×10^{-17}	46995
16	MMP15	rs12447804	0.195	T	-0.017	*0.009*	0.0802	-0.053	*0.009*	7.12×10^{-8}	35123
16	CFDP1	rs2865531	0.429	T	0.024	*0.007*	0.00063	0.039	*0.007*	2.3×10^{-8}	47594
21	KCNE2	rs9978142	0.144	T	-0.012	*0.009*	0.247	-0.048	*0.009*	8.23×10^{-7}	44577

Effect estimates and standard deviation of the 26 selected SNPs were extracted from stage 1 analysis of Soler Artigas et al.[3] We only included SNPs that were analyzed using at least 50% of the total sample at stage 1 (N>24,100). A1 is the coded allele.

* *From 1000Genomes European population.*

[a] *SNP rs1032296 was used instead of rs11100860 for FEV_1.*

[b] *SNP rs1985524 was used instead of rs11168048 for FEV_1.*

[c] *This locus is adjacent to the originally implicated GPR126 gene.*

Supplementary Table 2. Significance of univariate interaction effects for the 26 selected SNPs.

SNP ID	FEV_1				FEV_1/FVC			
	Smoking status		*Pack-year*		*Smoking status*		*Pack-year*	
	beta	*P-val*	*beta*	*P-val*	*beta*	*P-val*	*Beta*	*P-val*
rs2284746	0.005	0.66	$3.4x10^{-5}$	0.61	-0.003	0.78	$3.9x10^{-5}$	0.56
rs993925	-0.036	**0.0070**	$-1.9x10^{-5}$	0.80	-0.011	0.44	$-3.4x10^{-5}$	0.68
rs12477314	-0.004	0.81	$-9.0x10^{-6}$	0.92	0.003	0.87	$-1.9x10^{-4}$	**0.035**
rs2571445	-0.027	**0.040**	$4.1x10^{-5}$	0.56	-0.024	0.070	$1.1x10^{-5}$	0.88
rs1529672	-0.016	0.37	$7.5x10^{-5}$	0.40	-0.028	0.11	$2.1x10^{-4}$	**0.029**
rs1344555	-0.020	0.19	$7.5x10^{-5}$	0.36	-0.008	0.63	$-3.9x10^{-5}$	0.66
rs2045517	-0.008	0.54	$-9.2x10^{-5}$	0.18	-0.027	**0.039**	$-3.4x10^{-5}$	0.66
rs10516526	-0.035	0.16	$-2.2x10^{-4}$	0.12	-0.040	0.11	$-3.9x10^{-5}$	0.77
rs11100860[a]	0.008	0.53	$-1.2x10^{-5}$	0.87	-0.008	0.67	$-3.1x10^{-5}$	0.95
rs153916	-0.008	0.50	$1.1x10^{-4}$	0.090	-0.010	0.45	$-4.0x10^{-5}$	0.56
rs11134779	-0.012	0.36	$-4.6x10^{-5}$	0.51	-0.015	0.24	$6.0x10^{-5}$	0.42
rs11168048[b]	-0.010	0.44	$-2.4x10^{-5}$	0.72	-0.026	**0.028**	$-3.6x10^{-5}$	0.23
rs6903823	0.005	0.72	$4.1x10^{-5}$	0.59	-0.021	0.17	$4.5x10^{-5}$	0.58
rs2857595	-0.024	0.12	$-1.2x10^{-4}$	0.13	-0.028	0.080	$9.8x10^{-6}$	0.91
rs2798641	-0.002	0.89	$-1.7x10^{-4}$	**0.041**	-0.026	0.11	$-1.7x10^{-5}$	0.86
rs2070600	-0.022	0.46	$-1.1x10^{-4}$	0.39	0.019	0.51	$-7.0x10^{-5}$	0.62
rs262129	-0.001	0.97	$-8.6x10^{-5}$	0.25	0.001	0.95	$7.0x10^{-5}$	0.34
rs16909859	0.014	0.53	$2.2x10^{-6}$	0.99	0.032	0.17	$3.8x10^{-6}$	0.98
rs7068966	-0.005	0.66	$1.9x10^{-5}$	0.78	-0.021	0.090	$-1.6x10^{-4}$	**0.024**
rs11001819	0.001	0.94	$-3.3x10^{-5}$	0.62	0.022	0.080	$-2.0x10^{-5}$	0.77
rs11172113	0.011	0.41	$1.5x10^{-4}$	**0.033**	0.000	0.97	$-4.3x10^{-5}$	0.54
rs1036429	-0.005	0.72	$3.0x10^{-5}$	0.71	-0.022	0.14	$2.5x10^{-5}$	0.76
rs8033889	0.007	0.62	$-5.1x10^{-5}$	0.52	0.006	0.71	$-1.1x10^{-5}$	0.90
rs12447804	-0.005	0.78	$-5.2x10^{-4}$	0.28	-0.014	0.42	$-7.0x10^{-4}$	0.15
rs2865531	-0.011	0.39	$3.2x10^{-5}$	0.62	0.017	0.18	$9.3x10^{-5}$	0.19
rs9978142	0.017	0.33	$5.5x10^{-5}$	0.58	-0.020	0.26	$1.6x10^{-4}$	0.14

Nominally significant tests are indicated in bold. Betas were derived for the trait-decreasing alleles based on Table E1. SNPs are listed in order of chromosomal position as in Table S1.

[a] SNP rs1032296 was used instead of rs11100860 for FEV_1.

[b] SNP rs1985524 was used instead of rs11168048 for FEV_1.

Supplementary Table 3. Descriptive statistics of the 19 studies used in the initial screening.

Study (Country of origin)	Sample size *N (% female)*	Age (year) *Mean (SD)*	Height (cm) *Mean (SD)*	Never-smokers *N (%)*	Ever-smokers *N (%)*	Pack-years *Mean (SD)*	FEV_1 (mL) *Mean (SD)*	FVC (mL) *Mean (SD)*	FEV_1/FVC (%) *Mean (SD)*
AGES (Iceland)	1,696 *(59.4)*	76.2 *(5.6)*	166.7 *(9.4)*	813 *(47.9)*	883 *(52.1)*	24.5 *(21.9)*	2,128 *(690)*	2,865 *(848)*	73.9 *(10.5)*
ARIC (US)	8,934 *(52.7)*	54.3 *(5.7)*	168.8 *(9.4)*	3,620 *(40.5)*	5,314 *(59.5)*	28.9 *(21.6)*	2,943 *(744)*	3,993 *(980)*	73.7 *(7.9)*
B58C (UK)	4,605 *(50.3)*	44.5 *(0.4)*	169.2 *(9.3)*	1,376 *(29.9)*	3,229 *(70.1)*	15.7 *(12.1)*	3,288 *(757)*	4,164 *(980)*	79.5 *(8.1)*
CARDIA (US)	1,605 *(52.8)*	25.6 *(3.3)*	171.3 *(9.3)*	932 *(58.1)*	673 *(41.9)*	5.5 *(5.5)*	3,684 *(810)*	4,702 *(1,010)*	82.2 *(6.4)*
CHS (US)	3,140 *(61.0)*	72.3 *(5.4)*	164.6 *(9.4)*	1543 *(49.1)*	1597 *(50.9)*	33.2 *(26.9)*	2,116 *(659)*	3,005 *(866)*	70.5 *(10.5)*
ECRHS (EU)[1]	1,573 *(50.8)*	33.9 *(7.2)*	170.7 *(9.5)*	699 *(43.9)*	895 *(56.1)*	12.8 *(12.6)*	3,778 *(825)*	4,595 *(1029)*	82.6 *(6.6)*
EPIC obese cases (EU)[2]	1,084 *(57.8)*	59.1 *(8.8)*	165.93 *(9.24)*	489 *(44.3)*	595 *(54.9)*	18.2 *(14.1)*	2,355 *(694)*	2,839 *(872)*	83.8 *(10.2)*
EPIC population-based (EU)[2]	2,294 *(53.6)*	59.1 *(9.0)*	167.0 *(8.9)*	1,062 *(46.3)*	1,232 *(53.7)*	15.8 *(13.4)*	2,500 *(718)*	3,042 *(903)*	83.1 *(10.8)*
FHS (US)	7,694 *(53.9)*	51.9 *(14.6)*	168.5 *(9.7)*	3,556 *(46.2)*	4,138 *(53.8)*	22.8 *(21.5)*	3,038 *(944)*	4,025 *(1,144)*	75.1 *(8.0)*
Health ABC (US)	1,472 *(46.6)*	73.7 *(2.8)*	167.1 *(9.3)*	641 *(43.6)*	831 *(56.5)*	36.6 *(32.0)*	2,312 *(656)*	3,113 *(812)*	74.1 *(7.7)*
LifeLines (Netherlands)	2,616 *(59.9)*	54.2 *(9.5)*	173.0 *(9.1)*	981 *(37.7)*	1,621 *(52.3)*	14.5 *(12.6)*	3,172 *(804)*	4,233 *(1,007)*	75.0 *(7.5)*
MESA (US)	1,403 *(51.0)*	66.0 *(9.7)*	168.5 *(9.7)*	636 *(45.3)*	767 *(54.7)*	27.5 *(24.4)*	2,566 *(763)*	3,505 *(999.6)*	73.4 *(8.4)*
NFBC1966 (Finland)	3,564 *(50.5)*	31 *(0.0)*	171.5 *(9.3)*	1,648 *(46.2)*	1916 *(53.8)*	9.6 *(7.9)*	3,969 *(791)*	4,744 *(989)*	84.1 *(6.5)*
RS-I (Netherlands)	1,196 *(58.9)*	74.4 *(5.7)*	166.7 *(8.9)*	408 *(34.1)*	788 *(65.9)*	24.9 *(19.6)*	2,334 *(735)*	3,183 *(927)*	73.2 *(8.2)*
RS-II (Netherlands)	840 *(55.6)*	67.1 *(6.2)*	168.3 *(8.9)*	287 *(34.2)*	553 *(65.8)*	23.1 *(19.2)*	2,716 *(779)*	3,615 *(1,077)*	75.9 *(9.1)*
RS-III (Netherlands)	1,224 (56.8)	56.6 *(5.6)*	171.2 *(9.3)*	425 *(34.7)*	799 *(65.3)*	18.2 *(16.0)*	3,159 *(851)*	4,059 *(1,138)*	78.4 *(9.0)*
SAPALDIA Switzerland)	1,333 (52.6)	41.1 *(11.2)*	169.4 *(9.0)*	626 *(47.0)*	707 *(53.0)*	17.3 *(18.0)*	3,524 *(860)*	4,494 *(1,038)*	78.5 *(8.2)*
SHIP (Germany)	1,768 (51.2)	52.4 *(13.6)*	169.7 *(9.1)*	770 *(43.6)*	998 *(56.4)*	12.8 *(12.0)*	3,280 *(894)*	3,869 *(1,030)*	84.8 *(6.5)*
TwinsUK (UK)	2,006 (100)	54.2 *(14.1)*	161.8 *(6.4)*	1,242 *(61.9)*	764 *(38.1)*	13.7 *(21.4)*	2,599 *(606)*	3,251 *(650)*	79.7 *(7.7)*

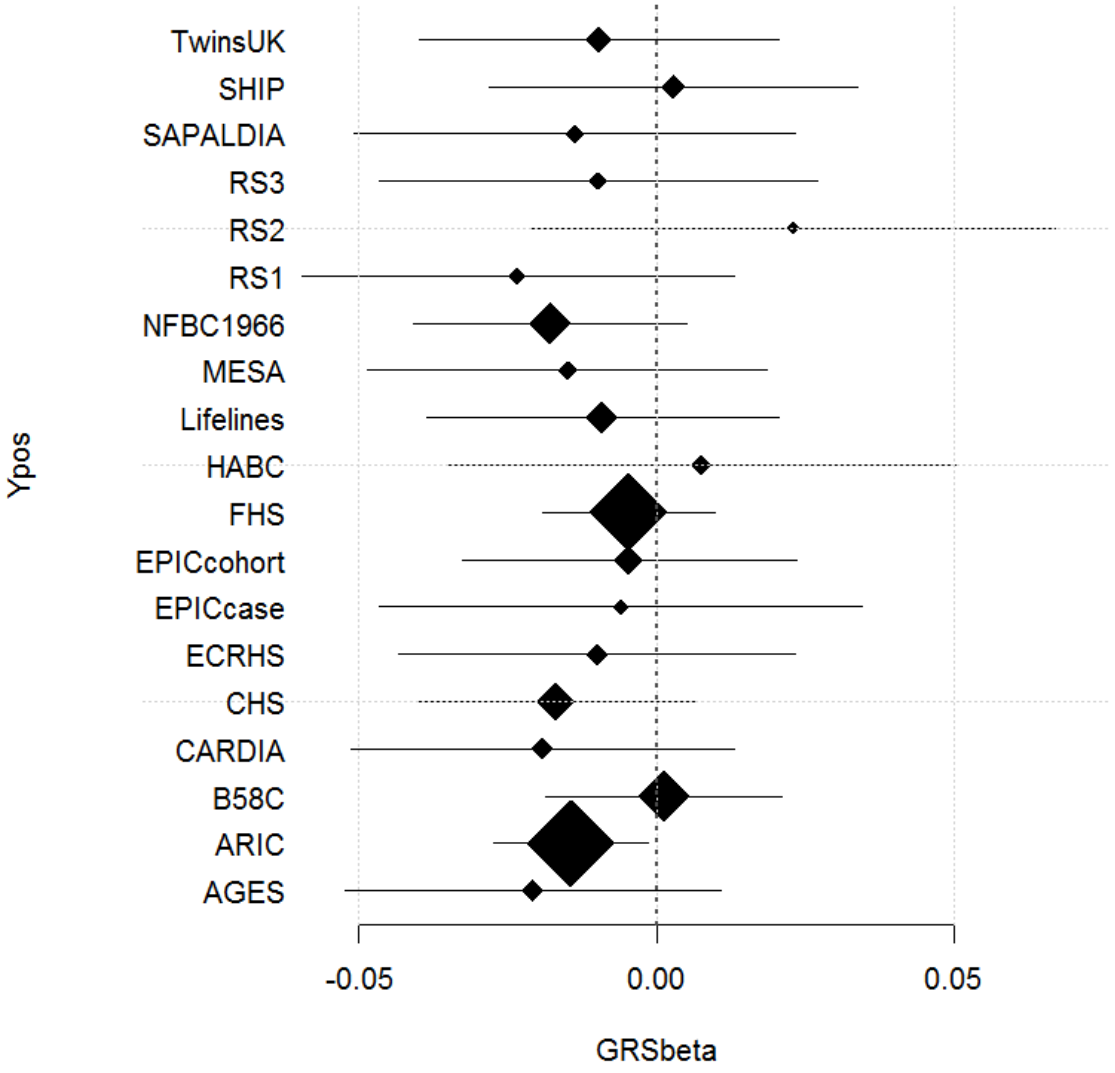
AGES, Age, Gene/Environment Susceptibility; ARIC, Atherosclerosis Risk in Communities; B58C, British 1958 Cohort; CARDIA, Coronary Artery Risk Development in Young Adults; CHS, Cardiovascular Health Study; ECRHS, European Community Respiratory Health Survey; EPIC, European Prospective Investigation into Cancer and Nutrition; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; FHS, Framingham Heart Study; Health ABC, Health, Aging, and Body Composition Study; MESA, Multi-Ethnic Study of Atherosclerosis; NFBC1966, Northern Finland Birth Cohort of 1966; RS, Rotterdam Study (cohorts I-III); SAPALDIA, Swiss Study on Air Pollution and Lung Diseases in Adults; SD, standard deviation; SHIP, Study of Health in Pomerania; SNP, single nucleotide polymorphism.

[1] The genetics data used in ECRHS include participants from 16 centers across 8 European countries (Estonia, France, Germany, Norway, Spain, Sweden, Switzerland, and UK).

[2] EPIC includes participants from 10 European countries: Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden, and the United Kingdom.

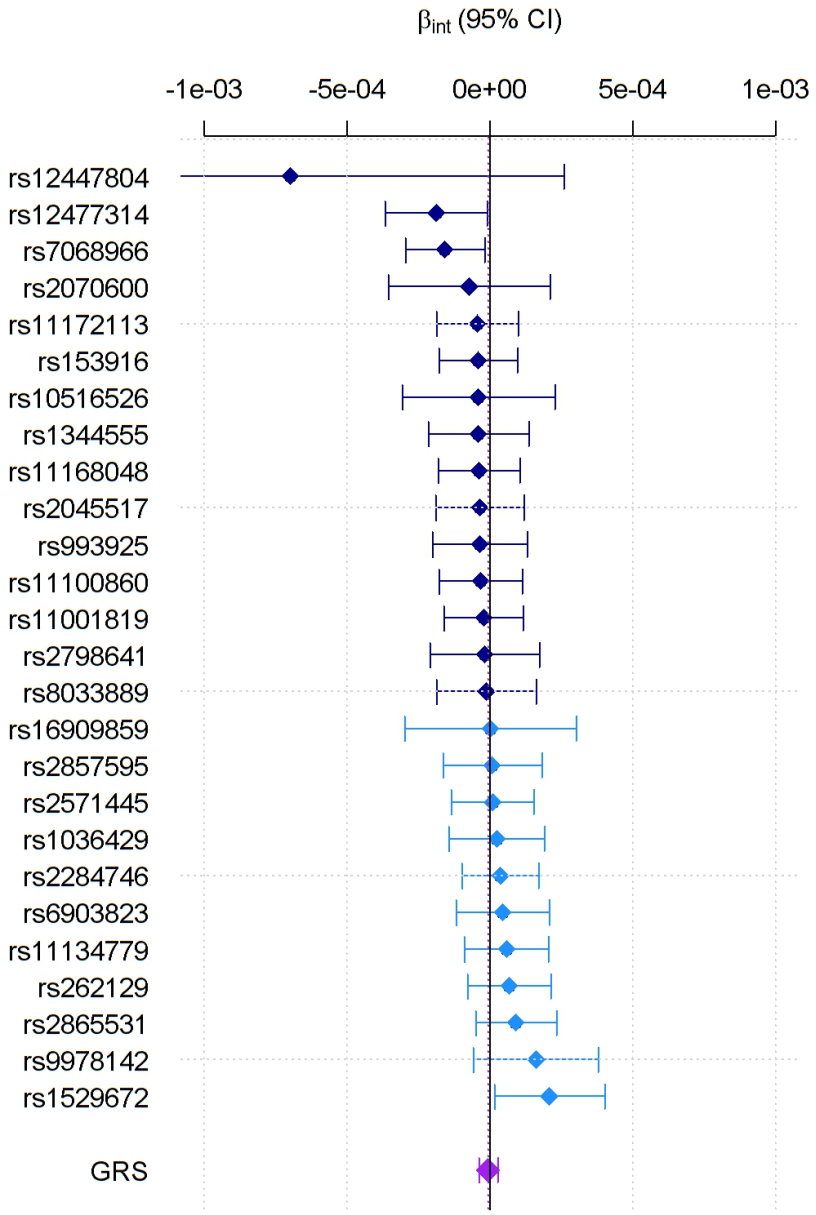
Supplementary Figure 1. Forest plot of the GRS-by-ever smoking effect on FEV_1/FVC.

Effect estimate and 95% confidence interval are plotted for each of the 19 studies.



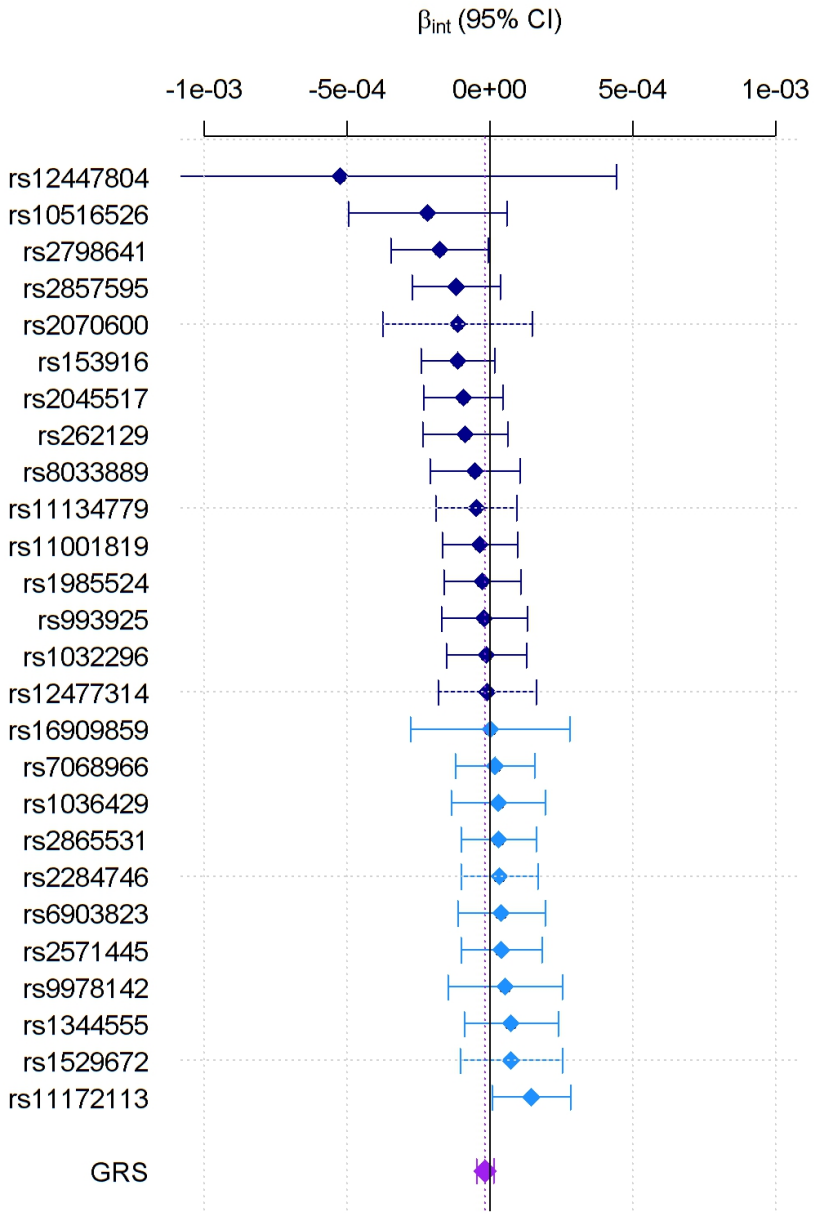
Supplementary Figure 2. Distribution of SNP-by-pack years interaction effects on FEV_1/FVC.

Single SNP risk allele-by-pack years interaction effect estimates (β_{int}) and 95% confidence intervals are plotted by increasing values. Negative and positive interactions are in dark blue and light blue, respectively. The unweighted GRS-by-pack years interaction is plotted in purple.



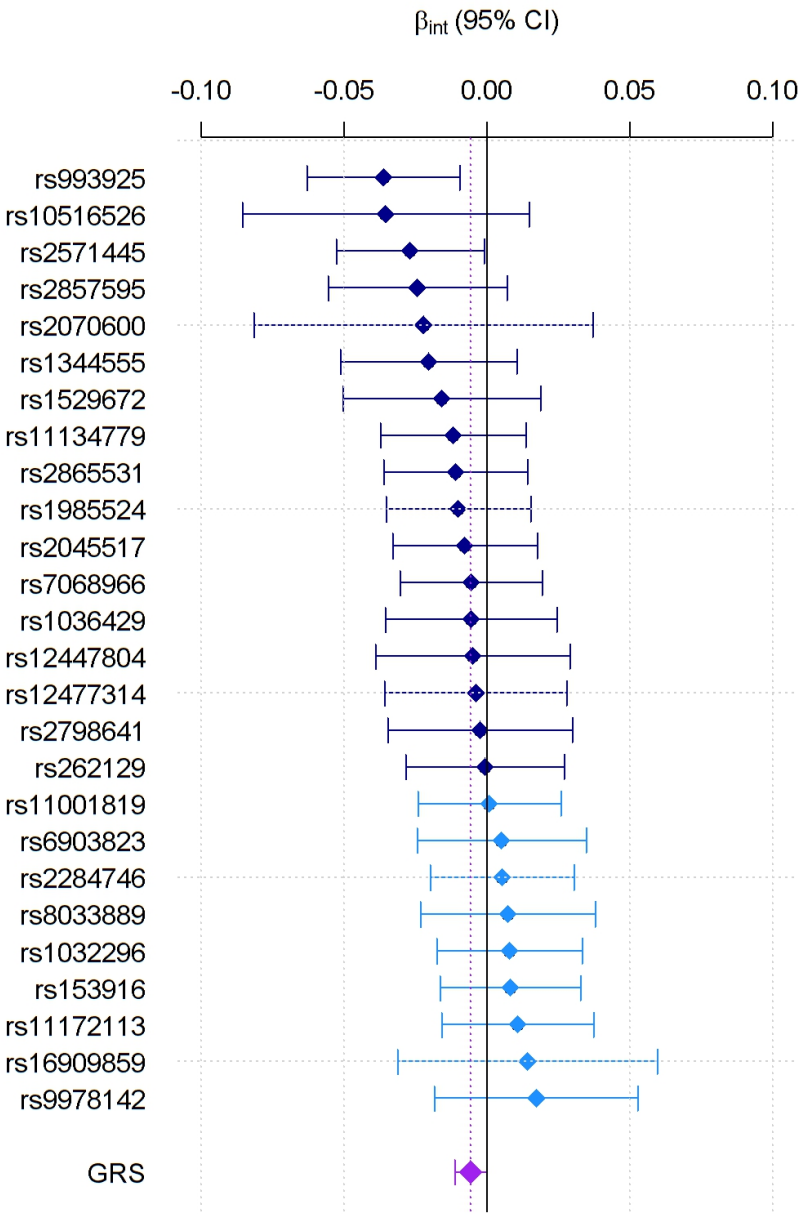
Supplementary Figure 3. Distribution of SNP-by-pack years interaction effects on FEV_1.

Single SNP risk allele-by-pack years interaction effect estimates (β_{int}) and 95% confidence intervals are plotted by increasing values. Negative and positive interactions are in dark blue and light blue, respectively. The unweighted GRS-by-pack years interaction is plotted in purple.



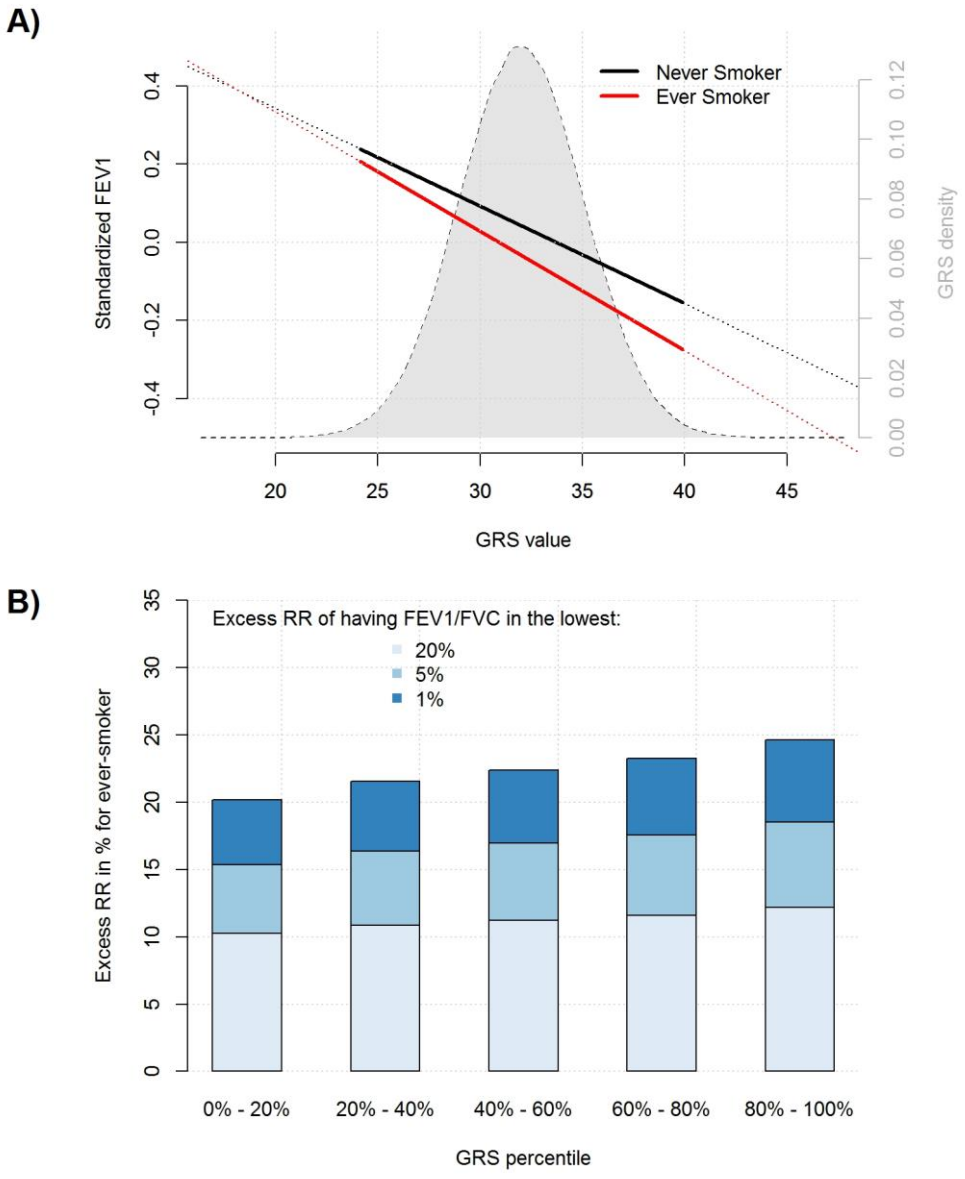
Supplementary Figure 4. Distribution of SNP-by-smoking status interaction effects on FEV_1.

Single SNP risk allele-by-smoking status (ever/never) interaction effect estimates (β_{int}) and 95% confidence intervals are plotted by increasing values. Negative and positive interactions are in dark blue and light blue, respectively. The unweighted GRS-by-smoking status interaction is plotted in purple.



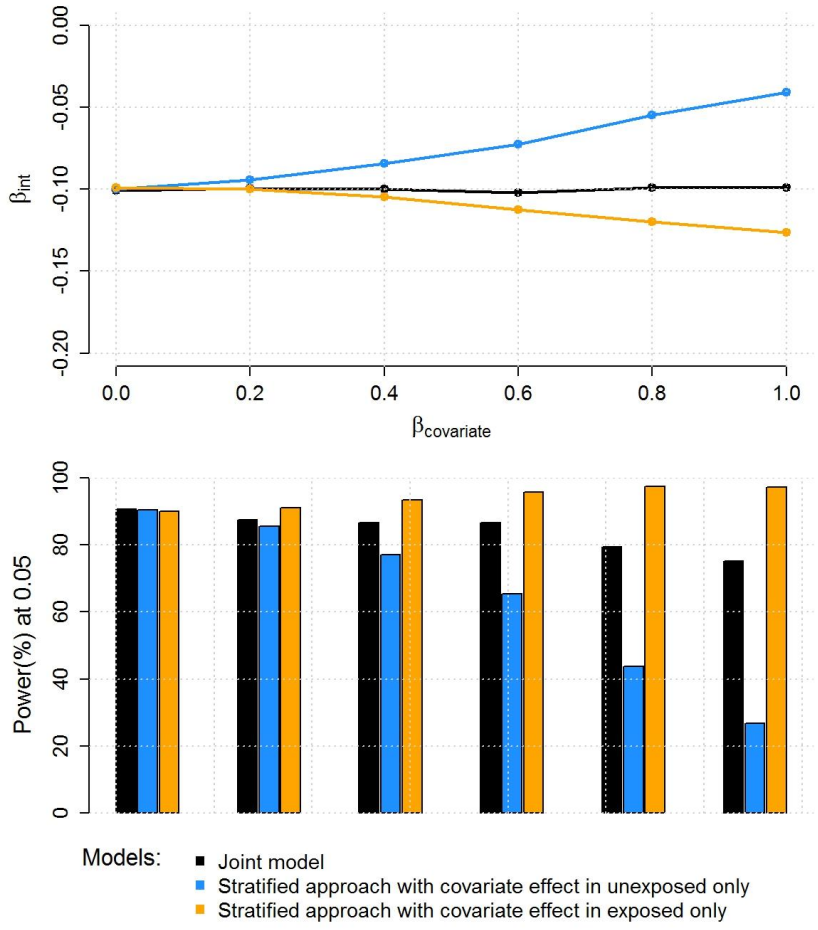
Supplementary Figure 5. Overview of the unweighted genetic risk score by smoking interaction effect on FEV_1.

Upper panel (A) presents the distribution of the unweighted genetic risk score (GRS, grey density plot) and the relationship between the unweighted GRS and standardized FEV_1 in ever smokers (red line) and never-smokers (black line). Lower panel (B) shows the excess relative risk (RR) of having FEV_1 in the lowest 1%, 5% and 20% of the population for ever smokers as compared to never smokers, stratified by GRS quintiles.



Supplementary Figure 6. Covariate-induced bias of interaction term in stratified analysis.

We simulated series of datasets where an outcome is defined as a function of a genetic variant, an exposure, and their interaction, and covariates present in unexposed or exposed individuals only. Upper panel presents the interaction effect estimated from the standard interaction test in the entire dataset (black) and from a two-steps stratified. The latter approach consisted of (i) normalizing the outcome using inverse-normal rank based transformation in each exposure strata separately, (ii) deriving marginal genetic effect in each strata separately, and (iii) inferring interaction effect from the marginal stratified result. When assuming a covariate effect in unexposed only (orange), the stratified approach shows overestimated interaction. Conversely, when assuming a covariate effect in exposed only (blue), interaction tend to be biased toward the null. Lower panel shows empirical statistical power observed for the three approaches.



References

1. Hancock DB, Artigas MS, Gharib SA, et al. Genome-wide joint meta-analysis of SNP and SNP-by-smoking interaction identifies novel loci for pulmonary function. *PLoS genetics* 2012;**8**(12): e1003098.
2. Aschard H. A perspective on interaction effects in genetic association studies. *Genet Epidemiol* 2016.
3. Soler Artigas M, Wain LV, Miller S, et al. Sixteen new lung function signals identified through 1000 Genomes Project reference panel imputation. *Nature communications* 2015;**6**: 8658.
4. Hancock DB, Soler Artigas M, Gharib SA, et al. Genome-wide joint meta-analysis of SNP and SNP-by-smoking interaction identifies novel loci for pulmonary function. *PLoS genetics* 2012;**8**(12): e1003098.
5. Soler Artigas M, Loth DW, Wain LV, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nature genetics* 2011 Nov;**43**(11): 1082-90.