Supplemental Material

Supplementary Cohort Information

Contents

1958 British Birth Cohort

The 1958 British Birth cohort (1958BC) includes all births during one week in March in England, Scotland and Wales (Power C, Elliott J. Cohort profile: 1958 British birth cohort (National Child Development Study). International journal of epidemiology 2006;35(1):34-41 doi: 10.1093/ije/dyi183). Participants in the study have been followed regularly, with information collected on a wide-range of factors related to health, lifestyle, growth and development. At age 45 years, 11,971 participants currently living in Britain were invited to take part in a biomedical survey, of whom 9,377 (78%) filled in a questionnaire and 8,302 (89%) also provided a blood sample. The 1958BC is largely a white European population (98%) and despite some data attrition, it has been evaluated to be broadly representative of the surviving cohort (Atherton K, Fuller E, Shepherd P, et al. Loss and representativeness in a biomedical survey at age 45 years: 1958 British birth cohort. Journal of epidemiology and community health 2008;62(3):216-23 doi: 10.1136/jech.2006.058966).

Genotyping

Genetic information was obtained from blood samples collected at 45y, through two sub-samples from case-control studies that had used the 1958BC as a source for population controls: 3000 samples were randomly selected as part of the Wellcome Trust Case Control Consortium [WTCCC2] (International Multiple Sclerosis Genetics C, Wellcome Trust Case Control C, Sawcer S, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011;476(7359):214-9 doi: 10.1038/nature10251) and 2592 distinct samples were selected as part of the Type 1 Diabetes Genetics Consortium [T1DGC] (Barrett JC, Clayton DG, Concannon P, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nature genetics 2009;41(6):703-7 doi: 10.1038/ng.381). The SNP rs16969968 was imputed both in T1DGC and in WTCCC2, with average posterior call rate >0.99 in both studies.

Measurements of blood pressure and resting heart rate

At age 44-45y, blood pressure (BP) was measured by a nurse during a home-based clinical assessment. Three BP measures were taken after the participant had been seated for five minutes using an Omron 705CP automated digital oscillometric sphygmomanometer (Omron, Tokyo, Japan); a large cuff was used if the mid-upper arm circumference was >32cm. Mean diastolic and systolic BP values were used. The use of blood pressure medication was based on self-report.

Smoking status

Cigarette smoking was recorded at age 42 by Computer Aided Personal Interviewing, and was classified as never, ex or current smoker. Reports of never smoking were verified using data from surveys at ages 23 and 33. Number of cigarettes smoked per day at 42 years was also reported for current smokers. Pipe and cigar smokers were excluded from analyses.
In the current analyses, we included 5,033 participants with rs1051730 genotype, blood pressure, resting heart rate and smoking data available.

Ethics

Written consent was obtained from participants for the use of information in medical studies. The 45-year biomedical survey and genetic studies were approved by the South-East Multi- Centre Research Ethics Committee (ref: 01/1/44) and the joint UCL/UCLH Committees on the Ethics of

Human Research (Ref: 08/H0714/40).

Acknowledgments and funding

Statistical analyses were funded by the Academy of Finland (Project 24300796 and SALVE/PREVMEDSYN). DNA collection was funded by MRC grant G0000934 and cell-line creation by Wellcome Trust grant 068545/Z/02. This research used resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases, National Human Genome Research Institute, National Institute of Child Health and Human Development, and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. This study makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of investigators who contributed to generation of the data is available from the Wellcome Trust Case-Control Consortium website. Funding for the project was provided by the Wellcome Trust under the award 076113. Great Ormond Street Hospital/University College London, Institute of Child Health receives a proportion of funding from the Department of Health's National Institute for Health Research (NIHR) ('Biomedical Research Centres' funding).

ALSPAC

The Avon Longitudinal Survey of Parents and Children is a prospective cohort study which recruited pregnant women residing in Avon, United Kingdom, with expected dates of delivery between 1 April 1991 and 31 December 1992. Full details of the study recruitment and methodology have been published previously (Fraser et al, Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol 2013;42:97-110). A total of 14,541 pregnancies were included in the initial sample, resulting in 14,062 live births and 13,988 children who were alive at one year of age. Detailed information on mothers and their partners (during and after pregnancy) and the children (since birth) has been collected from self-report questionnaires and attendance at clinics. Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee.

Please note that the study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bristol.ac.uk/alspac/researchers/data-access/datadictionary/).

ALSPAC mothers

Genotyping

Rs1051730 was directly genotyped as part of the genomewide SNP genotyping using the Illumina human660W-quad array. Genotypes were called with Illumina GenomeStudio. PLINK (v1.07) was used to carry out quality control measures on an initial set of 10,015 subjects and 557,124 directly genotyped SNPs. A total of 8,340 subjects and 526,688 SNPs passed quality control filters.

Measurements of blood pressure and resting heart rate

Between 2009 and 2011, ALSPAC mothers (N=11, 264 women) were invited to a research clinic when their participating children were between 16 and 20 years (median age 18 years).

Blood pressure and heart rate and BMI

Blood pressure and heart rate were measured using a Dinamap 9301 Vital Signs Monitor (Morton Medical, London, UK) using the correct cuff size. Two readings of systolic and diastolic BP (SBP and DBP) and heart rate were recorded, with the woman at rest and their arm supported, and the mean of the two measures was used in all analyses. All women were asked to report any medication (prescribed or purchased without prescription) that they were taking, including the dosage and timing and the reason they were taking the medication. Text medication names were coded to British National Formulary codes with checks made by AT and DAL. Weight and height were measured in light clothing and without shoes. Weight was measured to the nearest 0.1kg using Tanita scales, height to the nearest 0.1cm using a Harpenden stadiometer.

Smoking status

Information about smoking status was taken from a postal questionnaire which was also administered to women when their participating children were approximately 18 years of age. Women were asked whether they had ever smoked and whether they were currently a smoker. Former or current smokers who reported not being daily smokers were excluded from the analyses. No questions were asked about cigar or pipe smoking. Smoking heaviness (cigarettes per day) was reported as a continuous variable.

In the current analyses, we included 1,826 participants with rs1051730 genotype, blood pressure and heart rate and smoking data available.

ALSPAC children

Sample

A single individual from each family was used in the analysis. For twins, only the first born twin contributed to the analysis sample. Where there were multiple births by the same mother within the study, a single child was selected at random from each family.

Genotyping

DNA was extracted as described previously [12]. Genotyping was undertaken by KBioscience Ltd. (www.kbioscience.co.uk), using a proprietary competitive allele specific PCR system (KASPar) for SNP analysis.

Blood pressure and heart rate and BMI

Blood pressure and heart rate were measured using a Dinamap 9301 Vital Signs Monitor (Morton Medical, London, UK) using the correct cuff size. Two readings of systolic and diastolic BP (SBP and DBP) and heart rate were recorded, with the participant at rest and their arm supported, and the mean of the two measures was used in all analyses. Weight and height were measured in light clothing and without shoes. Weight was measured to the nearest 0.1kg using Tanita scales, height to the nearest 0.1cm using a Harpenden stadiometer.

Smoking status

At the age 17 clinic, participants also completed questions on a computer about their lifetime

smoking behaviour. From these, two categories of smoking status were created: never smokers and current daily smokers. Never smokers reported never having tried a cigarette in their lifetime and current daily smokers smoked at least one cigarette per day. Individuals reporting less frequent smoking were excluded from analyses.

In the current analyses, we included 1,574 participants with rs1051730 genotype, blood pressure and heart rate and smoking data available.

Ethics

Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bristol.ac.uk/alspac/researchers/data-access/data-dictionary/).

Acknowledgments and funding

The UK Medical Research Council and the Wellcome Trust (Grant ref: 092731) and the University of Bristol provide core support for ALSPAC. The genetic data for the ALSPAC mothers were funded by a grant from the Wellcome Trust (WT088806) and the blood pressure and pulse data by a grant from the British Heart Foundation (SP/07/008/24066). We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. AET, MRM and MEF are members of the UK Centre for Tobacco and Alcohol Studies, a UKCRC Public Health Research: Centre of Excellence. Funding from British Heart Foundation, Cancer Research UK, Economic and Social Research Council, Medical Research Council, and the National Institute for Health Research, under the auspices of the UK Clinical Research Collaboration, is gratefully acknowledged. This work was supported by the Wellcome Trust (grant number 086684) and the Medical Research Council (grant numbers MR/J01351X/1, G0800612, G0802736, MC_UU_12013/1, MC_UU_12013/5, MC_UU_12013/6).

BRHS

The British Regional Heart Study (BRHS) recruited 7,735 men aged 40–59 years in 1978–80; full details are reported elsewhere (Walker M, Whincup PH, Shaper AG. The British Regional Heart Study 1975-2004. International journal of epidemiology 2004;33(6):1185-92 doi: 10.1093/ije/dyh295. Men were recruited from 24 medium-sized British towns; at that time, very few eligible subjects were of non-European ancestry. Twenty years later, when aged 60–79 years, n = 4,252 participants were remeasured. The BRHS has local (from each of the districts in which the study was based) and multicentre ethical committee approvals. All men provided informed written consent to the investigation, which was performed in accordance with the Declaration of Helsinki.

Genotyping

DNA was extracted from a whole-blood sample taken at the twenty year follow up. Genotyping of rs1051730 was performed by KBioscience (http://www.kbioscience.co.uk), using KASPar chemistry, which is a competitive allele-specific PCR SNP genotyping system.

Measurements of blood pressure and resting heart rate

Seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the physical examinations at baseline and after 20 years of follow-up. Blood pressure was measured at baseline using The London School of Hygiene and Tropical Medicine (LSTHM) sphygmomanometer and at the 20th year re-examination using the Dinamap 1846SX vital signs monitor (Critikon Inc, Tampa, FL, USA). The mean of two successive readings, adjusted for observer variation, was used. A previous study (ref) found significant systematic overestimation by the Dinamap 1846 of 8mmHg for SBP, but no significant difference between the two apparatus for DBP. As in all previous analyses of these data from the 20th year re-examination, the SBP measurements have been adjusted by 8mmHg downwards. Reference: Whincup PH, Bruce NG, Cook DG, Shaper AG. The Dinamap 1846SX automated blood pressure recorder: comparison with the Hawksley random zero sphygmomanometer under field conditions. J Epidemiol Community Health 1992 ;46(2):164-9.

Smoking status

At the 20th year re-examination where anthropometric measures were made, at the same time participants answered a questionnaire including being asked “do you currently smoke cigarettes?” and “if no, have you ever smoked cigarettes?”, thus defining whether participants could be classed as never, former or current smokers. Men were also asked “how many cigarettes per day do you smoke?”

In the current analyses, we included 3,855 participants with rs1051730 genotype, blood pressure and smoking data available.

Ethics

The BRHS has local (from each of the districts in which the study was based) and multi-centre ethical committee approvals.

Acknowledgments and funding

The British Regional Heart Study is a British Heart Foundation (BHF) Research Group.

CaPS

The Caerphilly Prospective Study (CaPS) comprises 2,512 men—89% of all men aged between 45 and 59 years at baseline data collection (1979–1983) living in Caerphilly and adjoining villages (Caerphilly and Speedwell collaborative heart disease studies. The Caerphilly and Speedwell Collaborative Group. Journal of epidemiology and community health 1984;38(3):259-62). The men have been followed an additional 4 times. During phase 2, an additional 447 men of the same age group living in the same area entered the study for the first time.

Ethics approval was obtained from the MRC Epidemiology Unit (Cardiff), the South Glamorgan Area Health Authority, the Gwent REC, and the South Wales Research Ethics Committee D, and each subject signed their agreement to be involved.

Genotyping

Genotyping of rs16969968 was undertaken by KBioscience Ltd. (www.kbioscience.co.uk), using a proprietary competitive allele specific PCR system (KASPar) for SNP analysis. The call rate was 98%, with 100% duplicate concordance.

Blood pressure and heart rate

At phase 2, blood pressure was measured while seated at room temperature using a Hawksley random zero sphygmomanometer. Heart rate was measured from ECG rhythm trace.

Smoking status

Information on smoking (never, ex, and current smoker of 1–14, 15–24, or ≥25 cigarettes per day was collected by questionnaire at phase 2. Men who reported smoking cigars or pipes were excluded from all analyses.

In the current analyses, we included 1,160 participants with rs1051730 genotype, blood pressure and heart rate and smoking data available.

Ethics

Ethics approval was obtained from the MRC Epidemiology Unit (Cardiff), the South Glamorgan Area Health Authority, the Gwent REC, and the South Wales Research Ethics Committee D, and each subject signed their agreement to be involved.

Acknowledgments and funding

The Caerphilly Prospective Study was conducted by the former MRC Epidemiology Unit (South Wales). The Caerphilly archive is now maintained by the School of Social and Community Medicine in Bristol University. We thank the Health and Social Care Information Centre (HSCIC) for helping us maintain long term follow-up with the cohort. We thank all the men who have given their time to be participants in CaPS.

CoLaus/PsyCoLaus

This is a population-based study of 6,188 individuals (CoLaus), aged 35–75 years, randomly selected from the list of residents in Lausanne, Switzerland, between 2003 and 2006. Risk factors for cardiovascular diseases were assessed and DNA and plasma samples were collected for the study of genetic variants and biomarkers. Between 2004 and 2008, all 35- to 66-year-old individuals of the CoLaus sample were invited to participate in an extensive psychiatric evaluation (PsyCoLaus). Detailed descriptions of recruitment procedures and assessments have been provided previously (Firmann M, Mayor V, Vidal PM, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC cardiovascular disorders 2008;8:6 doi: 10.1186/1471-2261-8-6), (Preisig M, Waeber G, Vollenweider P, et al. The PsyCoLaus study: methodology and characteristics of the sample of a population-based survey on psychiatric disorders and their association with genetic and cardiovascular risk factors. BMC psychiatry 2009;9:9 doi: 10.1186/1471-244X-9-9). Colaus and PsyCoLaus were approved by the

Institutional Ethic's Committee of the University of Lausanne.

Genotyping

Genotyping was performed on whole blood samples. The rs1051730 variant was genotyped as part of genome-wide SNP genotyping using the Affymetrix 500 K SNP chip. Full details of this have been described previously (Freathy RM, Ring SM, Shields B, et al. A common genetic variant in the 15q24 nicotinic acetylcholine receptor gene cluster (CHRNA5-CHRNA3-CHRNB4) is associated with a reduced ability of women to quit smoking in pregnancy. Human molecular genetics 2009;18(15):2922-27 doi: Doi 10.1093/Hmg/Ddp216). The SNP rs1051730 was imputed in the study sample with a posterior call rate of 0.97.

Measurements of blood pressure and resting heart rate

Blood pressure was measured by a nurse in triplicate on the right arm, with an appropriately sized cuff, after at least 10 minute rest in the seated position, using an OmronR HEM-907 automated oscillometric sphygmomanometer (Matsusaka, Japan). Values averaged between the last two readings are reported. Antihypertensive drug treatment was assessed by asking the participants if they currently took any medicine to treat hypertension. The information was further controlled by systematically checking all medicines (prescribed or self-acquired) brought by the participants to the recruitment center.

Smoking status

Smoking information was collected by self-rating questionnaires, approximately 1 year before the psychiatric evaluation.Ever smokers were those who had smoked regularly at some point in their life. Of these, individuals who smoked at least one cigarette daily at the time of interview were classified as current smokers, whereas the rest were classified as former smokers. Pipe and cigar smokers (who do not smoke cigarettes) were excluded. Smoking heaviness was collected as a continuous variable.

In the current analyses, we included 2,085 participants with rs1051730 genotype, blood pressure, resting heart rate, and smoking data available.

Ethics

Colaus and PsyCoLaus were approved by the Institutional Ethic's Committee of the University of Lausanne.

Acknowledgments and funding

The CoLaus/PsyCoLaus study was supported by four grants of the Swiss National Science Foundation (#105993, 118308, 139468 and 122661), two unrestricted grants from GlaxoSmithKline as well as by the Faculty of Biology and Medicine of the University of Lausanne.

The Dan-monica10 study

In 1982–1984, a random sample of 4,807 persons from the referral area of Glostrup County Hospital, Copenhagen, was invited to participate in the Danish MONICA I health survey (1). The study was a

part of an international World Health Organization (WHO) co-ordinated study, MONItoring of trends and determinants in CArdiovascular Diseases (MONICA). The sample was selected to represent an equal number of men and women born in 1922, 1932, 1942 or 1952 (age 30, 40, 50 and 60 years). A total of 226 Individuals, who were not of Danish nationality, were excluded. Of the remaining 4581 Danes, 3608 participated (79%). We used data from a follow-up examination performed in 1993-1994, called Dan-MONICA10 (2). Since the first examination, 428 subjects had died and 23 had moved and could not be reached. The remaining 4130 Danes were invited to a new examination, when the participants were aged 41, 51, 61, or 71 years old. A total of 2656 (64%) participated in the Dan-MONICA10 follow-up study. Informed written consent was obtained from all participants. The study was approved by the Ethical Committee of Copenhagen. The health examinations took place a Research Centre for Prevention and Health, Glostrup. Participants completed a self-administered questionnaire on cardiovascular risk factors, medical history and lifestyle habits, including smoking and physical activity. A physical examination was performed by trained staff and included anthropometric measurements (weight, height, waist and hip circumference).

Measurements of blood pressure and resting heart rate

Blood pressure was measured in the sitting position after 5 min of rest using a standard mercury phygmomanometer, and the mean of two values was used for systolic and for diastolic pressure. Systolic blood pressure was read at the first Korotkoff sound and diastolic blood pressure at the disappearance of the Korotkoff sounds (phase V). The deflation rate was 2 mmHg/s. Before the investigation, the manometer was calibrated, and the nurse performing the measurements was tested for normal hearing. The analyses showed no digit preference. Resting heart rate was calculated by counting the radial pulse in 15 s and multiplying by 4.

Smoking status

Information on smoking status was collected by a self-administered questionnaire to be filled in at home prior to the health examination. Smoking status was recorded as never, former, occasional (<1 cigarette, cheroot, cigar, or pipe per day) and daily smokers. Occasional smokers as well as daily smokers, who exclusively smoke cheroots, cigars, or pipe, were excluded from all analyses. Smoking heaviness among daily smokers was recorded as number of cigarettes per day.

Genotyping

Genotyping of rs1051730 was performed using KBiosciences allele-specific PCR (KASPar) (KBioscience, Hoddesdon, UK).

Ethics

Ethics approval was given by the local research ethics committee. All participants gave written consent and the study was conducted in accordance with the Second Helsinki Declaration.

Acknowledgments and funding

The Dan-MONICA10 was sponsored by The Danish Heart Foundation; the Danish Medical Research Council; The Danish Hospital Foundation of Medical Research, region of Copenhagen, the Faroe Islands and Greenland; The Danish Health Insurance Foundation; The Foundation of E. & M. Wedel-Wedellsborg; Landsforeningen til Bekampelse af Kredslobssygdomme; The Augustinus Foundation; The Becket Foundation; and The Foundation of senior registrar J. & L. Boserup.

References

1. Association between alcohol consumption and aeroallergen sensitization in Danish adults. Linneberg A, Hertzum I, Husemoen LL, Johansen N, Jørgensen T. Clin Exp Allergy. 2006 Jun;36(6):714-21.
2. Normal values for ambulatory blood pressure and differences between casual blood pressure and ambulatory blood pressure: results from a Danish population survey. Rasmussen SL, Torp-Pedersen C, Borch-Johnsen K, Ibsen H. J Hypertens. 1998 Oct;16(10):1415-24.

ELSA

The English Longitudinal Study of Ageing (ELSA) is a population based study of adults aged ≥50 years living in the UK. The original sample was recruited in 2002 and consisted of 11,391 individuals (Steptoe A, Breeze E, Banks J, et al. Cohort Profile: The English Longitudinal Study of Ageing. International journal of epidemiology 2012 doi: 10.1093/ije/dys168). The sample has been followed up every 2 years and data have been collected via computer-assisted personal interviews and self-completion questionnaires. ELSA has been approved by the National Research Ethics Service and all participants have given informed consent.

Genotyping

In Wave 2 (2004/5) of the study, 5,633 participants provided blood samples for DNA extraction. Genotyping of rs16969968 was performed by KBioscience using in-house KaSPAR technology.

Measurements of blood pressure and resting heart rate

At Wave 2, blood pressure and heart rate were measured whilst the participant was seated using the Omron HEM 907 blood pressure monitor. Participants were asked to sit quietly for five minutes before the first measurement was taken. Measurements were taken three times and an average of the measures used in the analysis. Use of blood pressure medication was self-reported at Wave 2.

Smoking status

Smoking status was defined according to information collected at Wave 2. Individuals were classified as current smokers if they reported smoking at least one cigarette per day or at least one gram of tobacco per day on weekdays. Former smokers were individuals who reported ever having smoked cigarettes but who were not current smokers. Never smokers were individuals who had never reported smoking cigarettes. Current or former pipe and cigar smokers who did not also smoke cigarettes were excluded from all analyses.

In the current analyses, we included 4,464 participants with rs16969968 genotype, blood pressure, resting heart rate, and smoking data available.

Ethics

ELSA has been approved by the National Research Ethics Service and all participants have given informed consent.

Acknowledgments and funding

ELSA is funded by the National Institute on Aging in the US (R01 AG017644;R01AG1764406S1) and by a consortium of UK Government departments (including: Department for Communities and Local Government, Department for Transport, Department for Work and Pensions, Department of Health, HM Revenue and Customs and Office for National Statistics).

FINRISK

The National FINRISK Study is a large population survey on risk factors of non-communicable diseases in Finland. Every five years since 1972, area, sex and age stratified random samples of population have been drawn from the Population Register. In these analyses data from FINRISK 1992, 1997, 2002 and 2007 surveys were used. Age range of the participants was from 25 to 64 years in study years 1992 and 1997 and from 25 to 74 years in study years 2002 and 2007. Surveys have included a self-administered questionnaire, physical examination and blood draw for laboratory analyses and extraction of DNA. A history of hypertension diagnosis and current use of antihypertensives was obtained.

Genotyping

DNA was derived from whole blood samples, which were frozen immediately at the clinical study sites. The samples were transferred to the National Institute of Health and Welfare, where the DNA was extracted. Genotyping of rs16969968 (CHRNA5 D398N) was done under standard protocols of iPLEX Gold technology on the MassARRAY System (Sequenom, San Diego, CA, USA). The success rate was >0.99 and it was in HWE. Minor allele frequency was 0.32.

Measurements of blood pressure and resting heart rate

Blood pressure (BP) was measured on the right arm using a mercury sphygmomanometer after the study subject had rested in a sitting position for at least 5 minutes. The size of the arm cuff bladder was 14 cm x 40 cm. Systolic blood pressure (SBP) was recorded as the pressure at which the first sound of Korotkoff phase I was heard. Diastolic blood pressure (DBP) was recorded as the pressure at which the regular pulsating sounds disappeared (Korotkoff phase V). Two BP measurements were done in FINRISK 1992, 1997 and three BP measurements were done in FINRISK 2002, 2007 with a one minute break between the measurements. The mean of the first two measures was used for analyses. Pulse was measured between the first and second BP measurement by palpating from the wrist during 30 seconds.

Smoking status

In the same questionnaire, respondents were asked whether they had ever smoked. Those stating that they had never smoked were categorized as never smokers and skipped the other smokingrelated questions. Ever smokers were defined as those who had smoked at least 100 cigarettes in their lifetime. Further questions were used to classify ever smokers as current and former smokers. Former smokers reported having been either regular or occasional smokers but were not smoking currently. For the present analysis, only those who had quit over 6 months ago were included in the former smoker category. Current smokers reported regular or daily smoking having smoked on the

day of the assessment or the previous day. Exclusive pipe or cigar smokers were excluded from all analyses.

In order to create a variable for smoking quantity the participants were asked to indicate the average number of both manufactured and self-rolled cigarettes they smoked or had smoked per day before quitting. Manufactured and self-rolled cigarettes were totalled for the analysis.

Ethics

Surveys have obtained permissions from the local Ethics Committees that have varied over time, such as the Coordinating Ethics Committee for the Uusimaa Hospital District in 2007. From 1997 onwards a written informed consent has been obtained from each participant.

Acknowledgments and funding

This study was supported by the Academy of Finland Center of Excellence in Complex Disease Genetics (grant numbers 213506, 129680), the Academy of Finland (grant numbers 139635, 129494, 136895, 263836 and 141054), the Sigrid Juselius Foundation , and ENGAGE – European Network for Genetic and Genomic Epidemiology, FP7-HEALTH-F4-2007, grant agreement number 201413. Finnish Foundation for Cardiovascular Research.

The Danish GEMINAKAR study

The study is a nationwide Danish project investigating the genetic epidemiology of a wide variety of phenotypes among Danish twins, who were recruited from births from 1931–1982. Invitations to take part in a full day clinical investigation were sent to 2,585 randomly chosen twin pairs. In 1,098 complete twin pairs (42.5%) both were willing and able to participate. A stratified sample of 756 twin pairs underwent an extensive full day clinical examination of a variety of phenotypes. The examinations were run from 1997 to 2000. The twins in a pair were examined on the same day, and included measures of weight, height, waist and hip circumference.

Measurements of blood pressure

Systolic and diastolic blood pressure were measured after 30 min rest using a conventional mercury sphygmomanometer and a hands-free stethoscope. Measurements were taken three times and the mean was used for analysis.

Ethics

The study was approved by the relevant Danish Ethics Committee (baseline, S-VF-19970271) and Danish Data Protection Board (baseline, 1999-1200-441). All participants provided written informed consent.

Acknowledgments and funding

The GEMINAKAR study was supported by grants from the Medical Research Fund, the Danish Diabetes Association, the NOVO Foundation, and the Danish Heart Foundation.

Generation Scotland

The Generation Scotland: Scottish Family Health Study (GS:SFHS) recruited 24,084 participants aged 18–100 years between 2006–11; full details are reported elsewhere (Smith BH, Campbell A, Linksted P, et al. Cohort Profile: Generation Scotland: Scottish Family Health Study (GS:SFHS). The study, its participants and their potential for genetic research on health and illness. International journal of epidemiology 2013;42(3):689-700 doi: 10.1093/ije).

Participants came from across Scotland, with some family members from further afield. The sample was 59% female, with a wide range of ages and socio-demographic characteristics. Most (87%) participants were born in Scotland and 96% in the UK or Ireland. Mean family size (excluding 1,400 singletons without any relations in the study) was 4.07 members; median was 3 (IQR 2–5). The largest family had 36 participating members, and participants were grouped in 5,621 families. Although GS:SFHS is a family-based study, the current analysis only included subjects who were unrelated. Relatedness was defined using the pedigree data. An important feature of GS:SFHS is the breadth and depth of phenotype information, including clinical and physical measures and detailed data on cognitive function, personality traits and mental health.

Genotyping

Genome-wide genotype data for nearly one million genetic variants have been measured on 10,000 selected participants. Blood samples (or saliva from postal and a few clinical participants) from GS:SFHS participants were collected, processed and stored using standard operating procedures and managed through a laboratory information management system at the Wellcome Trust Clinical Research Facility Genetics Core, Edinburgh (Kerr SM, Campbell A, Murphy L, et al. Pedigree and genotyping quality analyses of over 10,000 DNA samples from the Generation Scotland: Scottish Family Health Study. BMC medical genetics 2013;14:38 doi: 10.1186/1471-2350-14-38). The yield of DNA was measured using picogreen and normalised to 50ng/µl before genotyping. Genotyping was performed using the Illumina HumanOmniExpressExome-8 v1.0 DNA Analysis BeadChip and Infinium chemistry (Gunderson KL. Whole-genome genotyping on bead arrays. Methods in molecular biology 2009;529:197-213 doi: 10.1007/978-1-59745-538-1_13). In summary, this consists of three steps: (i) whole genome amplification, (ii) fragmentation followed by hybridisation, and (iii) single-base extension and staining. For each of the samples, 4µl of DNA normalised to 50ng/µl was used. The Arrays were imaged on an Illumina HiScan platform and genotypes were called automatically using GenomeStudio Analysis software v2011.1.

Measurements of blood pressure and resting heart rate

Blood pressure and heart rate were recorded during the participants' main research clinic visit, by trained and validated personnel, according a Standard operating Procedure (available on request). Using an Omron HEM-7051T digital BP monitor, with a cuff size appropriate to the participant's arm circumference, measurement was taken after a minimum of 5 minutes' rest (longer if any recent tobacco or caffeine ingestion), with the subject seated. This was repeated after a minimum of 3 further minutes, and the results recorded on the Clinic Report Form. The mean of these two readings was used.

Information was collected about medications, and where the participant was known to be taking a BP lowering drug the values were adjusted according to the analysis plan(systolic +15, diastolic +10, pulse pressure +5).

Smoking status

Smoking status was assessed via a paper-based, self-report questionnaire. Participants were asked a series of questions on smoking history, from which current smokers, former smokers and never smokers were defined. Pipe or cigar smokers who smoked no cigarettes were excluded from the analyses. Heaviness of smoking was assessed using the average number of cigarettes smoked per day.

In the current analyses, we included 6,888 participants with rs1051730 genotype, blood pressure, and smoking data available.

Ethics

Ethics approval for the study was given by the NHS Tayside committee on research ethics (reference 05/s1401/89).

Acknowledgments and funding

Generation Scotland has received core funding from the Chief Scientist Office of the Scottish Government Health Directorates CZD/16/6 and the Scottish Funding Council HR03006. Genotyping of the GS:SFHS samples was carried out by the Genetics Core Laboratory at the Wellcome Trust Clinical Research Facility, Edinburgh, Scotland and was funded by the UK's Medical Research Council.

Genomics of Overweight Young Adults (GOYA) male

Among 362 200 Caucasian men examined at the mean age of 20 years at the draft boards in Copenhagen and its surroundings during 1943–77, a randomly selected control group of 1 in every 100 men (n=3601) and all obese men (n=1930) were manually identified. Obesity was defined as 35% overweight relative to a local standard in use at the time, and this corresponds to a BMI of at least 31.0 kg/m2 , which proved to be above the 99th percentile. All obese men and half of the random sample, still living in the region, were invited to a follow-up survey in 1992–94 at the mean age of 46 years. The criteria for invitation to the follow-up surveys and the participation have been described previously. A total of 1441 men (661 obese and 780 randomly selected) were included in the present study with BMI, smoking status and rs1051730 genotype data available.

Genotype

Genome-wide SNP genotyping was performed using the Illumina 610 K Quad SNP chip, and this included rs1051730. The rs1051730 SNP had an overall call rate of 99.9% and the Hardy–Weinberg P-values were 0.21 and 0.44 in the controls and obese cases, respectively.

Measurements of blood pressure and resting heart rate

A London School of Hygiene sphygmomanometer was used. Run down of the mercury column was set to 2 mm/s. During the 5 min, the questionnaire was completed and the participant was asked to sign a form of consent. The blood pressure was classified as elevated, if the systolic blood pressure (SBP) was more than 110 mmHg plus the age of the participant, and this figure exceeded 145, or the

diastolic blood pressure (DBP) was higher than 100 mmHg regardless of age.
A 12-lead (I, II, III, aVR, aVL, aVF, V1–6) ECG was performed to record the resting heart rate in the supine position.

Ethics

The study was approved by the regional scientific ethics committee and by the Danish Data Protection Board.

Acknowledgments and funding

The GOYA study was conducted as part of the activities of the Danish Obesity Research Centre (DanORC, www.danorc.dk) and The MRC centre for Causal Analyses in Translational Epidemiology (MRC CAiTE). The genotyping for GOYA was funded by the Wellcome Trust (WT 084762). GOYA is a nested study within The Danish National Birth Cohort which was established with major funding from the Danish National Research Foundation. Additional support for this cohort has been obtained from the Pharmacy Foundation, the Egmont Foundation, The March of Dimes Birth Defects Foundation, the Augustinus Foundation, and the Health Foundation. TSA was supported by the Gene Diet Interactions in Obesity (GENDINOB, www.gendinob.dk) postdoctoral fellowship grant. LP is funded by an MRC Population Health Scientist Fellowship (MR/J012165/1).

Health2006

The Health2006 study took place during 2006-2008 and consisted of a random sample of 7,931 Danish (Danish nationality and born in Denmark) men and women aged 18-69 years invited to participate in a health examination. A total of 3,471 (43.8%) persons participated. Potential participants living in the Copenhagen area were identified in the central Danish Civil Registration System, and then recruited by invitation. The aim was to investigate the prevalence and risk factors of chronic diseases such as mental health, asthma, allergies, CVD, and diabetes. Informed written consent was obtained from all participants. The studies have been approved by the Ethical Committee of Copenhagen.

Genotyping

Blood samples were taken from all participants as part of their health examination. The buffy coat was frozen for DNA extraction, and later genomic DNA was extracted using a Qiagen AutoPure LS system. Genotyping was performed using KBiosciences allele-specific PCR (KASPar) (KBiosciences, Hoddesdon, UK). The call rate for this SNP (rs1051730) was > 99.2%. No errors were observed in 370 duplicate samples.

Measurements of blood pressure and resting heart rate

Blood pressure was measured in the sitting position after 5 minutes of rest using a Mercury300 mercury phygmomanometer, and the mean of two values was used for systolic and for diastolic pressure. Systolic blood pressure was read at the first Korotkoff sound and diastolic blood pressure at the disappearance of the Korotkoff sounds (phase V). The deflation rate was 2 mmHg/second. Before the investigation, the manometer was calibrated. Subsequently, resting heart rate was measured in sitting position by counting the radial pulse in 30 seconds and multiplying by 2. The

measurement was repeated and the mean of the two measurements of heart rate was calculated.

Smoking status

Information on smoking status was collected by a self-administered questionnaire to be filled in at home prior to the health examination. Smoking status was recorded as never, former, occasional (<1 cigarette, cheroot, cigar, or pipe per day) and daily smokers. Occasional smokers as well as daily smokers smoking exclusively cheroots, cigars, or pipe were excluded from all analyses. Smoking heaviness among daily smokers was recorded as number of cigarettes per day. In the current analyses, we included 2,758 participants with rs1051730 genotype, blood pressure, resting heart rate, and smoking data available.

Ethics

The Health2006 study was approved by the Ethical Committee of Copenhagen (KA-20060011) and the Danish Data Protection Agency. Informed written consent was obtained from all participants.

Acknowledgments and funding

The Health2006 study was financially supported by grants from the Velux Foundation; the Danish Medical Research Council, Danish Agency for Science, Technology and Innovation; the Aase and Ejner Danielsens Foundation; ALK-Abello´ A/S (Horsholm, Denmark), Timber Merchant Vilhelm Bangs Foundation, MEKOS Laboratories (Denmark) and Research Centre for Prevention and Health, the Capital Region of Denmark.

Key reference

Thuesen BH, Cerqueira C, Aadahl M, Ebstrup JF, Toft U, Thyssen JP, Fenger RV, Hersoug LG, Elberling J, Pedersen O, Hansen T, Johansen JD, Jorgensen T, Linneberg A. Cohort Profile: The Health2006 cohort, Research Centre for Prevention and Health. Int J Epidemiol. 2014;43:568-75.

The Helsinki Birth Cohort Study (HBCS)

The Helsinki Birth Cohort Study (HBCS) is composed of 13 345 individuals born between the years 1934-44 in one of the two main maternity hospitals in Helsinki, Finland. Between 2001 and 2003, a randomly selected sample of 928 males and 1,075 females participated in a clinical follow-up study with a focus on cardiovascular, metabolic and reproductive health, cognitive function and depressive symptoms. There were 1,709 women and men (43% men) with valid genotype and phenotype data. The mean age of the participants in the follow-up was 61.5 years (SD=2.9). In 2004, a psychological questionnaire was mailed to the participants, and in 2010-2011 a third follow-up was conducted. Detailed information on the selection of the HBCS participants and on the study design can be found elsewhere (Barker DJ, Osmond C, Forsen TJ, et al. Trajectories of growth among children who have coronary events as adults. The New England journal of medicine 2005;353(17):1802-9 doi: 10.1056/NEJMoa044160), (Eriksson JG, Osmond C, Kajantie E, et al. Patterns of growth among children who later develop type 2 diabetes or its risk factors. Diabetologia 2006;49(12):2853-8 doi: 10.1007/s00125-006-0459-1). (Rikkonen K, Pesonen AK, Heinonen K, et al. Infant growth and hostility in adult life. Psychosomatic medicine 2008;70(3):306-13 doi: 10.1097/PSY.0b013e3181651638). The research plan of the HBCS was approved by the Institutional

Review Board of the National Public Health Institute and all participants have signed an informed consent.

Genotype

DNA was extracted from the blood samples and genotyping was performed with the modified Illumina 610k chip by the Wellcome Trust Sanger Institute, Cambridge, UK according to the standard protocols. rs1051730 was directly genotyped and did not deviate from the HWE ($p = 0.52$). Frequency of the A-allele was 34.0%.

Measurements of blood pressure and resting heart rate

Blood pressure was measured from the right arm while the subject was in the sitting position and was recorded as the mean of two successive readings from a standard sphygmomanometer after 10 minutes of rest. Pulse rate was counted from the radial artery during 30 seconds and the results was multiplied by two.

Smoking status

Smoking status was self-reported on a following scale: never smoked, quitted smoking, smoke only on weekends, smoke 1-5 cigarettes/day, smoke 6-20 cigarettes /day, smoke >20 cigarettes /day. Current smokers were defined as individuals who reported smoking at least one cigarette per day. Data on pipe and cigar smoking were not available.

In the current analyses, we included 1,713 participants with rs1051730 genotype, blodd pressure, and pulse rate and smoking data available.

Ethics

The research plan of the HBCS was approved by the Institutional Review Board of the National Public Health Institute and all participants have signed an informed consent.

Acknowledgments and funding

Helsinki Birth Cohort Study has been supported by grants from the Academy of Finland, the Finnish Diabetes Research Society, Samfundet Folkhalsan, Novo Nordisk Foundation, Finska Lakaresallskapet, Signe and Ane Gyllenberg Foundation, University of Helsinki, Ministry of Education, Ahokas Foundation, Emil Aaltonen Foundation.

HUNT

The second wave of the HUNT Study in Norway took place in 1995-97, where all adults aged 20 years and older in Nord Trondelag County were invited to participate. A total of 65,438 (70%) accepted to the invitation and gave written informed consent to use the data for medical research. The data collection included questionnaires, clinical measurements and blood samples (http://www.ntnu.edu/hunt/data/que).

Genotyping

Altogether 56,664 participants were genotyped for the rs1051730 single nucleotide polymorphism variant. DNA was extracted from blood samples for all participants of the HUNT 2 study and stored at the HUNT biobank. The rs1051730 polymorphism was genotyped at the HUNT biobank using TaqMan genotyping assays (Applied Biosystems, Foster City, CA, USA) and performed on an Applied Biosystems 7900HT Fast real-Time PCR System using 10 ng of genomic DNA. The call rate cut-off was set to 90%, and the genotype frequencies were in agreement with HapMap data.

Measurements of blood pressure and resting heart rate

Blood pressure and resting heart rate were measured three times at one-minute intervals using an automated noninvasive blood pressure monitor based on oscillometry (Dinamap 845XT; Critikon, Tampa, Florida, USA). Measurements were performed after the participant had been seated for two minutes with the cuff on the arm and the arm resting on a table. Cuff size was adjusted after measuring the arm circumference. The mean values of the second and third measurements of blood pressure and resting heart rate were used in the analyses.

Smoking status

Smoking status was measured with self-completed questionnaire data with a categorical variable, and the participants were classified as never smokers, former smokers or current smokers. Current smokers were asked how many cigarettes they smoked per day. Exclusive pipe and/or cigar smokers were excluded from the analyses.

In the current analyses, we included 55,634 participants with rs1051730 genotype, blood pressure and smoking data available.

Ethics

Use of data in the present study was approved by the Regional Committee for Medical Research Ethics (Reference nr. 2013/1127/REK midt).

Acknowledgments and funding

Nord-Trondelag Health Study (The HUNT Study) is a collaboration between HUNT Research Centre (Faculty of Medicine, Norwegian University of Science and Technology NTNU), Nord-Trondelag County Council and the Norwegian Institute of Public Health.

Inter99

The Inter99 study is a randomised controlled trial (CT00289237, ClinicalTrials.gov) investigating the effects of lifestyle intervention on CVD (N=61,301) (Jorgensen T, Borch-Johnsen K, Thomsen TF, et al. A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology 2003;10(5):377-86 doi: 10.1097/01.hjr.0000096541.30533.82). We used baseline data from a random subsample of 12,934 men and women aged approximately 30, 35, 40, 45, 50, 55, or 65 years invited to participate in a

health examination during 1999-2001. Participants were living in the Copenhagen area and were identified in the central Danish Civil Registration System, and recruited by invitation. A total of 6,784 (52.5%) participated. A subsampe of 1,948 individuals examined in the period 15th February 2000 to 31st January 2001 were asked to complete a shortened version of the Danish SCL-90-R beforehand (1,837 accepted). Only participants with a Northern European origin (Denmark, Norway, Sweden, Iceland, and Faeroe Islands) were included (n=1,770). Informed written consent was obtained from all participants. The study was approved by the Ethical Committee of Copenhagen.

Genotyping

DNA was extracted from blood samples taken from all participants as part of their health examination. Genotyping was performed using KBiosciences allele-specific PCR (KASPar) (KBiosciences, Hoddesdon, UK). The call rate for this SNP (rs1051730) was > 98.8%. No errors were observed in 353 duplicate samples.

Measurements of blood pressure and resting heart rate

All participants had their blood pressure measured twice with a mercury sphygmomanometer (Mercuro 300; Speidel & Keller GmbH & Co, Jungingen, Germany) with appropriate cuff size, after 5 min of rest, in the lying position. Trained staff at the Research Centre for Prevention and Health performed all measurements. If SBP 140 mmHg at least or DBP 90 mmHg at least, the measurements were repeated twice to minimize the 'white-coat' effect, with the two lowest values being recorded and the average of the recorded measurements was used. Resting heart rate was averaged over all beats in a 10-second 12 lead ECG.

Smoking status

Information on smoking status was collected by a self-administered questionnaire to be filled in at home prior to the health examination. Smoking status was recorded as never, former, occasional (<1 cigarette, cheroot, cigar, or pipe per day) and daily smokers. Occasional smokers as well as daily smokers smoking exclusively cheroots, cigars, or pipe were excluded from all analyses. Smoking heaviness among daily smokers was recorded as number of cigarettes per day.

In the current analyses, we included 1,493 participants with rs1051730 genotype, blood pressure, resting heart rate, and smoking data available.

Ethics

The studies have been approved by the Ethical Committee of Copenhagen.

Acknowledgments and funding

Data collection in the Inter99 study was supported economically by The Danish Medical Research Council, The Danish Centre for Evaluation and Health Technology Assessment, Novo Nordisk, Copenhagen County, The Danish Heart Foundation, The Danish Pharmaceutical Association, Augustinus foundation, Ib Henriksen foundation and Becket foundation. LLNH was supported by the Health Insurance Foundation (grant No. 2010 B 131).

MIDSPAN Family Study

This is one of the four MIDSPAN population cohort studies based in Scotland [Hart, C.L., et al., *The Midspan studies.* Int J Epidemiol, 2005. **34**(1): p. 28-34.]. The three original studies took place between 1964 and 1976. Twenty years later, in 1996, the next generation was studied when offspring of couples in the original Renfrew/ Paisley Study were recruited into the Family Study. This latter group is the subject of the present analysis. Details of the study have been described previously [Upton, M. N., et al., *Intergenerational 20 year trends in the prevalence of asthma and hay fever in adults: the MIDSPAN family study surveys of parents and offspring.* BMJ, 2000. **321**: p. 88-92.].

Genotyping

Genotyping was performed on an ABI PRISM 7900HT sequence detection system using a Taqman assay (Assay ID: C_9510307_20, Applied Biosystems), followed by allelic discrimination using software from Applied Biosystems (SDS V2.0).3

Measurements of blood pressure and resting heart rate

Blood pressure and heart rate measurements were obtained using an automated Dinamapp 8100 instrument with the correctly sized cuff applied to the left arm while the subject was in the sitting position. After 5 minutes of rest, 3 readings were taken and the mean of the last 2 was used.

Smoking status

The participants completed a questionnaire which included questions on smoking habit. Three categories of smoking habit were defined: never smoker, current smoker, and former smoker.

Ethics

Ethics approval was obtained from the Argyll and Clyde Health Board Local Research Ethics

Funding

The MIDSPAN Family Study was funded as part of the NHS Research and Development Cardiovascular Research Programme.

NFBC1966

The Northern Finland Birth Cohort 1966 (NFBC1966) Study was initiated in 1965 by enrolling mothers living in the two Northernmost provinces of Finland, Oulu and Lapland, and with expected dates of delivery in 1966 (Rantakallio P. Groups at risk in low birth weight infants and perinatal mortality. Acta paediatrica Scandinavica 1969;193:Suppl 193:1). Altogether 12,231 children were born into the cohort, 12,058 of them live-born. The original data have been supplemented by data collected by postal questionnaires at the ages of 1, 14, 31 and 46 years (on-going) and data from various hospital records and national register data. At the 31 year follow up, those living in the

original target area (Northern Finland) or in the capital (Helsinki) area were invited to a clinical examination, in which 71% (N=6,033) participated. The University of Oulu Ethics Committee and the Ethical Committee of Northern Ostrobothnia Hospital District have approved the study. Participants provided written informed consent.

Genotyping

Blood samples were drawn at age 31 and DNA was extracted successfully for 5,753 subjects. Illumina's HumanCNV370-Duo DNA Analysis BeadChip was used to obtain genome-wide genetic data. It contains an informative set of tag SNPs derived from the HapMap European-derived (CEU) sample. Imputation was performed on 328,007 SNPs using IMPUTE software version 0.3.1 58, applying information threshold of >0.4 and MAF threshold of >1%. The rs1051730 SNP was extracted from GWAS data. The University of Oulu Ethics Committee and the Ethical Committee of Northern Ostrobothnia Hospital District have approved the study. Participants provided written informed consent.

Measurements of blood pressure and resting heart rate

Blood pressure (SBP, DBP mmHg) was measured in a sitting position after 15 min of rest, from the right upper arm using a mercury sphygmomanometer. The appropriate cuff size was determined by the subject's arm circumference. Two readings were taken 2 min apart, and the average of the measurements was used. Heart rate was taken manually from the radial artery over 30 seconds in the sitting position, multiplied by 2, then again after 15 minutes rest.

Smoking status

Smoking habits were enquired via a postal questionnaire as part of the 31-year follow-up study. The questionnaire was returned by 75% (N=8,767) of the individuals. Smoking heaviness was measured on a continuous scale. The exclusive pipe or cigar smokers excluded were excluded from all analyses. In the current analyses, we included 3,919 participants with rs1051730 genotype, blood pressure and resting heart rate and smoking data available.

Ethics

The University of Oulu Ethics Committee and the Ethical Committee of Northern Ostrobothnia Hospital District have approved the study. Participants provided written informed consent.

Acknowledgments and funding

NFBC1966 and NFBC1986 received financial support from the Academy of Finland (project grants 104781, 120315, 129269, 1114194, 24300796, 141042 Center of Excellence in Complex Disease Genetics and SALVE), University Hospital Oulu, Biocenter, University of Oulu, Finland (75617), NHLBI grant 5R01HL087679-02 through the STAMPEED program (1RL1MH083268-01), NIH/NIMH (5R01MH63706:02), the European Commission (EURO-BLCS, Framework 5 award QLG1-CT-2000-01643), ENGAGE project and grant agreement HEALTH-F4-2007-201413, EU FP7 EurHEALTHAgeing -277849, the Medical Research Council, UK (G0500539, G0600705, G1002319, PrevMetSyn/SALVE) and the MRC, Centenary Early Career Award. The DNA extractions, sample quality controls, biobank up-keeping and aliquotting was performed in the National Public Health Institute, Biomedicum Helsinki, Finland and supported financially by the Academy of Finland and Biocentrum Helsinki. We thank the late Professor Paula Rantakallio (launch of NFBCs), and Ms Outi Tornwall and Ms Minttu

Jussila (DNA biobanking). The authors would like to acknowledge the contribution of the late Academian of Science Leena Peltonen.

NFBC1986

The Northern Finland Birth Cohort 1986 (NFBC1986) includes all births in the two northernmost provinces of Finland, Oulu and Lapland, between July 1st 1985 and June 30th 1986 (Jarvelin MR, Elliott P, Kleinschmidt I, et al. Ecological and individual predictors of birthweight in a northern Finland birth cohort 1986. Paediatric and perinatal epidemiology 1997;11(3):298-312). Altogether 9,479 children were born into the cohort, 9,432 of them live-born. The original data have been supplemented by data collected by postal questionnaires at the ages of 7, 8 and 16 years and data from various hospital records and national register data. A clinical examination was performed at age 16 years in which 74% (N=6,798) of the cohort members participated. The University of Oulu Ethics Committee and the Ethical Committee of Northern Ostrobothnia Hospital District have approved the study. Participants provided written informed consent.

Genotyping

Blood samples were collected as part of the 16-year follow-up and DNA was extracted for 6266 individuals. The DNA samples were processed at Imperial College London, UK and custom genotyping was performed at LGC Genomics Ltd, Hoddesdon, Herts, UK (formerly Kbioscience). The University of Oulu Ethics Committee and the Ethical Committee of Northern Ostrobothnia Hospital District have approved the study. Participants provided written informed consent.

Measurements of blood pressure and resting heart rate

Blood pressure (SBP, DBP mmHg) was measured in a sitting position after 15 min of rest, from the right upper arm using an OMRON blood pressure monitor (OMRON Matsusaka Co. Ltd, Japan). The appropriate cuff size was determined by the subject's arm circumference. Two readings were taken 2 min apart, and the average of the measurements was used. Heart rate was taken manually from the radial artery over 30 seconds in the sitting position, multiplied by 2, then again after 15 minutes rest.

Smoking status

Smoking habits were enquired via a postal questionnaire as part of the 16-year follow-up study. The questionnaire was returned by 80% (N=7,344) of the adolescents. Two categories of smokers were created, never smokers who reported never having tried a cigarette and current smokers who reported smoking at least one cigarette daily. Individuals who did not fall into this category were excluded from the analyses.

In the current analyses, we included 1,143 participants with rs1051730 genotype, blood pressure and resting heart rate and smoking data available.

Ethics

The University of Oulu Ethics Committee and the Ethical Committee of Northern Ostrobothnia Hospital District have approved the study. Participants provided written informed consent.

Acknowledgments and funding

NFBC1966 and NFBC1986 received financial support from the Academy of Finland (project grants 104781, 120315, 129269, 1114194, 24300796, 141042 Center of Excellence in Complex Disease Genetics and SALVE), University Hospital Oulu, Biocenter, University of Oulu, Finland (75617), NHLBI grant 5R01HL087679-02 through the STAMPEED program (1RL1MH083268-01), NIH/NIMH (5R01MH63706:02), the European Commission (EURO-BLCS, Framework 5 award QLG1-CT-2000-01643), ENGAGE project and grant agreement HEALTH-F4-2007-201413, EU FP7 EurHEALTHAgeing -277849, the Medical Research Council, UK (G0500539, G0600705, G1002319, PrevMetSyn/SALVE) and the MRC, Centenary Early Career Award. The DNA extractions, sample quality controls, biobank up-keeping and aliquotting was performed in the National Public Health Institute, Biomedicum Helsinki, Finland and supported financially by the Academy of Finland and Biocentrum Helsinki. We thank the late Professor Paula Rantakallio (launch of NFBCs), and Ms Outi Tornwall and Ms Minttu Jussila (DNA biobanking). The authors would like to acknowledge the contribution of the late Academian of Science Leena Peltonen.

NHANES

The National Health and Nutrition Examination Survey (NHANES) (http://www.cdc.gov/nchs/nhanes.htm) is a program of health surveys run by the National Center for Health Statistics, part of the Centers for Disease Control and Prevention in the United States. The third National Health and Nutrition Examination Survey (NHANES III) was conducted from October 1988 through October 1994 in two phases, each of which comprised a national probability sample. The first phase was conducted from October 18, 1988, through October 24, 1991, at 44 locations. The second phase was conducted from September 20, 1991, through October 15, 1994, at 45 different locations. In NHANES III, 39,695 persons were selected over the six years; of those, 33,994 (86%) were interviewed in their homes. All interviewed persons were invited to the Medical examination centre (MEC) for a medical examination. Seventy-eight percent (30,818) of the selected persons were examined in the MEC, and an additional 493 persons were given a special, limited examination in their homes (U.S. Department of Health and Human Services (DHHS). National Center for Health Statistics. NHANES III reference manuals and reports (CD-ROM). Hyattsville, MD: Centers for Disease Control and Prevention, 1996. Available from National Technical Information Service (NTIS), Springfield, VA. Data collection for NHANES was approved by the NCHS Research Ethics Review Board. Analysis of deidentified data from the survey is exempt from the federal regulations for the protection of human research participants. Analysis of restricted data through the NCHS Research Data Center is also approved by the NCHS ERB.

Genotype

The genetic data available through the NCHS is from 7,159 specimens collected during Phase-2 of the Third National Health and Nutrition Examination Survey. 2,630 specimens were collected from Non-Hispanic White individuals. Genotyping of rs1051730 was carried out using the iPLEX Gold (Sequenom, San Diego, CA) at Vanderbilt University 's DNA Resources Core. Average SNP call rate was 99.5%. This genotype is a restricted variable and therefore these data were accessed through the Research Data Center.

Measurements of blood pressure and resting heart rate

Blood pressure was measured by trained physicians in the mobile examination center. Measurements were taken in triplicate using a mercury sphygmomanometer (W. A. Baum Co., Inc, Copiague, NY) according to the standardized blood pressure measurement protocols recommended by the American Heart Association (Frohlich, 1988). Full details of the methodology can be found at http://www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/phys.pdf. Use of blood pressure medication was self-reported at the household examination.

Smoking status

Smoking status was collected in Household Adult Questionnaire administered during the household interview. Current smokers were defined as individuals who reported smoking at least one cigarette per day. Former smokers were defined as individuals who reported having smoked at least 100 cigarettes in their lifetime but did not currently smoke cigarettes. Never smokers were individuals who reported having smoked less than 100 cigarettes in their lifetime. Pipe and cigar smokers who did not also report cigarette smoking were excluded from analyses.

Sample analysis

Due to the survey design of the NHANES data, analyses were performed using survey commands available in Stata (version 11). Taylor series linearization was implemented to estimate variances (Woodruff R. A simple method for approximating the variance of a complicated estimate. Journal of the American Staistical Association 1971;66:411-14). Analyses were weighted using the genetic weights provided by NHANES, as described previously (Chang MH, Lindegren ML, Butler MA, et al. Prevalence in the United States of selected candidate gene variants: Third National Health and Nutrition Examination Survey, 1991-1994. American journal of epidemiology 2009;169(1):54-66 doi: 10.1093/aje/kwn286). Analyses were restricted to individuals of non- Hispanic white ethnic origin. In the current analyses, we included 694 participants with rs1051730 genotype, blood pressure and resting heart rate and smoking data available.

Ethics

Data collection for NHANES was approved by the NCHS Research Ethics Review Board. Analysis of deidentified data from the survey is exempt from the federal regulations for the protection of human research participants. Analysis of restricted data through the NCHS Research Data Center is also approved by the NCHS ERB. The findings and conclusions in this paper are those of the author(s) and do not necessarily represent the views of the Research Data Center, the National Center for Health Statistics, or the Centers for Disease Control and Prevention.

Acknowledgments and funding

The National Health and Nutrition Examination Survey (NHANES) (http://www.cdc.gov/nchs/nhanes.htm) is a program of health surveys run by the National Center for Health Statistics, part of the Centers for Disease Control and Prevention in the United States.

MRC NSHD

The Medical Research Council National Survey of Health and Development (NSHD) is an on-going

prospective birth cohort study consisting of all births in England, Scotland and Wales in one week in March 1946 (Wadsworth M, Kuh D, Richards M, et al. Cohort Profile: The 1946 National Birth Cohort (MRC National Survey of Health and Development). International journal of epidemiology 2006;35(1):49-54 doi: 10.1093/ije/dyi201). The sample includes single, legitimate births whose fathers were in non-manual or agricultural occupations and a randomly selected one in four of all others, whose fathers were in manual labour. The original cohort, now 68 years of age, comprised 2,547 women and 2,815 men who have been followed-up over 20 times since their birth. The data collected to date include repeat cognitive function, physical, lifestyle and anthropomorphic measures, as well as blood analytes and other measures. The cohort recently completed a particularly intensive phase of clinical assessment and biological sampling with blood and urine sampling and analysis, and cardiac and vascular imaging (Kuh D, Pierce M, Adams J, et al. Cohort profile: updating the cohort profile for the MRC National Survey of Health and Development: a new clinic-based data collection for ageing research. International journal of epidemiology 2011;40(1):e1-9 doi: 10.1093/ije/dyq231). Ethical approval was given by the Central Manchester Research Ethics Committee. Bona fide researchers can apply to access the NSHD data via a standard application procedure (further details available at: http://www.nshd.mrc.ac.uk/data.aspx).

Genotyping

DNA was extracted from blood samples collected in 1999 (Rousseau K, Vinall LE, Butterworth SL, et al. MUC7 haplotype analysis: results from a longitudinal birth cohort support protective effect of the MUC7*5 allele on respiratory function. Ann Hum Genet 2006;70(Pt 4):417-27 doi: 10.1111/j.1469-1809.2006.00250.x). Genotyping of rs16969968 was carried out by LGC Genomics (Hoddesdon, UK; www.lgcgenomics.com) using fluorescence-based competitive allele-specific PCR (KASPar). Call rate was >95%.

Measurements of blood pressure and resting heart rate

At age 53yrs, blood pressure and resting heart rate was measured twice by a trained nurse in the participant's home while the survey member was seated and after 5 min rest. The 2nd blood pressure reading and 2nd resting heart rate measurement were used for this analysis. All measurements were performed using an automated digital oscillometric sphygmomanometer (Omron HEM-705; Omron Corp., Tokyo, Japan).

Smoking status

Smoking status was collected during a home interview at age 53 years by trained interviewers (Clennell S, Kuh D, Guralnik JM, et al. Characterisation of smoking behaviour across the life course and its impact on decline in lung function and all-cause mortality: evidence from a British birth cohort. Journal of epidemiology and community health 2008;62(12):1051-6 doi:10.1136/jech.2007.068312). Current cigarette smoking status (“yes”, “no”) and the number of cigarettes smoked per day was obtained. Study members who provided an affirmative response to being current cigarette smokers, regardless of the quantity of cigarettes smoked per day, were classified as “smokers”, while those who provided a negative response were classified as “nonsmoker”. Pipe and cigar smokers who did not also report cigarette smoking were excluded from analyses. In the current analyses, we included 2,576 participants with rs16969968 genotype, blood pressure and resting heart rate and smoking data available.

Ethics

Ethics approval was given by the Central Manchester Research Ethics Committee.

Acknowledgments and funding

We are very grateful to the members of this birth cohort for their continuing interest and participation in the study. We would like to acknowledge the Swallow group at University College London, who performed the DNA extractions. This work was funded by the Medical Research Council [MC_UU_12019/1].

The Netherlands Twin Register (NTR)

Participants were registered with the Netherlands Twin Register (NTR). Longitudinal survey collection in twins and their parents started in 1991. Participants were invited about every 3 years to complete a survey containing questions about health, lifestyle, personality and psychopathology. In addition, subjects were invited to participate in specific research projects like a biobank project (Willemsen, de Geus et al. 2010) and/or ambulatory measurements (De Geus, Kupper et al. 2007; Neijts, Van Lien et al. 2014).

Genotyping

Genotyping at the rs16969968 locus was performed on different platforms. Further details regarding DNA collection, genotyping, imputation and quality control checks in the NTR can be found elsewhere (Nivard, Mbarek et al. 2014)

Measurements of blood pressure and resting heart rate

Blood pressure data from in total 11 studies were pooled. Studies 1 and 2 correspond to the twins respectively parents included in the study by Boomsma et al. (Boomsma, Orbeleke et al. 1991). Study 3 included adult twins from the study by Snieder et al., 1995 [REF Snieder et al., Developmental genetic trends in blood pressure levels and blood pressure reactivity to stress. In J. R. Turner, L. R. Cardon, & J. K. Hewitt (Eds.), Behavior genetic approaches in behavioral medicine (pp. 105–130). New York: Plenum Press]. In study 4 (Kupper, Willemsen et al. 2005), ambulatory blood pressure was measured in twins and siblings. For studies 1-4, methods to obtain the blood pressure data as used in the current study have been described in the publication by Hottenga et al., 2007 (Hottenga, Whitfield et al. 2007). In study 5, brachial systolic and diastolic blood pressure (SBP resp. DBP) of seated subjects were measured twice in a sound-attenuated, electrically shielded cabin using an oscillometric technique (Dinamap 845XT) during a four-minute period of sitting at rest followed by an extended session of electroencephalographic recording. In studies 6 and 7, embedded in protocols involving prolonged cognitive testing three resp. four recordings were obtained using the Dinamap 845XT after sitting quietly for two to four minutes. In study 8, blood pressure measurements were performed with an automatic blood pressure meter (Dinamap procare 100, KP medical B.V., Houten, The Netherlands) in the last 15 minutes of a 45-minute supine baseline that preceded an extensive test of pancreatic beta-cell function by repeated insulin/glucose sampling. In study 9, 10, and 11, blood pressure recordings were obtained twice using the OMRON HEM907 oscillometric device. In study 9, the measurements were performed with a 60-sec interval during the break of an extended protocol involving prolonged cognitive testing, after sitting quietly for 4 minutes. In study 10, the measurements were performed while sitting at rest before resp. after blood sampling. In study 11, after arrival at the home of the participants and an explanation of the ambulatory electrocardiographic recording procedure, subjects sat down for a resting four-minute baseline during which the two blood pressure

recordings were taken measurements (De Geus, Kupper et al. 2007; Neijts, Van Lien et al. 2014). In all studies, for each participant we obtained the average of the all resting blood pressure measurements.

Smoking status

Data on smoking behaviour were collected at the same time as the blood pressure measurements. Subjects were asked whether they (ever) smoked and current smokers were asked how many cigarettes per day they smoked (continuous measure). Daily cigarette smokers were selected for all analyses, subjects who only smoked pipe or cigar smokers were excluded.

In the current analyses, we included 404 unrelated participants with genotypes at the rs16969968 locus for whom data on blood pressure, resting heart rate, and smoking were available.

Ethics

The NTR study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center, Amsterdam (IRB number IRB-2991 under Federalwide Assurance 3703; IRB/institute code 03-180), and all subjects provided written informed consent.

Acknowledgments and funding

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the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)

PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden) (PMID:10569329 and PMID:12457784). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5,804 subjects were randomly assigned to pravastatin or placebo. All subjects were given a clinical examination at baseline before randomization, which included drawing of venous blood samples, measurement of weight, height, and BMI (weight/height2).

Genotyping

The PHASE project is a genome-wide association study (GWAS) in the participants of the PROSPER study, to investigate the genetic variation responsible for the individual variation in drug response, and has been described previously (PMID:21977987). In brief, genotyping was conducted using the Illumina 660-Quad beadchips following manufacturer's instructions. After a stringent quality control (call rate< 95%) 557,192 SNPs in 5244 participants were available for analysis 17. Those SNPs were imputed up to 2.5 million autosomal CEPH HapMap SNPs using MaCH imputation software based on the HapMap built 36 release 22.

Measurements of blood pressure and resting heart rate

Systolic and diastolic blood pressure were measured at baseline and repeated every three months. Blood pressure was measured in sitting position using a fully automatic electronic sphygmomanometer (Omron M4R). All measurements were performed in the same clinical setting. Resting heart rate (b.p.m.) was automatically measured from a 12-lead ECG recorded in the morning as part of the first enrolment visit, along with fasting venous blood sample collection, to limit circadian variability. All ECGs were interpreted using the same software, which produced all measurements including heart rate.

Smoking status

Smoking status was assessed at baseline using a questionnaire. Smoking status was coded as current, former, or never smoker.

Ethics

The protocol of the PROSPER study was approved by the medical ethics committees of each participating institution. Written informed consent was obtained from all participating subjects.

Acknowledgments and funding

The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trial was supported by an investigator initiated grant from Bristol-Myers Squibb, USA. The study was conducted, analysed, and reported independently of the company. The GWAS project PHASE has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement HEALTH-F2-2009-223004. A part of the genotyping was funded by The Netherlands Consortium for Healthy Ageing (NGI: 05060810). JWJ is an established clinical investigator of The Netherlands Heart Foundation (2001 D 032).

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Whitehall II

Whitehall II recruitment of 10,308 participants (70% men) between 1985 and 1988 involved 20

London based Civil service departments (Marmot M, Brunner E. Cohort Profile: the Whitehall II study. International journal of epidemiology 2005;34(2):251-6 doi: 10.1093/ije/dyh372). Genetic samples were collected in 2004 from over 6,000 participants. The study is highly phenotyped for

cardiovascular and other ageing related health outcomes, with 9 phases of follow up (5 with clinical assessment and biological sampling), over 20 years of follow up. A wide variety of health behaviour and environmental data are also collected and the participants are consented for linkage to recorded clinical data such as Hospital Episode Statistics (HES), the Office of National Statistics mortality data and the national registry of acute coronary syndromes in England and Wales (Myocardial Ischaemia National Audit Project). Ethical approval for the Whitehall II study was obtained from the University College London Medical School committee on the ethics of human research. Informed consent was gained from every participant.

Genotyping

Genotyping of rs16969968 was performed as part of genotyping using the Metabochip (Voight BF, Kang HM, Ding J, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. PLoS genetics 2012;8(8):e1002793 doi: 10.1371/journal.pgen.1002793)

Measurements of blood pressure and resting heart rate

During the first phase of data collection, systolic blood pressure and diastolic blood pressure were measured twice in the sitting position after 5 minutes of rest with OMRON HEM 907. The average of the 2 readings was taken to be the measured systolic and diastolic blood pressures. Use of blood pressure medication was self-reported at phase 1.

Smoking status

Information on smoking status was collected by questionnaire during the first phase of data collection. Individuals who reported smoking cigars or pipes but not cigarettes were excluded from all analyses.

In the current analyses, we included 2,916 participants with rs16969968 genotype and phenotype data available.

Ethics

The University College London Medical School committee on the ethics of human research reviewed and approved the Whitehall II study. Informed consent was obtained from every participant.

Acknowledgments and funding

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Supplementary Table S1: Descriptive statistics of study populations

Study	Smoking status	N (%)	Male, N (%)	Age, median (range)	SBP, Mean (SD)	DBP, Mean (SD)	Resting heart rate, Mean (SD)	Hypertension, N (%)	Severe Hypertension, N (%)
1958BC	never	2,356(46.9)	1,194(50.7)	45	127.3(17.4)	79.4(11.6)	70.2(10.4)	583(24.7)	194(8.2)
	former	1,446(28.8)	753(52.1)	45	127.3(17.1)	79.5(11.2)	70.8(10.4)	373(25.8)	132(9.1)
	current	1,222(24.3)	585(47.9)	45	127.7(17.4)	79.0(11.1)	74.5(10.8)	306(25.0)	105(8.6)
	ever	2,668(53.1)	1,338(50.1)	45	127.5(17.2)	79.3(11.1)	72.5(10.7)	679(25.4)	237(8.9)
	Total	5,024	2,532(50.4)	45	127.4(17.3)	79.3(11.3)	71.4(10.6)	1,262(25.1)	431(8.6)
ALSPAC children	never	1,268(80.6)	626(49.4)	17.7(16.3-19.2)	118.6(11.1)	63.3(6.2)	63.9(10.0)	-	-
	current	306(19.4)	128(41.8)	17.7(16.8-19.5)	117.8(10.8)	64.1(7.3)	63.9(9.9)	-	-
	Total	1,574	754(47.9)	17.7(16.3-19.5)	118.4(11.0)	63.5(6.5)	63.9(10.0)	-	-
ALSPAC mothers	never	1,190(65.2)	-	48.0(37.0-61.0)	120.7(14.0)	72.3(9.3)	67.0(9.1)	146(12.3)	69 (5.8)
	former	480(26.3)	-	48.0(37.0-60.0)	121.4(14.5)	72.4(9.4)	66.5(9.4)	59(12.3)	31 (6.5)
	current	156(8.5)	-	46.0(35.0-57.0)	117.5(12.9)	71.5(9.3)	67.8(9.6)	17(10.9)	9 (5.8)
	ever	636(34.8)	-	48.0(35.0-60.0)	120.4(14.2)	72.1(9.3)	66.8(9.5)	76(11.9)	40 (6.3)
	Total	1,826	-	48.0(35.0-61.0)	120.6(14.1)	72.3(9.3)	67.0(9.2)	222(12.2)	109 (6.0)
BRHS	never	1,120(29.1)	1,120(100.0)	66.9(58.3-80.8)	148.1(23.6)	85.9(11.1)	-	788(70.4)	491(43.8)
	former	2,234(58.0)	2,234(100.0)	69.3(58.4-81.1)	150.2(24.1)	85.2(11.1)	-	1,701(76.1)	1,150(51.5)
	current	501(13.0)	501(100.0)	67.8(58.4-80.0)	147.3(25.7)	83.8(11.6)	-	350(69.9)	232(46.3)
	ever	2,735(70.9)	2,735(100.0)	68.9(58.4-81.1)	149.6(24.4)	84.9(11.2)	-	2,051(75.0)	1,382(50.5)
	Total	3,855	3,855(100.0)	68.4(58.3-81.1)	149.2(24.2)	85.2(11.2)	-	2,839(73.6)	1,873(48.6)
CaPS	never	249(21.5)	249(100.0)	55.0(49.0-65.0)	145.9(22.9)	85.9(11.4)	64.6(10.1)	144(57.8)	65(26.1)
	former	499(43.0)	499(100.0)	57.0(48.0-66.0)	147.2(22.7)	85.4(11.7)	64.4(11.1)	293(58.7)	139(27.9)
	current	412(35.5)	412(100.0)	56.0(47.0-67.0)	144.9(22.2)	83.2(10.8)	66.5(12.0)	224(54.4)	91(22.1)
	ever	911(78.5)	911(100.0)	57.0(47.0-67.0)	146.1(22.5)	84.4(11.3)	65.4(11.6)	517(56.8)	230(25.2)
	Total	1,160	1,160(100.0)	57.0(47.0-67.0)	146.1(22.6)	84.8(11.4)	65.2(11.3)	661(57.0)	295(25.4)
CoLaus	never	2,013(42.1)	761(37.8)	53.2(35.0-75.4)	130.5(19.0)	80.6(10.9)	67.8(9.4)	716(35.6)	448(22.3)
	former	1,529(32.0)	786(51.4)	54.7(35.1-74.9)	132.4(20.0)	80.9(11.7)	67.6(10.1)	622(40.7)	392(25.6)
	current	1,235(25.9)	595(48.2)	49.6(34.9-75.3)	126.1(18.6)	78.3(11.1)	69.1(9.7)	343(27.8)	192(15.5)
	ever	2,764(57.9)	1,381(50.0)	52.3(34.9-75.3)	129.6(19.6)	79.7(11.5)	68.3(10.0)	965(34.9)	584(21.1)

Study	Smoking status	N (%)	Male, N (%)	Age, median (range)	SBP, Mean (SD)	DBP, Mean (SD)	Resting heart rate, Mean (SD)	Hypertension, N (%)	Severe Hypertension, N (%)
	Total	4,777	2,142(44.8)	52.8(34.9-75.4)	130.0(19.4)	80.1(11.3)	68.1(9.7)	1,681(35.2)	1,032(21.6)
Dan-Monica10	never	655(28.6)	235(35.9)	50.0(40.0-70.0)	132.3(20.5)	86.0(12.0)	66.9(10.6)	257(39.2)	117(17.9)
	former	587(25.6)	380(64.7)	60.0(40.0-70.0)	136.1(21.3)	86.4(12.4)	66.1(11.2)	266(45.3)	121(20.6)
	current	1,050(45.8)	553(52.7)	50.0(40.0-70.0)	126.2(19.9)	80.0(11.7)	64.9(10.7)	266(25.3)	112(10.7)
	ever	1,637(71.4)	933(57.0)	50.0(40.0-70.0)	129.8(21.0)	82.3(12.3)	65.3(10.9)	532(32.5)	233(14.2)
	Total	2,292	1,168(51.0)	50.0(40.0-70.0)	130.5(20.9)	83.4(12.3)	65.8(10.8)	789(34.4)	350(15.3)
ELSA	never	1,588(35.6)	528(33.2)	64.0(52.0-99.0)	136.7(19.8)	76.7(11.1)	59.3(15.1)	667(42.0)	326(20.5)
	former	2,366(53.0)	1,267(53.6)	66.0(52.0-99.0)	137.3(19.8)	76.3(11.7)	60.3(15.1)	1,067(45.1)	519(21.9)
	current	510(11.4)	234(45.9)	61.0(52.0-88.0)	136.1(20.3)	77.3(12.1)	58.1(14.4)	228(44.7)	113(22.2)
	ever	2,876(64.4)	1,501(52.2)	65.0(52.0-99.0)	137.0(19.9)	76.5(11.8)	59.9(15.0)	1,295(45.0)	632(22.0)
	Total	4,464	2,029(45.5)	65.0(52.0-99.0)	136.9(19.9)	76.5(11.6)	59.7(15.1)	1,962(44.0)	958(21.5)
FINRISK	never	9,755(47.9)	3,279(33.6)	51.0(25.0-74.0)	138.9(22.8)	82.1(12.4)	69.0(11.4)	4,459(45.8)	2,449(25.1)
	former	5,493(27.0)	3,135(57.1)	51.0(25.0-74.0)	139.4(22.3)	83.0(12.7)	68.0(11.6)	2,671(48.7)	1,532(28.0)
	current	5,120(25.1)	2,939(57.4)	44.0(25.0-74.0)	134.9(20.2)	80.4(12.7)	73.4(12.4)	1,954(38.2)	889(17.4)
	ever	10,613(52.1)	6,074(57.2)	47.0(25.0-74.0)	137.2(21.4)	81.8(12.7)	70.6(12.3)	4,625(43.6)	2,421(22.8)
	Total	20,368	9,353(45.9)	49.0(25.0-74.0)	138.0(22.1)	81.9(12.6)	69.9(11.9)	9,084(44.7)	4,870(23.9)
GEMINAKAR	never	549(49.3)	268(48.8)	36.0(18.0-67.0)	117.5(13.6)	69.3(10.2)	-	34 (6.2)	5 (0.9)
	former	185(16.6)	89(48.1)	40.0(18.0-63.0)	120.6(16.8)	70.8(10.6)	-	15 (8.1)	6 (3.2)
	current	379(34.1)	183(48.3)	38.0(18.0-65.0)	113.8(13.2)	65.9(9.6)	-	12 (3.2)	1 (0.3)
	ever	564(50.7)	272(48.2)	39.0(18.0-65.0)	116.0(14.8)	67.5(10.2)	-	27 (4.8)	7 (1.2)
	Total	1,113	540(48.5)	37.0(18.0-67.0)	116.7(14.2)	68.4(10.2)	-	61 (5.5)	12 (1.1)
GOYA male	never	178(22.5)	178(100.0)	44.0(34.0-66.0)	138.2(16.4)	90.8(10.3)	67.8(13.0)	91(51.1)	43(24.2)
	former	219(27.7)	219(100.0)	47.0(34.0-73.0)	140.7(17.9)	91.5(10.9)	68.9(13.7)	125(57.1)	51(23.3)
	current	394(49.8)	394(100.0)	47.0(34.0-71.0)	140.7(18.6)	90.5(11.7)	73.5(13.2)	227(57.6)	97(24.6)
	ever	613(77.5)	613(100.0)	47.0(34.0-73.0)	140.7(18.4)	90.9(11.4)	71.9(13.5)	352(57.4)	148(24.1)
	Total	791	791(100.0)	46.0(34.0-73.0)	140.1(18.0)	90.9(11.2)	70.9(13.5)	443(56.0)	191(24.1)
Generation Scotland	never	3,727(54.1)	1,424(38.2)	57.0(18.0-95.0)	137.6(19.6)	83.0(10.9)	68.8(11.3)	1,664(44.6)	843(22.6)
	former	2,273(33.0)	1,037(45.6)	59.0(18.0-99.0)	140.5(20.8)	83.5(11.0)	68.3(11.3)	1,143(50.3)	617(27.1)

Study	Smoking status	N (%)	Male, N (%)	Age, median (range)	SBP, Mean (SD)	DBP, Mean (SD)	Resting heart rate, Mean (SD)	Hypertension, N (%)	Severe Hypertension, N (%)
	current	888(12.9)	365(41.1)	53.0(18.0-84.0)	133.2(19.5)	80.9(11.0)	72.5(11.1)	319(35.9)	164(18.5)
	ever	3,161(45.9)	1,402(44.4)	57.0(18.0-99.0)	138.5(20.7)	82.8(11.0)	69.5(11.4)	1,462(46.3)	781(24.7)
	Total	6,888	2,826(41.0)	57.0(18.0-99.0)	138.0(20.1)	82.9(11.0)	69.1(11.3)	3,126(45.4)	1,624(23.6)
HBCS	never	745(43.5)	202(27.1)	61.1(56.7-69.8)	154.5(24.2)	93.3(12.7)	70.0(10.7)	569(76.4)	385(51.7)
	former	578(33.7)	336(58.1)	61.1(56.9-69.3)	154.1(24.2)	93.2(12.8)	68.2(11.7)	435(75.3)	320(55.4)
	current	390(22.8)	195(50.0)	60.3(56.8-69.1)	150.4(24.1)	91.9(13.0)	73.5(12.8)	274(70.3)	183(46.9)
	ever	968(56.5)	531(54.9)	60.7(56.8-69.3)	152.7(24.2)	92.7(12.9)	70.3(12.5)	709(73.2)	503(52.0)
	Total	1,713	733(42.8)	60.9(56.7-69.8)	153.5(24.2)	92.9(12.8)	70.2(11.7)	1,278(74.6)	888(51.8)
HUNT	never	24,377(43.8)	10,050(41.2)	47.0(19.0-97.0)	140.6(24.5)	81.0(13.6)	72.2(12.6)	10,595(43.5)	5,771(23.7)
	former	14,199(25.5)	8,478(59.7)	53.0(19.0-99.0)	142.1(23.5)	83.5(13.3)	71.8(12.8)	7,088(49.9)	3,860(27.2)
	current	17,058(30.7)	7,914(46.4)	46.0(19.0-94.0)	135.0(21.1)	80.1(12.4)	75.5(12.2)	6,003(35.2)	2,674(15.7)
	ever	31,257(56.2)	16,392(52.4)	49.0(19.0-99.0)	138.2(22.5)	81.6(12.9)	73.8(12.6)	13,091(41.9)	6,534(20.9)
	Total	55,634	26,442(47.5)	48.0(19.0-99.0)	139.3(23.4)	81.3(13.2)	73.1(12.6)	23,686(42.6)	12,305(22.1)
Health2006	never	1,385(43.0)	616(44.5)	47.8(18.6-71.7)	132.0(20.3)	83.1(12.0)	66.1(9.9)	475(34.3)	263(19.0)
	former	1,080(33.5)	495(45.8)	53.4(19.8-71.8)	135.6(20.2)	85.1(11.8)	66.3(10.2)	456(42.2)	278(25.7)
	current	755(23.4)	319(42.3)	50.1(19.4-71.7)	130.9(19.7)	81.5(11.5)	70.4(10.7)	241(31.9)	144(19.1)
	ever	1,835(57.0)	814(44.4)	52.1(19.4-71.8)	133.7(20.1)	83.7(11.8)	68.0(10.6)	697(38.0)	422(23.0)
	Total	3,220	1,430(44.4)	50.5(18.6-71.8)	133.0(20.2)	83.4(11.9)	67.2(10.4)	1,172(36.4)	685(21.3)
Health2008	never	280(44.9)	133(47.5)	44.1(30.5-61.0)	121.7(15.6)	78.1(10.3)	66.3(9.3)	49(17.5)	30(10.7)
	former	221(35.4)	87(39.4)	50.0(31.4-60.8)	125.6(18.1)	80.6(11.6)	66.2(9.7)	54(24.4)	31(14.0)
	current	123(19.7)	55(44.7)	47.5(30.7-60.7)	120.5(17.9)	75.9(11.3)	66.4(9.7)	25(20.3)	18(14.6)
	ever	344(55.1)	142(41.3)	49.2(30.7-60.8)	123.8(18.2)	78.9(11.7)	66.3(9.7)	79(23.0)	49(14.2)
	Total	624	275(44.1)	46.8(30.5-61.0)	122.9(17.1)	78.5(11.1)	66.3(9.5)	128(20.5)	79(12.7)
Inter99	never	2,109(36.4)	971(46.0)	45.0(30.0-60.0)	133.5(18.4)	84.7(12.1)	67.5(11.3)	762(36.1)	463(22.0)
	former	1,522(26.3)	786(51.6)	50.0(30.0-60.0)	134.4(19.5)	85.0(12.3)	66.8(10.8)	579(38.0)	334(21.9)
	current	2,156(37.3)	1,062(49.3)	45.0(30.0-60.0)	128.2(18.9)	80.6(12.1)	66.6(11.1)	630(29.2)	378(17.5)
	ever	3,678(63.6)	1,848(50.2)	45.0(30.0-60.0)	130.8(19.4)	82.4(12.4)	66.7(11.0)	1,209(32.9)	712(19.4)
	Total	5,787	2,819(48.7)	45.0(30.0-60.0)	131.8(19.1)	83.3(12.3)	67.0(11.1)	1,971(34.1)	1,175(20.3)

Study	Smoking status	N (%)	Male, N (%)	Age, median (range)	SBP, Mean (SD)	DBP, Mean (SD)	Resting heart rate, Mean (SD)	Hypertension, N (%)	Severe Hypertension, N (%)
MIDSPAN	never	823(45.2)	358(43.5)	44.0(30.0-59.0)	127.5(16.4)	75.3(12.1)	74.7(11.9)	156(19.0)	45 (5.5)
	former	513(28.2)	260(50.7)	47.0(30.0-59.0)	130.1(16.3)	77.0(12.0)	74.2(12.3)	130(25.3)	35 (6.8)
	current	484(26.6)	213(44.0)	45.0(30.0-59.0)	128.2(17.6)	76.1(12.3)	76.7(12.4)	114(23.6)	30 (6.2)
	ever	997(54.8)	473(47.4)	46.0(30.0-59.0)	129.1(17.0)	76.6(12.1)	75.4(12.4)	244(24.5)	65 (6.5)
	Total	1820	831(45.7)	45.0(30.0-59.0)	128.4(16.7)	76.0(12.1)	75.1(12.2)	400(22.0)	110 (6.0)
NFBC1966	never	1,810(47.8)	792(43.8)	31.2(30.4-32.4)	125.9(14.2)	78.3(11.7)	70.8(11.7)	374(20.7)	97 (5.4)
	former	582(15.4)	271(46.6)	31.1(30.4-32.4)	125.1(12.8)	77.6(10.9)	69.4(10.7)	98(16.8)	27 (4.6)
	current	1,393(36.8)	804(57.7)	31.2(30.4-32.3)	124.7(13.7)	76.5(11.6)	68.5(10.8)	241(17.3)	66 (4.7)
	ever	1,975(52.2)	1,075(54.4)	31.2(30.4-32.4)	124.8(13.4)	76.8(11.4)	68.8(10.8)	339(17.2)	93 (4.7)
	Total	3,785	1,867(49.3)	31.2(30.4-32.4)	125.3(13.8)	77.5(11.6)	69.8(11.3)	713(18.8)	190 (5.0)
NFBC1986	never	755(64.1)	375(49.7)	16.3(16.0-17.0)	117.6(12.8)	69.2(7.3)	69.4(11.9)	41 (5.4)	2 (0.3)
	current	423(35.9)	187(44.2)	16.3(16.0-16.9)	114.6(12.6)	67.1(7.6)	68.1(10.1)	14 (3.3)	0 (0.0)
	Total	1,178	562(47.7)	16.3(16.0-17.0)	116.5(12.8)	68.4(7.5)	68.9(11.3)	55 (4.7)	2 (0.2)
NHANES	never	1,083(48.8)	35.9[2]	40.0(29.0-57.0)[1]	122.4(15.1)[2]	74.5(7.6)[2]	75.1(8.9)[2]	21.32[2]	13.34[2]
	former	656(29.5)	57.2[2]	51.0(38.0-67.0)[1]	128.5(15.5)[2]	76.5(7.8)[2]	74.0(9.2)[2]	28.21[2]	20.41[2]
	current	481(21.7)	53.4[2]	37.0(27.0-49.0)[1]	118.9(11.1)[2]	74.0(6.6)[2]	78.2(8.5)[2]	12.45[2]	7.03[2]
	ever	1,137(51.2)	55.3[2]	43.0(32.0-58.0)[1]	123.8(13.8)[2]	75.3(7.3)[2]	76.0(9.0)[2]	20.61[2]	13.95[2]
	Total	2,220	46.4[2]	42.0(30.0-58.0)[1]	123.2(14.4)[2]	74.9(7.5)[2]	75.6(9.0)[2]	20.93[2]	13.67[2]
NSHD	never	1,102(41.7)	465(42.2)	53	136.9(21.6)	85.2(13.1)	67.1(11.3)	506(46.6)	276(25.4)
	former	913(34.6)	535(58.6)	53	139.8(21.9)	87.0(14.0)	66.9(11.1)	476(52.5)	262(28.9)
	current	626(23.7)	312(49.8)	53	137.9(21.1)	85.1(11.9)	71.3(11.4)	313(50.4)	151(24.3)
	ever	1,539(58.3)	847(55.0)	53	139.0(21.6)	86.2(13.2)	68.7(11.4)	789(51.6)	413(27.0)
	Total	2,641	1,312(49.7)	53	138.1(21.6)	85.8(13.2)	68.0(11.4)	1,295(49.5)	689(26.3)
NTR	Never	205(50.7)	63(30.7)	37.0(16.0-66.2)	123.7(13.1)	76.5(11.0)	-	35(17.1)	12(5.9)
	Former	94(23.3)	43(45.7)	40.5(22.2-75.5)	126.0(15.0)	78.5(11.5)	-	22(23.4)	11(11.7)
	Current	105(26.0)	48(45.7)	41.0(19.5-62.5)	125.0(15.8)	79.1(12.2)	-	21(20.0)	11(10.5)
	Ever	199(49.3)	91(45.7)	40.5(19.5-75.5)	125.4(15.4)	78.8(11.9)	-	43(21.6)	22(11.1)
	Total	404	154(38.1)	39.0(16.0-75.5)	124.6(14.3)	77.7(11.5)	-	78(19.3)	34(8.4)

Study	Smoking status	N (%)	Male, N (%)	Age, median (range)	SBP, Mean (SD)	DBP, Mean (SD)	Resting heart rate, Mean (SD)	Hypertension, N (%)	Severe Hypertension, N (%)
PROSPER	Never	1,795(34.2)	1,369(76.3)	75.8(70.2-83.4)	169.3(22.1)	97.7(12.9)	66.4(11.4)	1,734(96.6)	1,657(92.3)
	Former	2,056(39.2)	773(37.6)	75.1(69.4-83.3)	167.7(22.9)	96.0(12.7)	65.3(11.9)	1,968(95.7)	1,824(88.7)
	Current	1,392(26.5)	578(41.5)	73.9(70.2-83.3)	158.0(23.5)	89.2(13.9)	67.9(11.6)	1,146(82.3)	850(61.1)
	Ever	3,448(65.8)	1,351(39.2)	74.6(69.4-83.3)	163.8(23.6)	93.2(13.6)	66.4(11.8)	3,114(90.3)	2,674(77.6)
	Total	5,243	2,720(51.9)	75.0(69.4-83.4)	165.7(23.3)	94.8(13.5)	66.4(11.7)	4,848(92.5)	4,331(82.6)
Whitehall II	Never	1,549(53.1)	1,132(73.1)	42.0(34.0-56.0)	122.6(13.9)	76.5(10.1)	-	217(14.0)	51 (3.3)
	former	944(32.4)	745(78.9)	44.0(34.0-55.0)	123.4(14.6)	77.2(10.2)	-	146(15.5)	33 (3.5)
	current	423(14.5)	295(69.7)	42.0(35.0-55.0)	121.5(13.3)	75.3(9.5)	-	49(11.6)	6 (1.4)
	ever	1,367(46.9)	1,040(76.1)	43.0(34.0-55.0)	122.8(14.2)	76.6(10.0)	-	195(14.3)	39 (2.9)
	Total	2,916	2,172(74.5)	43.0(34.0-56.0)	122.7(14.0)	76.5(10.0)	-	412(14.1)	90 (3.1)

1. Age, median (Inter quartile range)
2. NHANES data are weighted percentages

SBP: Systolic blood pressure
DBP Diastolic blood pressure
SD: standard deviation
Percentages are row percentages

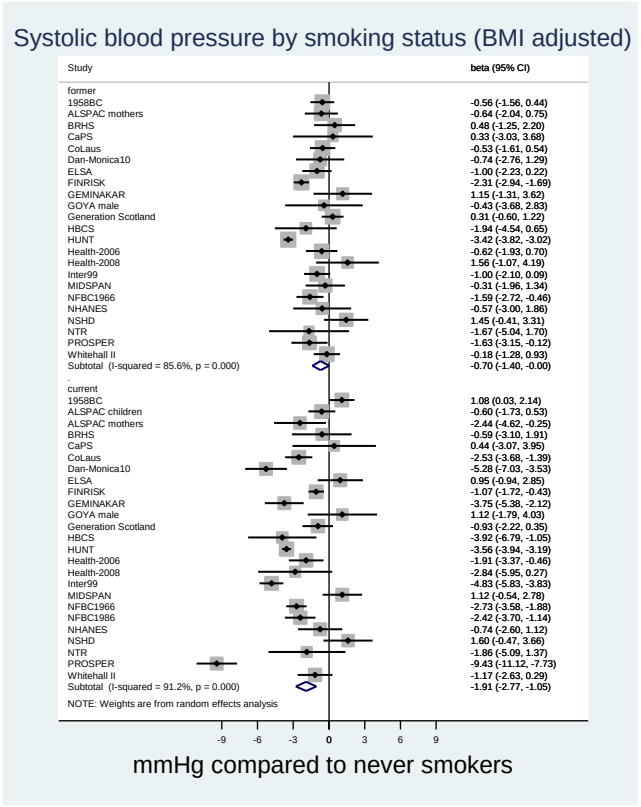
Table S2: Allele frequencies for rs1051730/rs16969968 in the CARTA studies

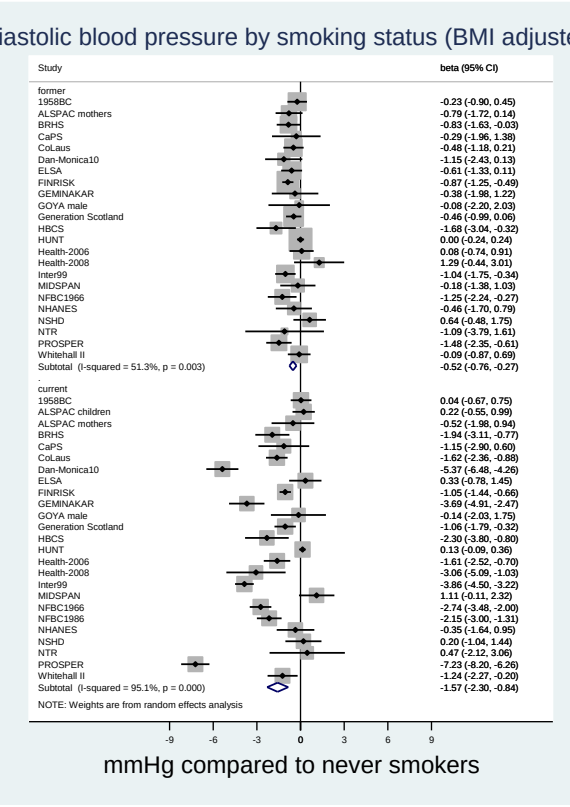
Study	Total	SNP	Major homozygotes	Heterozygotes	Minor homozygotes	MAF	HWE p-value[1]	BP_ adjusted[2]
1958BC	5,024	rs16969968	2,180	2,229	615	0.34	0.221	Yes
ALSPAC children	1,574	rs1051730	704	701	169	0.33	0.779	No
ALSPAC mothers	1,826	rs1051730	822	818	186	0.33	0.400	Yes
BRHS	3,855	rs1051730	1,760	1,666	429	0.33	0.247	Yes
CaPS	1,160	rs16969968	534	509	117	0.32	0.790	No
CoLaus	4,777	rs1051730	1,959	2,203	615	0.36	0.911	Yes
Dan-Monica10	2,292	rs1051730	1,013	1,025	254	0.33	0.826	Yes
ELSA	4,464	rs16969968	2,038	1,924	502	0.33	0.138	Yes
FINRISK	20,368	rs16969968	9,251	8,979	2,138	0.33	0.555	Yes
GEMINAKAR	1,113	rs1051730	529	472	112	0.31	0.657	No
GOYA male	791	rs1051730	349	341	101	0.34	0.242	Yes
Generation Scotland	6,888	rs1051730	3,067	3,080	741	0.33	0.447	Yes
HBCS	1,713	rs1051730_A	744	776	193	0.34	0.692	Yes
HUNT	55,634	rs1051730	24,666	24,656	6,312	0.34	0.204	No
Health2006	3,220	rs1051730	1,437	1,434	349	0.33	0.756	Yes
Health2008	624	rs16969968	291	269	64	0.32	0.875	Yes
Inter99	5,787	rs1051730	2,525	2,606	656	0.34	0.674	Yes
MIDSPAN	1,820	rs1051730	821	809	190	0.33	0.655	Yes
NFBC1966	3,785	rs1051730	1,740	1,634	411	0.32	0.366	Yes
NFBC1986	1,178	rs1051730	558	523	97	0.30	0.107	No
NHANES	2,220	rs1051730	0.407[3]	0.463[3]	0.130[3]	-	-	Yes
NSHD	2,642	rs16969968	1,223	1,164	255	0.32	0.360	Yes
NTR	404	rs16969968	206	160	38	0.29	0.398	Yes
PROSPER	5,243	rs1051730	2,456	2,278	509	0.31	0.586	Yes
Whitehall II	2,916	rs16969968	1,294	1,307	315	0.33	0.577	Yes

MAF: Minor allele frequency, HWE: Hardy Weinberg Equilibrium
1. P-value from chi-square test

2. Blood pressure values are adjusted if medication is used
3. Weighted percentages of genotypes are presented from NHANES as this is survey data

Figure S1. Association of smoking status with systolic and diastolic blood pressure, and resting heart rate. Former and current smoking status are compared to never smoking status; the difference was estimated by linear regression adjusted for sex, age, and body mass index (BMI).





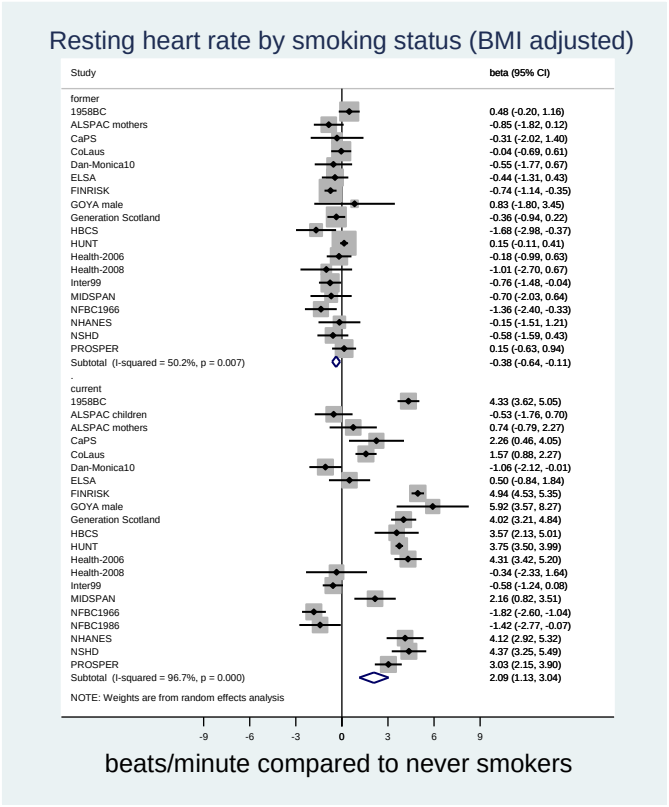
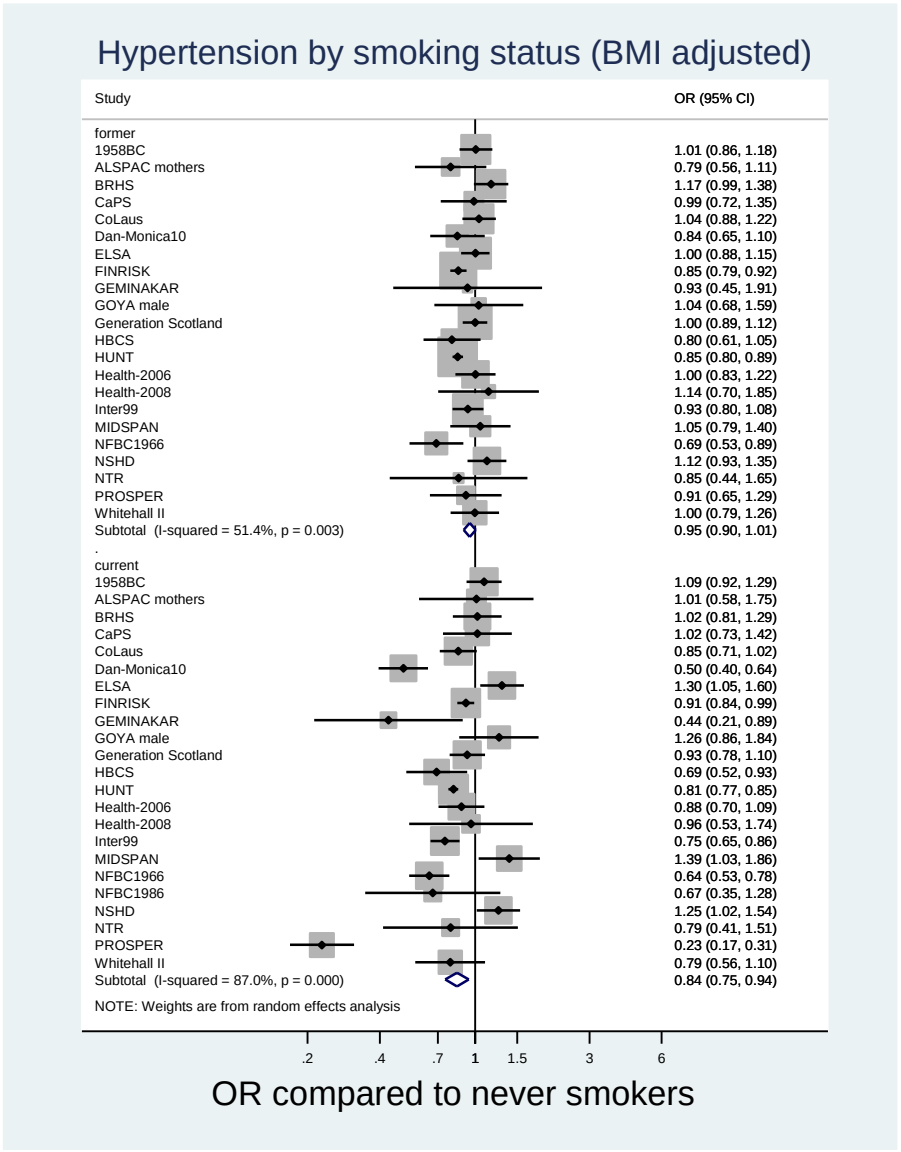


Figure S2. Association of smoking status with hypertension and severe hypertension. Former and current smoking status are compared to never smoking status; the difference was estimated by logistic regression adjusted for sex, age, and body mass index (BMI). OR, Odds ratio.



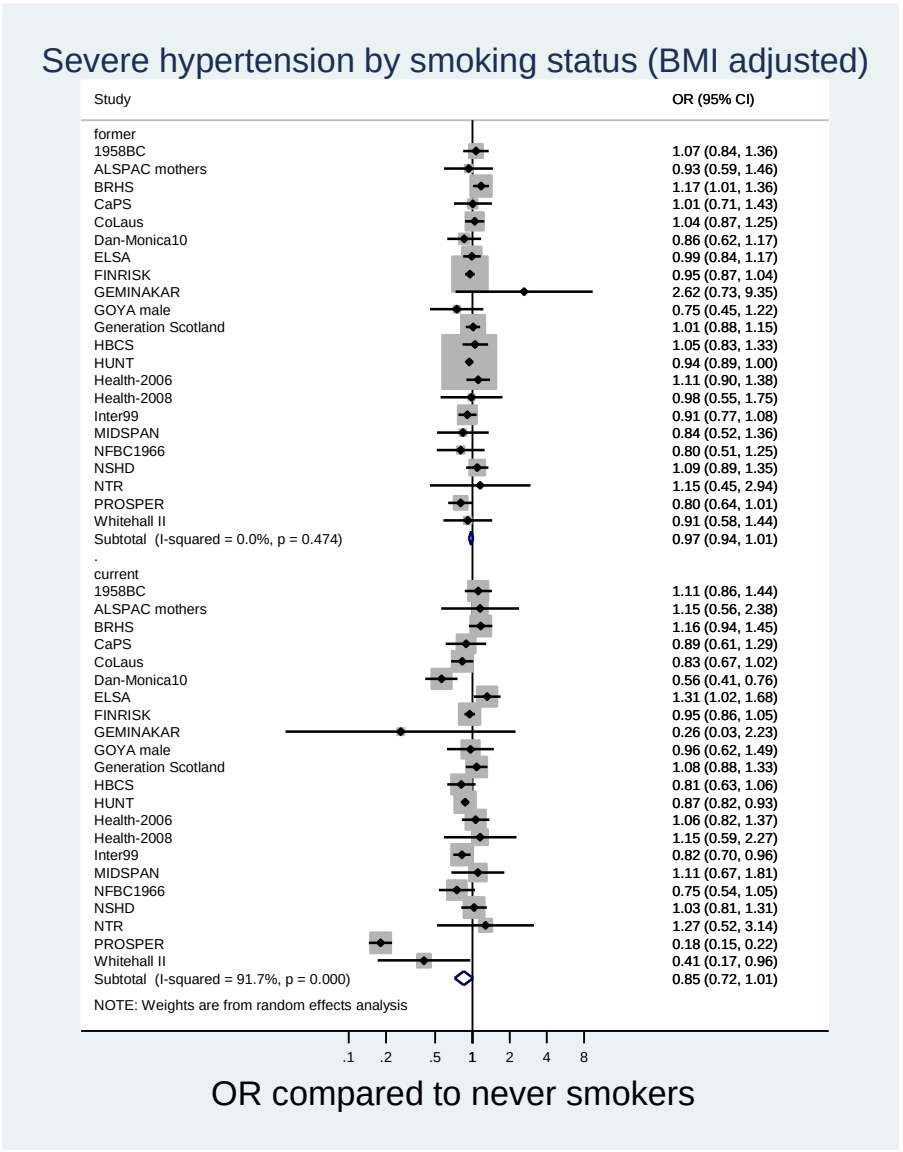


Figure S3. Association of smoking heaviness in current smokers with systolic and diastolic blood pressure and resting heart rate. The difference per one cigarette per day increase in smoking heaviness was estimated by linear regression adjusted for sex, age, and body mass index (BMI).

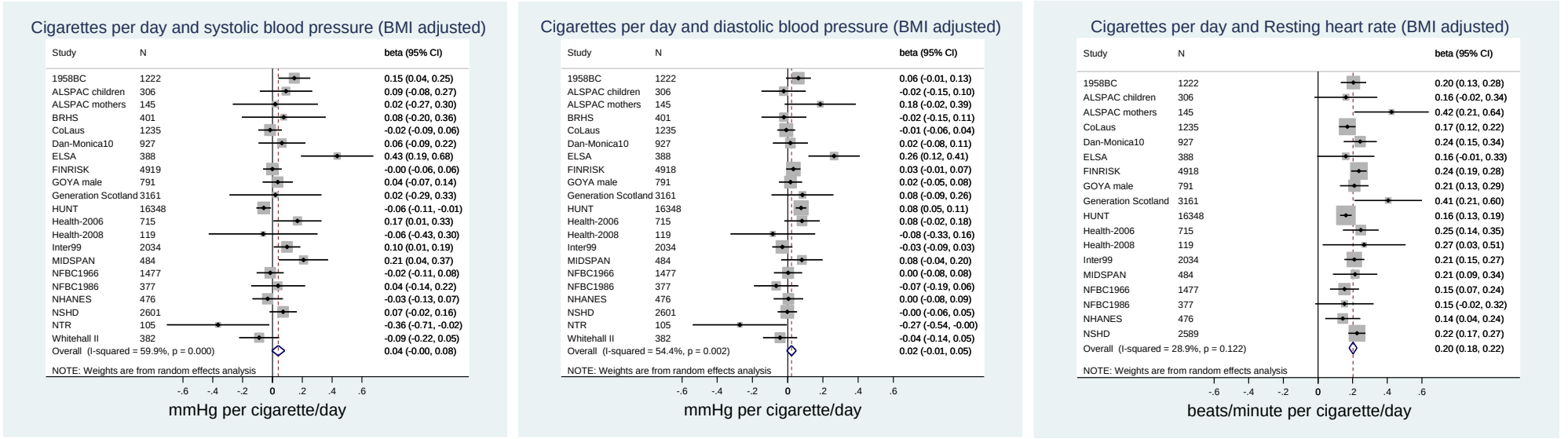
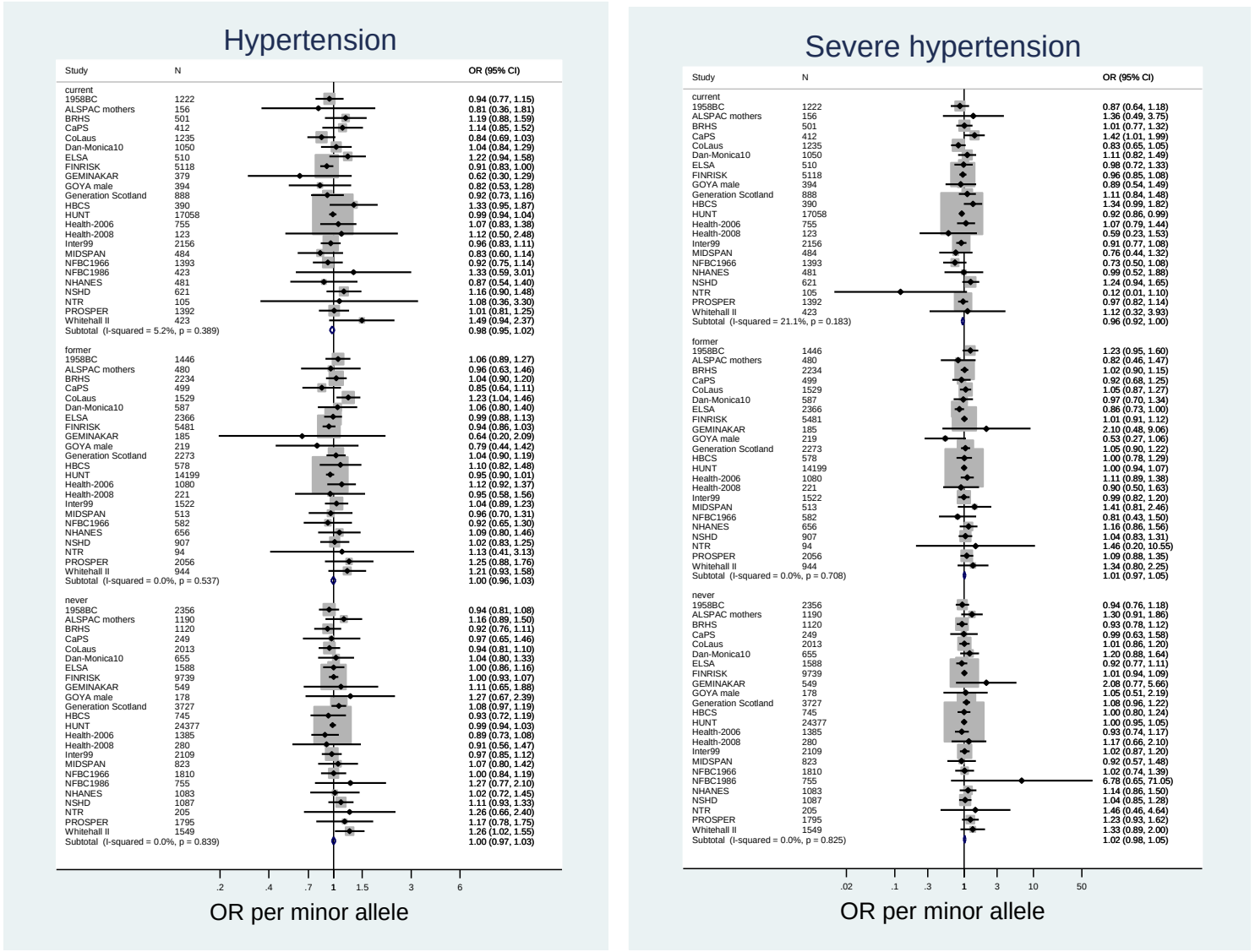
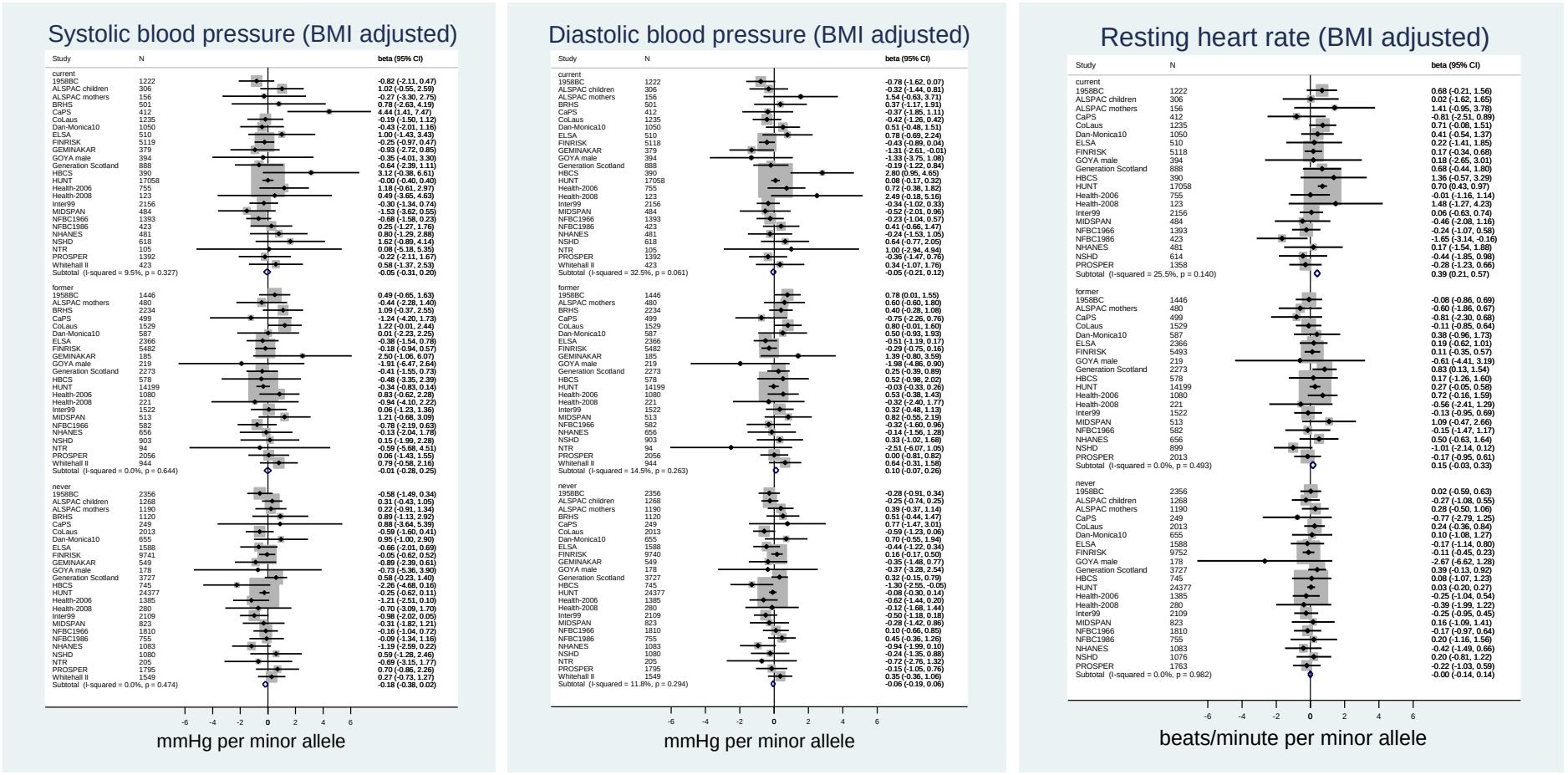


Figure S4. Mendelian randomisation analysis of the association of the smoking increasing allele (minor allele) of rs1051730/rs16969968 with hypertension and severe hypertension. Analyses were stratified by smoking status (current, former, and never smoking). The difference per one allele was estimated by logistic regression adjusted for sex and age. OR, Odds ratio.



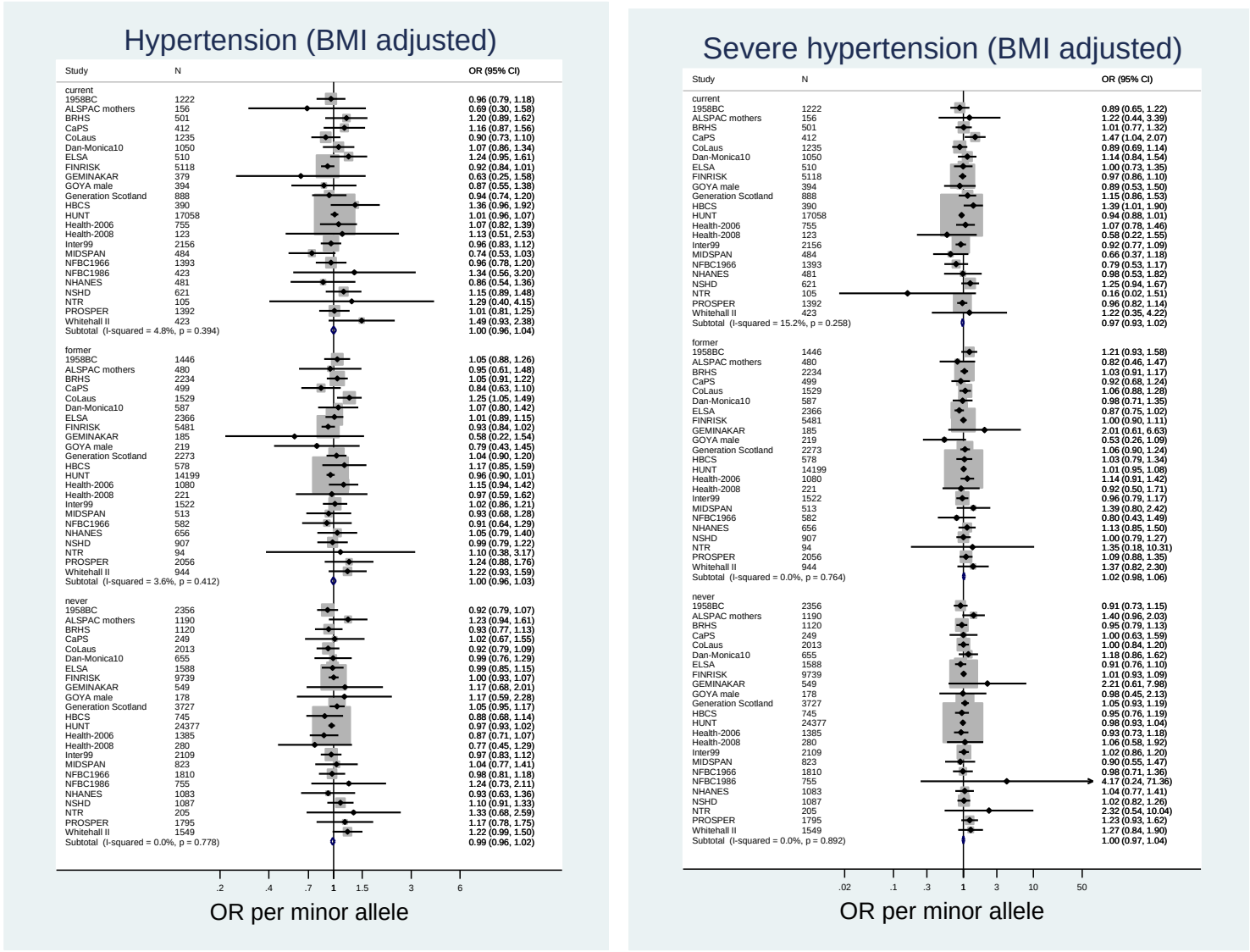
Hypertension: Overall test for heterogeneity by smoking status, P=0.655. Severe hypertension: Overall test for heterogeneity by smoking status, P=0.095.

Figure S5. Mendelian randomisation analysis of the association of the smoking increasing allele (minor allele) of rs1051730/rs16969968 with systolic and diastolic blood pressure and resting heart rate. Analyses were stratified by smoking status (current, former, and never smoking). The difference per one allele was estimated by linear regression adjusted for sex, age, and body mass index (BMI).



Systolic blood pressure: Overall test for heterogeneity by smoking status, P=0.562.
Diastolic blood pressure: Overall test for heterogeneity by smoking status, P=0.275.
Resting heart rate: Overall test for heterogeneity by smoking status, P=0.003.

Figure S6. Mendelian randomisation analysis of the association of the smoking increasing allele (minor allele) of rs1051730/rs16969968 with hypertension and severe hypertension. Analyses were stratified by smoking status (current, former, and never smoking). The difference per one allele was estimated by logistic regression adjusted for sex, age, and body mass index (BMI). OR, Odds ratio.



Hypertension: Overall test for heterogeneity by smoking status, P=0.857. Severe hypertension: Overall test for heterogeneity by smoking status, P=0. 395.

Figure S7. Mendelian randomisation analysis of the association of the smoking increasing allele (minor allele) of rs1051730/rs16969968 with smoking smoking status: ever versus never smoker and current versus former smoker. The difference per one allele was estimated by logistic regression. OR, Odds ratio.