

Effect of Smoking on Blood Pressure and Resting Heart Rate

A Mendelian Randomization Meta-Analysis in the CARTA Consortium

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Background—Smoking is an important cardiovascular disease risk factor, but the mechanisms linking smoking to blood pressure are poorly understood.

Methods and Results—Data on 141317 participants (62666 never, 40669 former, 37982 current smokers) from 23 population-based studies were included in observational and Mendelian randomization meta-analyses of the associations of smoking status and smoking heaviness with systolic and diastolic blood pressure, hypertension, and resting heart rate. For the Mendelian randomization analyses, a genetic variant rs16969968/rs1051730 was used as a proxy for smoking heaviness in current smokers. In observational analyses, current as compared with never smoking was associated with lower systolic blood pressure and diastolic blood pressure and lower hypertension risk, but with higher resting heart rate. In observational analyses among current smokers, 1 cigarette/day higher level of smoking heaviness was associated with higher (0.21 bpm; 95% confidence interval 0.19; 0.24) resting heart rate and slightly higher diastolic blood pressure (0.05 mmHg; 95% confidence interval 0.02; 0.08) and systolic blood pressure (0.08 mmHg; 95% confidence interval 0.03; 0.13). However, in Mendelian randomization analyses among current smokers, although each smoking increasing allele of rs16969968/rs1051730 was associated with higher resting heart rate (0.36 bpm/allele; 95% confidence interval 0.18; 0.54), there was no strong association with diastolic blood pressure, systolic blood pressure, or hypertension. This would suggest a 7 bpm higher heart rate in those who smoke 20 cigarettes/day.

Conclusions—This Mendelian randomization meta-analysis supports a causal association of smoking heaviness with higher level of resting heart rate, but not with blood pressure. These findings suggest that part of the cardiovascular risk of smoking may operate through increasing resting heart rate. (*Circ Cardiovasc Genet.* 2015;8:832-841. DOI: 10.1161/CIRCGENETICS.115.001225.)

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Smoking is a major risk factor for cardiovascular disease, but it is not clear by which mechanism smoking exerts its detrimental effects on cardiovascular disease. Generally, epidemiological studies have reported lower blood pressure levels among current smokers compared with nonsmokers.^{1–14} On the contrary, smoking cessation has been reported to be followed by a decrease in blood pressure.^{15,16} Whether the apparent association between smoking and lower blood pressure is causal or can be explained by confounding by lifestyle or socioeconomic factors related to both smoking and blood pressure remains an open question. Furthermore, smoking is causally associated with lower body mass index (BMI),¹⁷ and higher level of adiposity is strongly associated with elevated blood pressure and is also considered a major risk factor for hypertension.^{18,19} Hence, there is a strong possibility that the lower blood pressure observed in smokers could be explained by lower body weight caused by smoking. Data from observational epidemiological studies also suggest that smoking is associated with higher level of resting heart rate.²⁰ However, as for blood pressure, various confounding factors could explain this association.

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In Mendelian randomization, causal relationships in human populations are examined by using genetic variants as proxies for exposures of interest. The principle of Mendelian randomization relies on the basic laws of Mendelian genetics, segregation, and independent assortment. When these principles hold at a population level, genetic variants will not be associated with the confounding factors that generally distort conventional observational studies.²¹ In addition, an outcome measure cannot alter the germline genotype that an individual is born with, and so these analyses should not be affected by reverse causality. It can be applied in 2 ways—first, to establish whether an observational association is likely to be causal, and second, using formal instrumental variable methods to more accurately estimate the magnitude of this causal effect. Here we focus on the former approach.

A genetic variant, single nucleotide polymorphism (SNP), rs16969968, in the *CHRNA5-CHRNA3-CHRNA4* nicotinic receptor subunit gene cluster on chromosome 15 has demonstrated robust association with smoking heaviness within smokers.^{22–24} The rs16969968 variant is functional and leads to an amino acid change (D398N) in the nicotinic receptor alpha5 subunit protein.²⁵ The minor allele of the rs16969968 SNP is associated with an average increase in smoking amount of one cigarette per day in smokers and is even more strongly associated with serum cotinine (a metabolite of nicotine) levels.^{24,26} Importantly, the rs16969968 has not been robustly associated with smoking status and may, therefore, primarily be used as a marker of smoking heaviness in smokers. It is in perfect linkage disequilibrium with another SNP, rs1051730, and the 2 SNPs therefore represent the same genetic signal and can be used interchangeably. A large meta-analysis found that the minor allele of rs1051730 that increases smoking

in current smokers is associated with lower BMI in current smokers, supporting the hypothesis that smoking is causally related to lower BMI.¹⁷ As expected, the effect of rs1051730 on BMI differed with smoking status; the negative effect was observed in current smokers but not in never smokers, indicating that the association operate via smoking.¹⁷

A study in 5402 young Finnish adults examined the effects of rs1051730 on blood pressure.²³ The smoking increasing allele of rs1051730 tended to associate with lower systolic and diastolic blood pressure (SBP and DBP) among current smokers, but there was no strong statistical evidence for these associations. Furthermore, there was no evidence of interaction between smoking status and rs1051730 genotype. Åsvold et al looked at the association between rs1051730 and cardiovascular risk factors in the Nord-Trøndelag health study (HUNT) study.²⁷ In the total population, including never, former, and current smokers, they found that the smoking increasing allele of rs1051730 was associated with lower SBP, but there was no association with DBP. Unexpectedly, the association between the rs1051730 and SBP was mainly seen among never smokers, and there was no interaction between genotype and smoking status in relation to SBP possibly because of lack of power to detect interaction effects. Thus, it is not yet fully established whether smoking-related genetic variants are associated with blood pressure.

In this study, we investigated the associations between smoking and both blood pressure and resting heart rate, using both an observational and a Mendelian randomization approach. First, we meta-analyzed a total of 23 population-based studies participating in the consortium for Causal Analysis Research in Tobacco and Alcohol (CARTA).²⁸ Second, we examined the possible causal effects of smoking on blood pressure and resting heart rate by Mendelian randomization using rs16969968/rs1051730 as unconfounded and unbiased proxies of smoking heaviness in current smokers.

Methods

Study Populations

The study was performed within the CARTA consortium (<http://www.bris.ac.uk/expsych/research/brain/targ/research/collaborations/carta>).^{28,29} We used data on individuals (aged ≥16 years) of self-reported European ancestry from 23 studies: the 1958 British birth cohort (1958 BC), the Avon Longitudinal Study of Parents and Children (ALSPAC, including both mothers and children),^{30,31} the British Regional Heart Study (BRHS), the Caerphilly Prospective Study (CaPS), Cohorte Lausannoise (CoLaus/PsyCoLaus), the Danmonica10 study, the English Longitudinal Study of Ageing (ELSA), the National FINRISK Study (FINRISK), the Danish GEMINAKAR study (The importance of genes, familiar, and common environment for the development of insulin resistance, abdominal adiposity, and cardiovascular risk factors), Genetics of Overweight Young Adults (GOYA) male, Generation Scotland: the Scottish Family Health Study (GS:SFHS), Health2006, the Helsinki Birth Cohort Study (HBCS), the HUNT, Inter99, MIDSPAN Family Study, the Northern Finland Birth Cohorts (NFBC1966 and NFBC1986), the National Health and Nutrition Examination Survey (NHANES), the MRC National Survey of Health and Development (NSHD), the Netherlands Twin Registry (NTR), the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), and the Whitehall II study. All studies received

ethics approval from local research ethics committees. Further details of these studies are provided in Data Supplement. Descriptive statistics are presented in Table I in the Data Supplement.

Genotype

Within each study, individuals were genotyped for 1 of 2 SNPs in the *CHRNA5-A3-B4* nicotinic receptor subunit gene cluster, rs16969968 or rs1051730. These SNPs are in perfect linkage disequilibrium with each other in Europeans ($R^2=1.00$ in HapMap 3; <http://hapmap.ncbi.nlm.nih.gov/>) and, therefore, represent the same genetic signal. For studies with data available for both SNPs, the SNP that was genotyped in the largest number of participants was used. Details of genotyping methods within each study are provided in the Data Supplement.

Measurements of Blood Pressure and Heart Rate

Details on the methods used for measuring SBP and DBP (mm Hg) and resting heart rate (beats per minute) in each study are provided in the Data Supplement. In cohorts where information about use of blood pressure lowering medication was available, a constant was added to SBP (15 mm Hg) and DBP (10 mm Hg) in subjects on blood pressure lowering medication as recommended by Tobin et al.³² If this information was not available, SBP and DBP were analysed as they were. Availability of information on blood pressure lowering medication in each study is provided in Table I in the Data Supplement. The following dichotomous outcomes were defined: (1) hypertension as SBP >140 mm Hg or DBP >90 mm Hg or taking blood pressure lowering medication, (2) severe hypertension as SBP >160 mm Hg or DBP >100 mm Hg or taking blood pressure lowering medication. Thus, participants taking blood pressure lowering medication were defined as having both hypertension and severe hypertension. BMI was calculated as weight/height² (kg/m²). Age was calculated as years at the time of measurements of blood pressure and heart rate.

Smoking Status

Individuals were classified as current, former, or never cigarette smokers. Where information on smoking frequency was available, current smokers were restricted to individuals smoking at least 1 cigarette per day. Smokers (occasional smokers) who reported smoking less frequently than this were excluded from analyses on smokers. Where information on pipe and cigar smoking was available, individuals reporting being current or former smokers of pipes or cigars but not cigarettes were excluded from all analyses.

For studies with adolescent populations (ALSPAC children and NFBC 86), analyses were restricted to current daily smokers who reported smoking at least 1 cigarette per day (current smokers) and individuals who had never tried smoking (never smokers).

Data on smoking heaviness in current smokers, measured as cigarettes smoked per day, were collected in most studies as a continuous variable and in a few studies as a categorical variable. For full details of the smoking measures collected within each study, see the Data Supplement.

Statistical Analysis

Analyses were conducted within each contributing study using Stata and R software following the same prespecified analysis protocol. The analysis protocol is available on the CARTA website: <http://www.bris.ac.uk/expsych/research/brain/targ/research/collaborations/carta/>

Scripts for data analyses and output for Stata and R were developed and made available to ensure uniform analyses and minimize errors in data extraction. Thus, analyses were restricted to individuals with full data on blood pressure, heart rate, smoking status, and rs16969968/rs1051730 genotype. Within each study, genotype frequencies were tested for deviation from Hardy Weinberg Equilibrium using a chi-squared test.

In observational analyses, sex- and age-adjusted associations of smoking status (never, former, current) and smoking heaviness with continuous measures of SBP, DBP, and heart rate were assessed using

linear regression. For the smoking status analysis, never smokers were used as the reference group. The smoking heaviness analysis was restricted to current daily smokers, and beta estimates represent change in the outcome measure per additional cigarette consumed per day. Sex- and age-adjusted associations expressed as odds ratios (ORs) of smoking status (never, former, current) and smoking heaviness with binary measures of hypertension (hypertension and severe hypertension) were assessed using logistic regression. In additional analyses, the above models were further adjusted for BMI (continuous variable) to observe changes (if any) in the estimates for the effects of smoking.

Mendelian randomization analyses of the association between rs16969968/rs1051730 and continuous measures of blood pressure and heart rate were assessed using linear regression stratified by smoking status (never, former, current) and adjusted for age and sex. Mendelian randomization analyses of the association between rs16969968/rs1051730 and binary measures of hypertension (hypertension and severe hypertension) were assessed using logistic regression stratified by smoking status (never, former, current) adjusted for age and sex. An additive genetic model was assumed, so risk estimates represent the difference in risk of the outcome per additional copy of the minor (risk) allele. In additional analyses, the above models were further adjusted for BMI (continuous variable) observing changes in the estimates for the effects of the risk allele.

Results from individual studies were meta-analyzed in Stata (version 11) using the `metan` command. Results from observational analyses were combined by random effects model because of substantial heterogeneity ($I^2>50\%$). Results from Mendelian randomization analyses were combined in a fixed effects model, and the Cochran Q statistic was used to test for heterogeneity between genotype and smoking status in relation to the outcome measures.

Results

Descriptive Statistics

In total, data on 141 317 individuals were available for analysis, including 62 666 never smokers, 40 669 former smokers, and 37 982 current smokers. Overall, 49% of the combined study population was male. The median age within the contributing studies ranged from 16 to 75 years. Descriptive statistics for each of the study populations are found in the Table I in the Data Supplement. Minor allele frequency for rs16969968/rs1051730 ranged between 0.29 and 0.36 (Table II in the Data Supplement). There was no strong evidence for deviation from Hardy Weinberg Equilibrium in any of the studies (P values all ≥ 0.1 ; Table II in the Data Supplement).

Observational Analysis

The meta-analyzed estimates of the age- and sex-adjusted associations of smoking status (never, former, current smoking) with SBP, DBP, and resting heart rate are shown in Figure 1. Study-specific estimates are shown in the Data Supplement. Current as compared with never smoking was associated with lower SBP (-2.40 mm Hg; 95% confidence interval [CI] -3.39 ; -1.41) and DBP (-1.93 mm Hg; 95% CI -2.72 ; -1.15). Accordingly, current as compared with never smoking was associated with a lower risk of hypertension (OR 0.78; 95% CI 0.70; 0.88) and severe hypertension (OR 0.79; 95% CI 0.66; 0.94) (Figure 2).

The above associations were attenuated, but remained, when further adjusted for BMI (Figures I and II in the Data Supplement). For example, after additional adjustment for BMI, current as compared with never smoking was associated with a lower risk of hypertension (OR 0.84; 95% CI 0.73;

0.94) and severe hypertension (OR 0.85; 95% CI 0.72; 1.01; Figure II in the Data Supplement).

Current as compared with never smoking was associated with 1.93 bpm (95% CI 0.97; 2.89) higher resting heart rate (Figure 1), and this association was not attenuated by adjustment for BMI (Figure I in the Data Supplement).

The analysis of smoking heaviness (performed only in current smokers) showed that higher level of smoking heaviness was associated with slightly higher levels of SBP and DBP (Figure 3). In age- and sex-adjusted analyses, 1 cigarette per day higher level of smoking heaviness was associated with a 0.08 (95% CI 0.03; 0.13) and 0.05 (95% CI 0.02; 0.08) mm Hg higher SBP and DBP, respectively. These associations attenuated after further adjustment for BMI (Figure III in the Data Supplement). One cigarette per day higher level of smoking heaviness was associated with a 0.21 (95% CI 0.19; 0.24) beats per minute higher level of resting heart rate (Figure 3), and this association was not attenuated by adjustment for BMI (Figure III in the Data Supplement).

Mendelian Randomization Analysis

In meta-analyses stratified by smoking status, there was no strong evidence that rs16969968/rs1051730 was associated with either continuous measures of blood pressure (Table; Figure 4) or with binary measures of hypertension (hypertension and severe hypertension; Table; Figure IV in the Data Supplement). Associations were closer to the null after adjustment for BMI (Figures V and VI in the Data Supplement). There was no evidence that the effect of the *CHRNA5-A3-B4* variant differed between never, former, and current smokers (*P* values for heterogeneity from Cochran Q test all ≥ 0.1).

In contrast, in current smokers, the smoking increasing allele of rs16969968/rs1051730 was associated with a 0.36 (95% CI 0.18; 0.54) beats per minute higher heart rate per allele (Table; Figure 4). This estimate was 0.39 (95% CI 0.21; 0.57) after additional adjustment for BMI (Figure V in the Data Supplement). In addition, there was evidence of interaction (*P* value for heterogeneity from Cochran Q test 0.003) between smoking status and the *CHRNA5-A3-B4* variant in age-, sex-, and BMI-adjusted analyses, lending further support to a causal effect of smoking on resting heart rate.

The results of observational and Mendelian Randomization analyses stratified by 2 smoking status categories (never and ever smokers) were in line with the analyses stratified by 3 smoking categories (never, former, and current smokers; data not shown).

Additionally, sensitivity analyses were performed, excluding each of the following studies: HUNT, ALSPAC children, and Prosper. The reasons for this were the large number of participants contributed by 1 study (HUNT), relatedness between participants (mothers and children in ALSPAC), and results being outliers (Prosper). These analyses yielded generally results similar to results based on all studies. However, when excluding the HUNT study, the association of the smoking increasing allele of rs16969968/rs1051730 with resting heart rate in current smokers attenuated and became statistically insignificant.

Finally, we investigated the association of the smoking increasing allele of rs16969968/rs1051730 with smoking status and smoking heaviness. In current smokers, the smoking increasing allele of rs16969968/rs1051730 was associated with 0.9 cigarettes/day/allele increase in smoking heaviness (Figure 5). Our results confirmed previous findings in that the smoking increasing allele is not associated with smoking initiation (the allele is not associated with ever versus never smoking), but may be associated with smoking cessation (the allele is significantly associated with current versus former smoking; Figure VII in the Data Supplement).

Discussion

We performed a meta-analysis of 23 population-based studies, including a total of 141 317 individuals, using both observational and Mendelian randomization analyses. In observational analyses, we found that current smoking is associated with lower blood pressure and lower prevalence of hypertension. However, observational and Mendelian randomization analyses did not support a causal association between smoking heaviness in current smokers and blood pressure. In contrast, both observational and Mendelian randomization analyses consistently suggested that smoking heaviness is causally related to increasing resting heart rate among smokers.

In our meta-analyses, the risk estimate for the effect of rs16969968/rs1051730 on blood pressure in current smokers

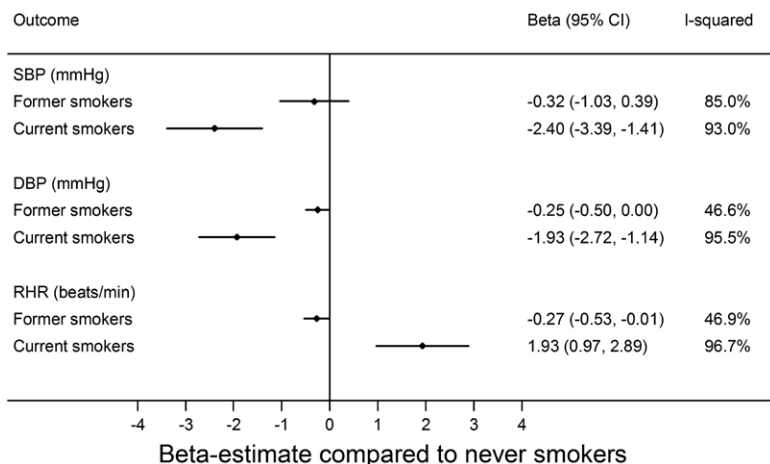


Figure 1. Association of smoking status with systolic blood pressure (SBP), diastolic blood pressure (DBP), and resting heart rate (RHR). Former and current smoking status are compared with never smoking status; the difference was estimated by linear regression adjusted for sex and age. CI indicates confidence interval.

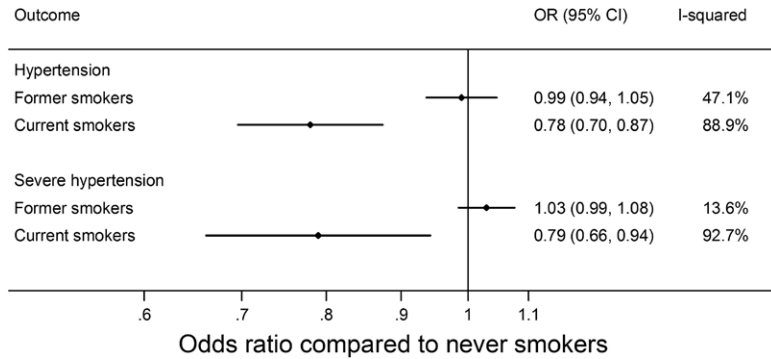


Figure 2. Association of smoking status with hypertension and severe hypertension. Former and current smoking status is compared with never smoking status; the difference was estimated by logistic regression adjusted for sex and age. CI indicates confidence interval; and OR, odds ratio.

was close to the null, with the confidence interval overlapping the null. Thus, the Mendelian randomization analysis did not support a causal effect of smoking heaviness on blood pressure. It should be stressed that the unadjusted analysis captures the overall effect of smoking on blood pressure. Given evidence from previous Mendelian randomization studies that smoking reduces BMI and BMI causes higher blood pressure, we might have expected to see a negative association between the smoking increasing allele and blood pressure in current smokers, unless smoking increases blood pressure through other pathways. There was some indication that adjustment for BMI in our Mendelian randomization analyses attenuated the estimates for the effects of the smoking increasing allele among current smokers even more toward the null, an observation that may suggest that any potential effect of smoking on blood pressure would be mediated through the BMI-decreasing effect of smoking.¹⁷ It should be stressed that if BMI acts as a mediator, adjustment for BMI would be inappropriate, and caution should be exercised when interpreting the BMI-adjusted results. Future studies could use a double Mendelian randomization approach using genetic makers of both smoking and BMI.

Among current smokers, results of our Mendelian randomization analysis supported that smoking heaviness is causally associated with increasing resting heart rate. These results thus corroborate results of our observational analysis and are furthermore in line with studies showing that resting heart rate tends to decrease after smoking cessation.^{16,33,34} The mechanisms by which smoking increases resting heart rate are not clear. Human experimental studies show that administration of nicotine increases resting heart rate, and that this is an acute effect.^{35,36} Thus, when nicotine is administered intravenously, the heart rate increasing effect peaks within the first minute after administration.³⁶ Interestingly, it has been observed that reductions in hospital admissions with acute myocardial infarction after smoking regulation and bans in public places seem to have a surprisingly rapid onset.³⁷

The clinical implications of smoking-induced higher resting heart rate are not clear. However, considering the well-documented detrimental effects of smoking on risk of cardiovascular disease and our finding that this might not involve a strong direct effect on blood pressure, it may be speculated that more attention should be paid to resting heart rate as a marker of cardiovascular health and risk prediction.³⁸ Several studies have shown that resting heart rate is a predictor of cardiovascular events even after controlling for other cardiovascular risk factors, such as blood pressure.³⁹⁻⁴⁴ Of potential relevance is that heart rate lowering drugs improve long-term survival of patients with myocardial infarction.⁴⁵ Our results roughly translated estimate that someone smoking 20 cigarettes per day could increase their resting heart rate by around 7 bpm, and vice versa that someone stopping smoking could have this meaningful reduction in HR.

The minor allele of the rs16969968/rs1051730 is known to be associated with smoking heaviness by ≈ 1 cigarette per day per copy of the allele in current smokers,⁴⁶ and we observed a 0.36 (95% CI 0.18; 0.54) beats per minute higher resting heart rate per one extra copy of the smoking increasing allele in current smokers (Figure 4). Using the *CHRNA5-A3-B4* variant (rs16969968/rs1051730) as an instrument for tobacco exposure may be advantageous compared with using a self-reported measure of tobacco exposure. First, self-reported tobacco use is likely subject to some degree of misclassification and reporting bias. Second, self-reported tobacco consumption does not take into account variation in smoking topography, such as the amount of a cigarette an individual smokes or the depth of inhalation.²⁴ Third, the *CHRNA5-A3-B4* variant is an instrument for lifetime cumulated tobacco exposure, and this is not fully captured by cigarettes per day. In further support of the genetic variant being a better measure of smoking heaviness, it has been shown that rs16969968/rs1051730 explains more of the variance (4%) in serum cotinine, a biomarker of tobacco exposure, than in self-reported number of cigarettes per day (1%).^{24,26} Notably, the effect (0.21 bpm increase per cigarette per day)

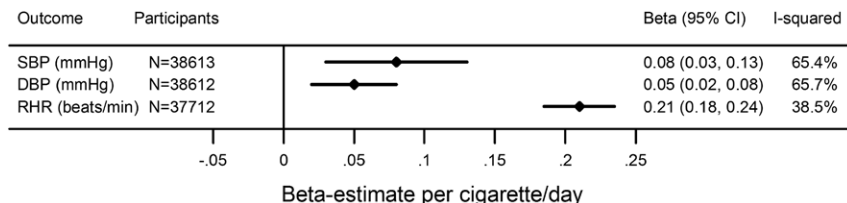


Figure 3. Association of smoking heaviness in current smokers with systolic blood pressure (SBP), diastolic blood pressure (DBP), and resting heart rate (RHR). The difference per one cigarette per day increase in smoking heaviness was estimated by linear regression adjusted for sex and age. CI indicates confidence interval.

Table. Mendelian Randomization Meta-Analysis: Results of Meta-Analysed Estimates of the Association of the Smoking Increasing Allele of rs16969968/rs1051730 With Continuous Measures of Blood Pressure, Binary Measures of Hypertension (Hypertension and Severe Hypertension), and the Continuous Measure of Resting Heart Rate

	Never Smokers	Former Smokers	Current Smokers
Systolic blood pressure, beta-coefficient* (95% CI), mm Hg/allele	-0.07 (-0.28; 0.13), <i>P</i> =0.479	-0.03 (-0.31; 0.25), <i>P</i> =0.824	-0.20 (-0.46; 0.06), <i>P</i> =0.136
Diastolic blood pressure, beta-coefficient* (95% CI), mm Hg/allele	0.01 (-0.12; 0.14), <i>P</i> =0.879	0.08 (-0.09; 0.26), <i>P</i> =0.331	-0.15 (-0.32; 0.02), <i>P</i> =0.079
Hypertension, odds ratio* (95% CI) per allele	1.00 (0.97; 1.03), <i>P</i> =0.923	1.00 (0.96; 1.03), <i>P</i> =0.811	0.98 (0.95; 1.02), <i>P</i> =0.277
Severe hypertension, odds ratio* (95% CI) per allele	1.02 (0.98; 1.05), <i>P</i> =0.362	1.01 (0.97; 1.05), <i>P</i> =0.543	0.96 (0.92; 1.00), <i>P</i> =0.061
Resting heart rate, beta-coefficient* (95% CI), bpm/allele	0.03 (-0.11; 0.17), <i>P</i> =0.686	0.15 (-0.03; 0.33), <i>P</i> =0.109	0.36 (0.18; 0.54), <i>P</i> <0.001†

Results were stratified by smoking status. CI indicates confidence interval.

*Associations have been adjusted for sex and age by linear (continuous outcomes) or logistic (binary outcomes) regression.

†Bonferroni-adjusted (15 tests) *P* value was 0.001.

of smoking heaviness on resting heart rate observed in our observational analyses based on self-reported smoking habits was smaller than indicated by the Mendelian randomization analyses, a finding that is consistent with the idea that the rs16969968/rs1051730 SNP is a more accurate marker of smoking heaviness than self reports. Of note, it has previously been shown that the use of instrumental variable analysis in Mendelian randomization studies estimate the magnitude of any causal effect of cigarette smoking, where measured self-reported cigarette consumption is used as the exposure, is likely inappropriate, and may lead to biased estimates.⁴⁷

We would not expect to see an effect of rs16969968/rs1051730 on resting heart rate in never smokers because the variant cannot be associated with smoking heaviness within these individuals without any exposure. Thus, this group can be used to test the assumption of no pleiotropy (ie, that the gene affects only one exposure) in Mendelian randomization analyses. Thus, our observation that there was no clear statistical evidence for an association of the rs16969968/rs1051730 variant with heart rate in never smokers and that we observed statistical evidence of heterogeneity of the effect of the variant between smoking categories lends further support to the notion that smoking is causally related to higher resting heart rate.

The principle of Mendelian randomization is based of assumptions, for example, (a) the genetic marker is associated with the exposure, (b) the genetic marker is independent of the outcome given the exposure and all confounders (pleiotropy, see above), and (c) the genetic marker is independent of factors that confound the exposure–outcome relationship. It should be recognized that these assumptions may not all be easy to evaluate.

Stratification of Mendelian randomization analyses may induce collider bias if the instrument is predictive of the stratification variable.⁴⁸ We do not think that this is a major concern because our instrument rs16969968/rs1051730 is primarily a genetic variant for smoking heaviness within smokers, and there is no clear evidence that it is associated with smoking initiation (ie, being an ever versus a never smoker). It does show some evidence for an association with smoking

cessation,⁴⁹ but analyses performed in ever smokers yielded results similar to analyses performed in current smokers. The rs16969968/rs1051730 variant has been used in a similar way (ie, stratified on smoking status) in several other Mendelian randomization studies to demonstrate the expected causal associations of smoking with increased all-cause mortality,⁵⁰ decreased lung function,⁵¹ and BMI. The fact that stratification by smoking status has been used to show that smoking is, as expected, causally related to these phenotypes supports our view that this approach is appropriate.

Conclusions

This large Mendelian randomization meta-analysis suggests that smoking is causally related to higher level of resting heart rate, but not to alterations in blood pressure and risk of hypertension. These findings are consistent with the hypothesis that smoking exerts its detrimental effects on cardiovascular disease at least partly via increasing resting heart rate.

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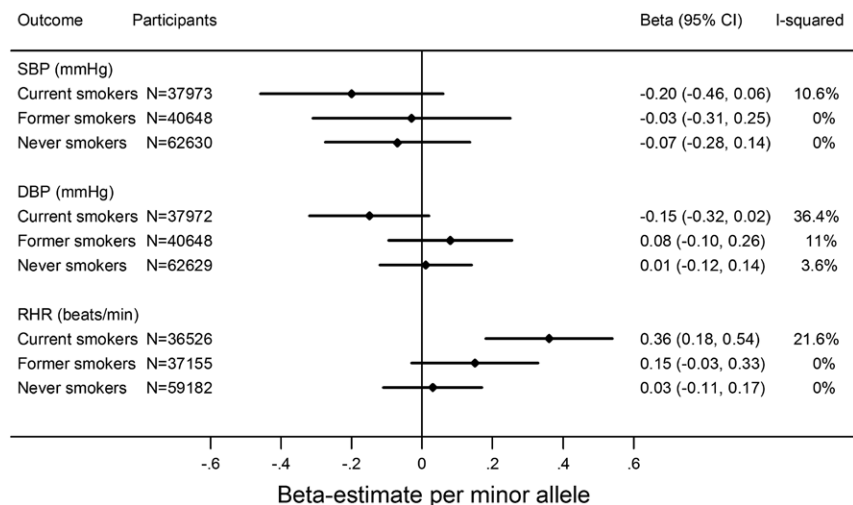


Figure 4. Mendelian randomization analysis of the association of the smoking increasing allele (minor allele) of rs1051730/rs16969968 with systolic blood pressure (SBP), diastolic blood pressure (DBP), and resting heart rate (RHR). Analyses were stratified by smoking status (current, former, and never smoking). The difference per one allele was estimated by linear regression adjusted for sex and age. Overall test for heterogeneity by smoking status: SBP, $P=0.656$; DBP, $P=0.138$; and RHR, $P=0.015$. CI indicates confidence interval.

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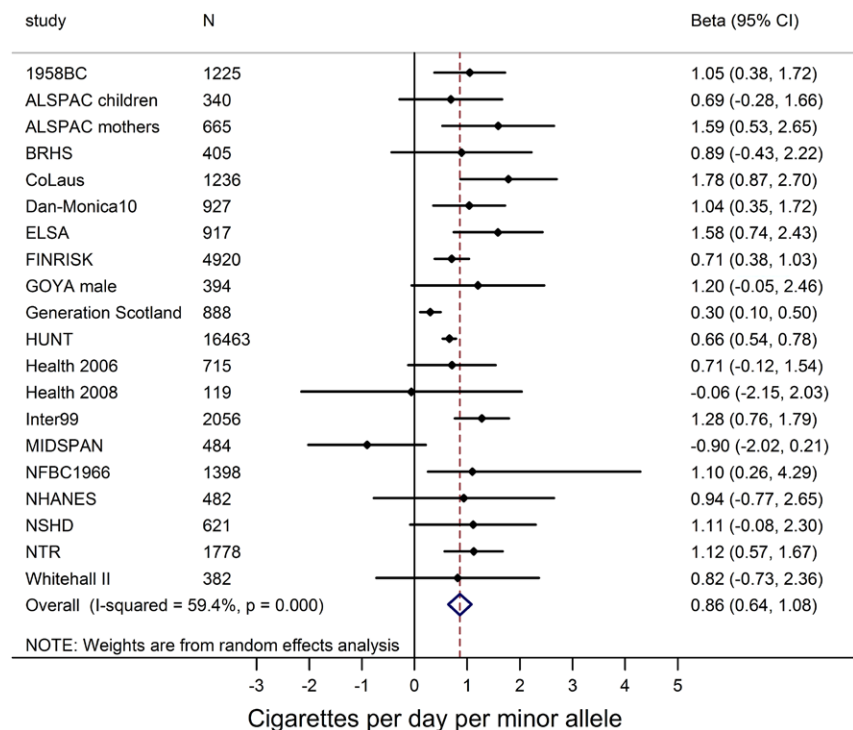


Figure 5. Mendelian randomization analysis of the association of the smoking increasing allele (minor allele) of rs1051730/rs16969968 with smoking heaviness in current smokers. The difference (increase in smoking quantity) per one allele was estimated by linear regression adjusted for sex and age. ALSPAC indicates Avon Longitudinal Study of Parents and Children; CI, confidence interval; ELSA, English Longitudinal Study of Ageing; GOYA, Genetics of Overweight Young Adults; HUNT, Nord-Trøndelag Health Study; NHANES, National Health and Nutrition Examination Survey; NSHD, National Survey of Health and Development; and NTR, Netherlands Twin Registry.

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Disclosures

Dr Korhonen consulted for Pfizer in 2011 to 2014 on nicotine dependence. Dr Kaprio consulted for Pfizer on nicotine dependence in 2013 and 2014. Dr Kuh has received funding from the Medical Research Council to pay for her salary and the National Survey of Health and Development (NSHD) data collection. Dr Whincup has received a research grant from the British Heart Foundation. Dr Waeber has received a research grant from the Swiss National Science Foundation for recruitment for the population-based study COLAUS. Dr Smith has received a research grant from the Scottish Government, Chief Scientist Office, for collection of data (Generation Scotland). The other authors report no conflicts.

Appendix

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CLINICAL PERSPECTIVE

Smoking is a major risk factor for cardiovascular disease, but it is not clear by which mechanism smoking exerts its detrimental effects on cardiovascular disease. Previous observational epidemiological studies show that smokers as compared with nonsmokers have lower blood pressure and risk of hypertension. However, these studies may have been hampered by confounding and biases. Mendelian randomization is an approach where genetic markers of exposures are used for testing causal associations. We performed a meta-analysis of 23 population-based studies, including a total of 141 317 individuals, using both observational and Mendelian randomization analyses. Our results suggested that smoking is causally related to higher level of resting heart rate, but not to significant alterations in blood pressure and risk of hypertension. These findings are consistent with the hypothesis that smoking exerts its detrimental effects on cardiovascular disease at least partly via increasing resting heart rate. Our results roughly translated estimate that someone smoking 20 cigarettes per day could increase their resting heart rate by around 7 beats per min, and vice versa that someone stopping smoking could have this meaningful reduction in heart rate. Considering the well-documented detrimental effects of smoking on risk of cardiovascular disease and our finding that this might not involve a strong direct effect on blood pressure, it may be speculated that more attention should be paid to resting heart rate as a marker of cardiovascular health and risk prediction.