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ASSOCIATION STUDIES ARTICLE

A multi-ancestry genome-wide study incorporating gene–smoking interactions identifies multiple new loci for pulse pressure and mean arterial pressure

Yun Ju Sung[1,*], Lisa de las Fuentes[2,1,*], Thomas W. Winkler[3,*], Daniel I. Chasman[4,*], Amy R. Bentley[5,*], Aldi T. Kraja[6,*], Ioanna Ntalla[7,*], Helen R. Warren[7,8,*], Xiuqing Guo[9,*], Karen Schwander[1], Alisa K. Manning[10,11], Michael R. Brown[12], Hugues Aschard[13,14], Mary F. Feitosa[6], Nora Franceschini[15], Yingchang Lu[16], Ching-Yu Cheng[17,18,19], Xueling Sim[20], Dina Vojinovic[21], Jonathan Marten[22], Solomon K. Musani[23], Tuomas O. Kilpeläinen[24,25], Melissa A Richard[26], Stella Aslibekyan[27], Traci M. Bartz[28], Rajkumar Dorajoo[29], Changwei Li[30], Yongmei Liu[31], Tuomo Rankinen[32], Albert Vernon Smith[33,34], Salman M. Tajuddin[35], Bamidele O. Tayo[36], Wei Zhao[37], Yanhua Zhou[38], Nana Matoba[39], Tamar Sofer[40,41], Maris Alver[42], Marzyeh Amini[43], Mathilde Boissel[44], Jin Fang Chai[20], Xu Chen[45], Jasmin Divers[46], Ilaria Gandin[47], Chuan Gao[48], Franco Giulianini[4], Anuj Goel[49,50], Sarah E. Harris[51,52], Fernando P. Hartwig[53,54], Meian He[55], Andrea R. V. R. Horimoto[56], Fang-Chi Hsu[46], Anne U. Jackson[57], Candace M. Kammerer[58], Anuradhani Kasturiratne[59], Pirjo Komulainen[60], Brigitte Kühnel[61,62], Karin Leander[63], Wen-Jane Lee[64], Keng-Hung Lin[65], Jian'an Luan[66], Leo-Pekka Lyytikäinen[67,68], Colin A. McKenzie[69], Christopher P. Nelson[70,71], Raymond Noordam[72], Robert A. Scott[66], Wayne H.H. Sheu[73,74,75,76], Alena Stančáková[77], Fumihiko Takeuchi[78], Peter J. van der Most[43], Tibor V. Varga[79], Robert J. Waken[1], Heming Wang[40,41], Yajuan Wang[80], Erin B. Ware[81,37], Stefan Weiss[82,83], Wanqing Wen[84], Lisa R. Yanek[85], Weihua Zhang[86,87], Jing Hua Zhao[66], Saima Afaq[86], Tamuno Alfred[16], Najaf Amin[21], Dan E. Arking[88], Tin Aung[17,18,19], R. Graham Barr[89], Lawrence F. Bielak[37], Eric Boerwinkle[12,90], Erwin P. Bottinger[16], Peter S. Braund[70,71], Jennifer A. Brody[91], Ulrich Broeckel[92], Brian Cade[41], Archie Campbell[93], Mickaël Canouil[44], Aravinda Chakravarti[88], Massimiliano Cocca[47], Francis S. Collins[94], John M. Connell[95], Renée de Mutsert[96], H. Janaka de Silva[97], Marcus Dörr[98,83], Qing Duan, Charles B. Eaton[100], Georg Ehret[88,101], Evangelos Evangelou[86,102], Jessica D. Faul[81], Nita G. Forouhi[66], Oscar H. Franco[21], Yechiel Friedlander[103], He Gao[86], Bruna Gigante[63], C. Charles Gu[1], Preeti Gupta[17], Saskia P Hagenaars[51,104], Tamara B. Harris[105], Jiang He[106,107], Sami Heikkinen[77,108], Chew-Kiat Heng[109,110], Albert Hofman[21], Barbara V. Howard[111,112], Steven C. Hunt[113,114], Marguerite R. Irvin[115], Yucheng Jia[9], Tomohiro Katsuya[116,117], Joel Kaufman[118], Nicola D. Kerrison[66], Chiea Chuen Khor[29,119], Woon-Puay Koh[120,20], Heikki A. Koistinen[121,122,123], Charles B. Kooperberg[124], Jose E. Krieger[56], Michiaki Kubo[125], Zoltan Kutalik[126,127], Johanna Kuusisto[77], Timo A. Lakka[108,60,128], Carl D. Langefeld[46], Claudia Langenberg[66], Lenore J. Launer[105], Joseph H. Lee[129], Benjamin Lehne[86], Daniel Levy[130,131], Cora E. Lewis[132], Yize Li[1], Lifelines Cohort Study[133], Sing Hui Lim[17], Ching-Ti Liu[38], Jianjun Liu[29,20], Jingmin Liu[134], Yeheng Liu[9], Marie Loh[86,135], Kurt K. Lohman[46], Tin Louie[136], Reedik Mägi[42]

[†]*Writing group.

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Koichi Matsuda[137], Thomas Meitinger[138,139], Andres Metspalu[42], Lili Milani[2], Yukihide Momozawa[140], Thomas H. Mosley, Jr[141], Mike A. Nalls[142], Ubaydah Nasri[9], Jeff R. O'Connell[143,144], Adesola Ogunniyi[145], Walter R. Palmas[146], Nicholette D. Palmer[147], James S. Pankow[148], Nancy L. Pedersen[45], Annette Peters[62,149], Patricia A. Peyser[37], Ozren Polasek[150,151,152], David Porteous[93], Olli T. Raitakari[153,154], Frida Renström[79,155], Treva K. Rice[1], Paul M. Ridker[4], Antonietta Robino[156], Jennifer G. Robinson[157], Lynda M. Rose[4], Igor Rudan[158], Charumathi Sabanayagam[17,18], Babatunde L. Salako[145], Kevin Sandow[9], Carsten O. Schmidt[159,83], Pamela J. Schreiner[148], William R. Scott[86,160], Peter Sever[161], Mario Sims[23], Colleen M. Sitlani[91], Blair H. Smith[162], Jennifer A. Smith[37,81], Harold Snieder[43], John M. Starr[51,163], Konstantin Strauch[164,165], Hua Tang[166], Kent D. Taylor[9], Yik Ying Teo[20,167,168,29,169], Yih Chung Tham[17], André G Uitterlinden[170], Melanie Waldenberger[61,62,66], Lihua Wang[6], Ya Xing Wang[171], Wen Bin Wei[172], Gregory Wilson[173], Mary K. Wojczynski[6], Yong-Bing Xiang[174], Jie Yao[9], Caizheng Yu[55], Jian-Min Yuan[175,176], Alan B. Zonderman[177], Diane M. Becker[85], Michael Boehnke[57], Donald W. Bowden[147], John C. Chambers[86,87], Yii-Der Ida Chen[9], David R. Weir[81], Ulf de Faire[63], Ian J. Deary[51,104], Tõnu Esko[42,178], Martin Farrall[49,50], Terrence Forrester[69], Barry I. Freedman[179], Philippe Froguel[44,180], Paolo Gasparini[47,114], Christian Gieger[61,62,181], Bernardo Lessa Horta[53], Yi-Jen Hung[182], Jost Bruno Jonas[172,183], Norihiro Kato[78], Jaspal S. Kooner[87,160], Markku Laakso[77], Terho Lehtimäki[67,68], Kae-Woei Liang[184,74,185], Patrik K.E. Magnusson[45], Albertine J. Oldehinkel[186], Alexandre C. Pereira[56,187], Thomas Perls[188], Rainer Rauramaa[60], Susan Redline[41], Rainer Rettig[83,189], Nilesh J. Samani[70,71], James Scott[160], Xiao-Ou Shu[84], Pim van der Harst[190], Lynne E. Wagenknecht[191], Nicholas J. Wareham[66], Hugh Watkins[49,50], Ananda R. Wickremasinghe[59], Tangchun Wu[55], Yoichiro Kamatani[39], Cathy C. Laurie[136], Claude Bouchard[32], Richard S. Cooper[36], Michele K. Evans[35], Vilmundur Gudnason[33,34], James Hixson[12], Sharon L.R. Kardia[37], Stephen B. Kritchevsky[192], Bruce M. Psaty[193,194], Rob M. van Dam[20,195], Donna K. Arnett[196], Dennis O. Mook-Kanamori[96,197], Myriam Fornage[26], Ervin R. Fox[198], Caroline Hayward[22], Cornelia M. van Duijn[21], E. Shyong Tai[195,20,120], Tien Yin Wong[17,18,19], Ruth J.F. Loos[16,199], Alex P. Reiner[124], Charles N. Rotimi[5], Laura J. Bierut[200], Xiaofeng Zhu[80], L. Adrienne Cupples[38], Michael A. Province[6], Jerome I. Rotter[9,*], Paul W. Franks[79,201,202,*], Kenneth Rice[136,*], Paul Elliott[86,*], Mark J. Caulfield[7,8,*], W. James Gauderman[203,*], Patricia B. Munroe[7,8,*], Dabeeru C. Rao[1,*] and Alanna C. Morrison[12,*]

[1]Division of Biostatistics, Washington University School of Medicine, St. Louis, MO 63110, USA, [2]Cardiovascular Division, Department of Medicine, Washington University, St. Louis, MO 63110, USA, [3]Department of Genetic Epidemiology, University of Regensburg, Regensburg 93051, Germany, [4]Preventive Medicine, Brigham and Womens Hospital, Boston, MA 02215, USA, [5]Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA, [6]Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, MO 63108, USA, [7]Clinical Pharmacology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK, [8]National Institute for Health Research Barts Cardiovascular Biomedical Research Centre, Queen Mary University of London, London, London EC1M 6BQ, UK, [9]Division of Genomic Outcomes, Department of Pediatrics, The Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA 90502, USA, [10]Center for Human Genetics Research, Massachusetts General Hospital, Boston, MA 02114, USA, [11]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA, [12]Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA, [13]Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA, [14]Centre de Bioinformatique, Biostatistique et Biologie Intégrative (C3BI), Institut Pasteur, Paris 75724, France, [15]Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC 27514, USA, [16]Icahn School of Medicine at Mount Sinai, The Charles Bronfman Institute for Personalized Medicine, New York, NY 10029, USA, [17]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore 169856, Singapore, [18]Ophthalmology & Visual Sciences Academic Clinical Program (Eye ACP), Duke-NUS Medical School, Singapore 169857, Singapore, [19]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore, [20]Saw Swee Hock School of Public Health, National University Health System and National University of Singapore, Singapore 117549, Singapore, [21]Department of Epidemiology, Erasmus University Medical Center, Rotterdam, 3000 CA, The Netherlands, [22]Medical Research Council Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK, [23]Jackson Heart Study, Department of Medicine, University of Mississippi Medical

Center, Jackson, MS 39213, USA,[24]Novo Nordisk Foundation Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen DK-2100, Denmark, [25]Department of Environmental Medicine and Public Health, The Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA,[26]Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA, [27]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA, [28]Cardiovascular Health Research Unit, Biostatistics and Medicine, University of Washington, Seattle, WA 98101, USA,[29]Genome Institute of Singapore, Agency for Science Technology and Research, Singapore 138672, Singapore, [30]Epidemiology and Biostatistics, University of Georgia at Athens College of Public Health, Athens, GA 30602, USA, [31]Public Health Sciences, Epidemiology and Prevention, Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA, [32]Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA, [33]Icelandic Heart Association, Kopavogur 201, Iceland, [34]Faculty of Medicine, University of Iceland, Reykjavik 101, Iceland, [35]Health Disparities Research Section, Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA, [36]Department of Public Health Sciences, Loyola University Chicago, Maywood, IL 60153, USA, [37]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI 48109, USA, [38]Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA, [39]Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan, [40]Department of Medicine, Harvard Medical School, Boston, MA, USA 02115, [41]Division of Sleep and Circadian Disorders, Brigham and Womens Hospital, Boston, MA 02115, USA, [42]Estonian Genome Center, Institute of Genomics, University of Tartu, Tartu 51010, Estonia, [43]Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen 9700 RB, The Netherlands, [44]CNRS UMR 8199, European Genomic Institute for Diabetes (EGID), Institut Pasteur de Lille, University of Lille, Lille 59000, France, [45]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Stockholm 17177, Sweden, [46]Biostatistical Sciences, Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA, [47]Department of Medical Sciences, University of Trieste, Trieste 34137, Italy, [48]Molecular Genetics and Genomics Program, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA, [49]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, Oxfordshire OX3 9DU, UK, [50]Wellcome Centre for Human Genetics, University of Oxford, Oxford, Oxfordshire OX3 7BN, UK, [51]Centre for Cognitive Ageing and Cognitive Epidemiology, The University of Edinburgh, Edinburgh EH8 9JZ, UK,[52]Medical Genetics Section, University of Edinburgh Centre for Genomic and Experimental Medicine and Medical Research Council Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh EH4 2XU, UK, [53]Postgraduate Programme in Epidemiology, Federal University of Pelotas, Pelotas, RS 96020220, Brazil, [54]Medical Research Council Integrative Epidemiology Unit, University of Bristol, Bristol, BS8 2BN, UK, [55]School of Public Health, Department of Occupational and Environmental Health and State Key Laboratory of Environmental Health for Incubating, Tongi Medical College, Huazhong University of Science and Technology, Wuhan, 430014, China, [56]Lab Genetics and Molecular Cardiology, Cardiology, Heart Institute, University of Sao Paulo, Sao Paulo, 01246903, Brazil, [57]Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI 48109, USA, [58]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, USA, [59]Department of Public Health, Faculty of Medicine, University of Kelaniya, Ragama, 11010 Sri Lanka, [60]Foundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio 70100, Finland, [61]Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg 85764, Germany, [62]Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg 85764, Germany, [63]Institute of Environmental Medicine, Karolinska Institutet, Stockholm 17177, Sweden, [64]Medical Research, Taichung Veterans General Hospital, Department of Social Work, Tunghai University, Taichung 40705, Taiwan, [65]Ophthalmology, Taichung Veterans General Hospital, Taichung 40705, Taiwan, [66]Medical Research Council Epidemiology Unit, University of Cambridge, Cambridge CB2 0QQ, UK, [67]Department of Clinical Chemistry, Fimlab Laboratories, Tampere 33520, Finland, [68]Department of Clinical Chemistry, Finnish Cardiovascular Research Center–Tampere, Faculty of Medicine and Life Sciences, University of Tampere, Tampere 33014, Finland, [69]Tropical Metabolism Research Unit, Tropical Medicine Research Institute, University of the West Indies, Mona JMAAW15, Jamaica, [70]Department of Cardiovascular Sciences, University of Leicester, Leicester LE3 9QP, UK, [71]National Institute for Health Research Leicester Biomedical Research Centre, Glenfield Hospital, Leicester LE3 9QP, UK, [72]Internal Medicine, Gerontology and Geriatrics, Leiden University Medical Center, Leiden 2300RC, The Netherlands, [73]Endocrinology and Metabolism, Internal Medicine, Taichung Veterans General Hospital, Taichung 40705, Taiwan, [74]School of Medicine, National Yang-ming University, Taipei 70705, Taiwan, [75]School of Medicine, National Defense Medical Center, Taipei 70705, Taiwan, [76]Institute of Medical Technology, National Chung-Hsing University, Taichung 70705, Taiwan, [77]Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland, Kuopio 70210, Finland, [78]Department of Gene Diagnostics and Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo 1628655, Japan, [79]Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University Diabetes Centre, Skåne University Hospital, Malmö SE-205 02, Sweden, [80]Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH 44106, USA, [81]Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor, MI 48104, USA, [82]Interfaculty Institute for Genetics and Functional Genomics, University Medicine Ernst Moritz Arndt University Greifswald, Greifswald 17487, Germany, [83]Deutsches Zentrum für Herz-Kreislaufforschung E.V. (German Centre for Cardiovascular Health), Partner Site Greifswald, Greifswald 17475, Germany, [84]Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37203, USA, [85]General Internal Medicine, GeneSTAR Research Program, Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA, [86]Medical Research Council-Public Health England Centre for Environment and Health, Department of Epidemiology and Biostatistics,

Imperial College London, London W2 1PG, UK, [87]Department of Cardiology, Ealing Hospital, Middlesex, UB1 3HW, UK, [88]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA, [89]Departments of Medicine and Epidemiology, Columbia University Medical Center, New York, NY 10032, USA, [90]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA, [91]Cardiovascular Health Research Unit, Medicine, University of Washington, Seattle, WA 98101, USA,[92]Section of Genomic Pediatrics, Department of Pediatrics, Medicine and Physiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA, [93]Centre for Genomic & Experimental Medicine, Institute of Genetics & Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK, [94]Medical Genomics and Metabolic Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA, [95]Ninewells Hospital & Medical School, University of Dundee, Dundee, Scotland DD1 9SY, UK, [96]Clinical Epidemiology, Leiden University Medical Center, Leiden 2300RC, The Netherlands, [97]Department of Medicine, Faculty of Medicine, University of Kelaniya, Ragama 11600, Sri Lanka, [98]Department of Internal Medicine B, University Medicine Greifswald, Greifswald 17475, Germany, [99]Department of Genetics, University of North Carolina, Chapel Hill, NC 27514, USA, [100]Department of Family Medicine and Epidemiology, Alpert Medical School of Brown University, Providence, RI 02912, USA, [101]Cardiology, Department of Specialties of Medicine, Geneva University Hospital, Geneva 1211, Switzerland, [102]Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina 45110, Greece, [103]Braun School of Public Health, Hebrew University-Hadassah Medical Center, Jerusalem 91120, Israel, [104]Psychology, The University of Edinburgh, Edinburgh EH8 9JZ, UK, [105]Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, USA, [106]Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112, USA, [107]Medicine, Tulane University School of Medicine, New Orleans, LA 70112, USA, [108]Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio Campus 70211, Finland, [109]Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore, [110]Khoo Teck Puat–National University Childrens Medical Institute, National University Health System, Singapore 119228, Singapore, [111]MedStar Health Research Institute, Hyattsville, MD 20782, USA, [112]Center for Clinical and Translational Sciences and Department of Medicine, Georgetown-Howard Universities, Washington, DC 20057, USA, [113]Division of Epidemiology, Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT 84132, USA, [114]Department of Genetic Medicine, Weill Cornell Medicine, Doha 26999, Qatar, [115]Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL 35294, USA, [116]Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, Suita 5650871, Japan, [117]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita 5650871, Japan, [118]Epidemiology, Occupational and Environmental Medicine Program, University of Washington, Seattle, WA 98105, USA, [119]Department of Biochemistry, National University of Singapore, Singapore 117596, Singapore, [120]Health Services and Systems Research, Duke National University of Singapore Medical School, Singapore 169857, Singapore, [121]Department of Public Health Solutions, National Institute for Health and Welfare, Helsinki 00271, Finland, [122]Department of Medicine and Abdominal Center: Endocrinology, University of Helsinki and Helsinki University Central Hospital, Helsinki 00271, Finland, [123]Minerva Foundation Institute for Medical Research, Biomedicum 2U, Helsinki 00290, Finland, [124]Fred Hutchinson Cancer Research Center, University of Washington School of Public Health, Seattle, WA 98109, USA, [125]RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan, [126]Institute of Social and Preventive Medicine, Lausanne University Hospital, Lausanne 1010, Switzerland, [127]Swiss Institute of Bioinformatics, Lausanne 1015, Switzerland, [128]Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio 70210, Finland, [129]Sergievsky Center, College of Physicians and Surgeons, Columbia University Mailman School of Public Health, New York, NY 10032, USA, [130]National Heart, Lung, and Blood Institute Framingham Heart Study, Framingham, MA 01702, USA, [131]The Population Sciences Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA, [132]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35205, USA, [133]Lifelines Cohort, Groningen 9700 RB, The Netherlands, [134]WHI CCC, Fred Hutchinson Cancer Research Center, Seattle, WA 98115, USA, [135]Translational Laboratory in Genetic Medicine, Agency for Science, Technology and Research, Singapore 138648, Singapore, [136]Department of Biostatistics, University of Washington, Seattle, WA 98105, USA, [137]Laboratory for Clinical Genome Sequencing, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Minato-ku 108-8639, Japan, [138]Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg 85764, Germany, [139]Institute of Human Genetics, Technische Universität München, Munich 80333, Germany, [140]Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences, Yokohama 230-0045, Japan, [141]Geriatrics, Medicine, University of Mississippi, Jackson, MS 39216, USA, [142]Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892, USA, [143]Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, MD 21201, USA, [144]Program for Personalized and Genomic Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA, [145]Department of Medicine, University of Ibadan, Ibadan 200001, Nigeria, [146]Internal Medicine, Columbia University, New York, NY 10032, USA, [147]Biochemistry, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA, [148]Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN 55454, USA, [149]DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Neuherberg 85764, Germany, [150]Department of Public Health, Department of Medicine, University of Split, Split 21000, Croatia, [151]Psychiatric Hospital “Sveti Ivan", Zagreb 10090, Croatia, [152]Gen-info Ltd, Zagreb 10000, Croatia, [153]Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku 20521, Finland, [154]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku 20520, Finland, [155]Department of Biobank Research, Umeå University, Umeå, Västerbotten SE-901 87, Sweden, [156]Institute for Maternal and Child Health–Istituto di Ricovero e Cura a Carattere Scientifico “Burlo Garofolo", 34137 Trieste, Italy,

[157]Department of Epidemiology and Medicine, University of Iowa, Iowa City, IA 52242, USA, [158]Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh EH8 9AG, UK, [159]Institute for Community Medicine, University Medicine Greifswald, Greifswald 17475, Germany, [160]National Heart and Lung Institute, Imperial College London, London W12 0NN, UK, [161]International Centre for Circulatory Health, Imperial College London, London W2 1PG, UK, [162]Division of Population Health Sciences, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, UK, [163]Alzheimer Scotland Dementia Research Centre, The University of Edinburgh, Edinburgh EH8 9JZ, UK, [164]Institute of Genetic Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg 85764, Germany,[165]Genetic Epidemiology, IBE, Faculty of Medicine, Ludwig Maximilian University, Munich 80539, Germany, [166]Department of Genetics, Stanford University, Stanford, CA 94305, USA, [167]Life Sciences Institute, National University of Singapore, Singapore 117456, Singapore, [168]NUS Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore 117456, Singapore, [169]Department of Statistics and Applied Probability, National University of Singapore, Singapore 117546, Singapore, [170]Department of Internal Medicine, Erasmus University Medical Center, Rotterdam 3000 CA, The Netherlands, [171]Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Ophthalmology and Visual Science Key Lab, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China, [172]Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China, [173]Jackson Heart Study, School of Public Health, Jackson State University, Jackson, MS 39213, USA, [174]State Key Laboratory of Oncogene and Related Genes & Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 20032, P.R. China, [175]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, USA, [176]Division of Cancer Control and Population Sciences, UPMC Hillman Cancer, University of Pittsburgh, Pittsburgh, PA 15232, USA, [177]Behavioral Epidemiology Section, Laboratory of Epidemiology and Population Sciences, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA, [178]Broad Institute of the Massachusetts Institute of Technology and Harvard University, Boston, MA 02142, USA, [179]Nephrology, Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA, [180]Department of Genomics of Common Disease, Imperial College London, London W12 0NN, UK, [181]German Center for Diabetes Research (DZD e.V.), Neuherberg 85764, Germany, [182]Endocrinology and Metabolism, Tri-Service General Hospital, National Defense Medical Center, Taipei City, Taipei 11490, Taiwan, [183]Department of Ophthalmology, Medical Faculty Mannheim, University Heidelberg, Mannheim, 68167, Germany, [184]Cardiovascular Center, Taichung Veterans General Hospital, Taichung 40705, Taiwan, [185]Department of Medicine, China Medical University, Taichung 40705, Taiwan, [186]Department of Psychiatry, University of Groningen, University Medical Center Groningen, Groningen 9700 RB, The Netherlands, [187]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA, [188]Geriatrics Section, Boston University Medical Center, Boston, MA 02118, USA, [189]Institute of Physiology, University of Medicine Greifswald, Greifswald 17495, Germany, [190]Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen 9700 RB, The Netherlands, [191]Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA, [192]Department of Internal Medicine, Section on Gerontology and Geriatric Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA, [193]Cardiovascular Health Research Unit, Epidemiology, Medicine and Health Services, University of Washington, Seattle, WA 98101, USA, [194]Kaiser Permanente Washington Health Research Institute, Seattle, WA 98101, USA, [195]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore, [196]Deans Office, University of Kentucky College of Public Health, Lexington, KY 40508, USA, [197]Public Health and Primary Care, Leiden University Medical Center, Leiden 2300RC, The Netherlands, [198]Cardiology, Medicine, University of Mississippi Medical Center, Jackson, MS 39216, USA, [199]Icahn School of Medicine at Mount Sinai, The Mindich Child Health and Development Institute, New York, NY 10029, USA, [200]Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110, USA, [201]Harvard T. H. Chan School of Public Health, Department of Nutrition, Harvard University, Boston, MA 02115, USA, [202]Department of Public Health & Clinical Medicine, Umeå University, Umeå, Västerbotten SE-901 85, Sweden and [203]Biostatistics, Preventive Medicine, University of Southern California, Los Angeles, CA 90032, USA

*To whom correspondence should be addressed at: Division of Biostatistics, Washington University School of Medicine, Campus Box 8067 660, S. Euclid Avenue, St. Louis, MO 63110-1093, USA. Tel: +314 3620053; Fax: +314 3622693; Email: yunju@wustl.edu

Abstract

Elevated blood pressure (BP), a leading cause of global morbidity and mortality, is influenced by both genetic and lifestyle factors. Cigarette smoking is one such lifestyle factor. Across five ancestries, we performed a genome-wide gene–smoking interaction study of mean arterial pressure (MAP) and pulse pressure (PP) in 129 913 individuals in stage 1 and follow-up analysis in 480 178 additional individuals in stage 2. We report here 136 loci significantly associated with MAP and/or PP. Of these, 61 were previously published through main-effect analysis of BP traits, 37 were recently reported by us for systolic BP and/or diastolic BP through gene–smoking interaction analysis and 38 were newly identified ($P < 5 \times 10^{-8}$, false discovery rate < 0.05). We also identified nine new signals near known loci. Of the 136 loci, 8 showed significant interaction with smoking status. They include *CSMD1* previously reported for insulin resistance and BP in the spontaneously hypertensive rats. Many of the 38 new loci show biologic plausibility for a role in BP regulation. *SLC26A7* encodes a chloride/bicarbonate exchanger expressed in the renal outer medullary collecting duct. *AVPR1A* is widely expressed, including in vascular smooth

muscle cells, kidney, myocardium and brain. *FHAD1* is a long non-coding RNA overexpressed in heart failure. *TMEM51* was associated with contractile function in cardiomyocytes. *CASP9* plays a central role in cardiomyocyte apoptosis. Identified only in African ancestry were 30 novel loci. Our findings highlight the value of multi-ancestry investigations, particularly in studies of interaction with lifestyle factors, where genomic and lifestyle differences may contribute to novel findings.

Introduction

Elevated blood pressure (BP), a leading cause of morbidity and mortality worldwide, is known to be influenced by both genetic and lifestyle factors. To date genome-wide association studies (GWAS) have identified over 1000 loci associated with BP and hypertension (1–10). The effects of genetic variants on BP may manifest differently depending on lifestyle exposures. Therefore, incorporating gene–environment (G×E) interactions may identify additional loci (11,12). We established the Gene–Lifestyle Interactions Working Group within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium in order to assess the impact of interactions with multiple lifestyle factors on the genetics of cardiovascular traits (13). Among many lifestyle factors, cigarette smoking influences BP in both acute (14) and chronic (15) fashion, motivating genetic association studies of gene-by-smoking interactions.

Recently we reported findings from a genome-wide association meta-analysis incorporating gene–smoking interactions for systolic BP (SBP) and diastolic BP (DBP) (16). In addition to SBP and DBP, BP can also be characterized as having both steady and pulsatile components, each determined by different physiologic properties of the heart and vasculature and differently related to cardiovascular outcomes. Mean arterial pressure (MAP) reflects the steady component of BP, which is predominantly determined by cardiac output and systemic vascular resistance and regulated by small artery and arteriole tone (17). MAP has been found to be more 'informative' than SBP and DBP in predicting mortality from cardiovascular disease including stroke and ischemic heart disease (18,19). Pulse pressure (PP) represents the pulsatile component of BP and is largely determined by cardiac stroke volume and large artery stiffness (17,20). PP has been found to be predictive of coronary heart disease risk and, in some cases, superior to both SBP and DBP, in particular for older adults (21,22). Thus, while SBP is prioritized as the primary treatment target for hypertension (23), MAP and PP continue to be relevant BP traits for investigation. Understanding their biological underpinnings may lead to discovery of new BP pathways.

In this study, we performed a genome-wide association meta-analysis of MAP and PP incorporating gene–smoking interactions (Fig. 1). The aim is to evaluate whether any of the previously identified BP loci are modified by smoking, whether interactions can be identified using a genome-wide approach and whether additional novel BP loci can be identified by accounting for potential single nucleotide polymorphism (SNP)–smoking interactions. Here, we report our findings through two degrees of freedom (DF) test that jointly evaluates genetic main and interaction effects (24) based on 610 091 individuals across five ancestries.

Results

Overview

Across five ancestries, we performed a genome-wide gene–smoking interaction study of MAP and PP in 129 913 individuals in stage 1 and follow-up analysis in 480 178 additional individuals in stage 2: summary information is in Table 1 (Supplementary Materials, Tables S1–S6). Through genome-wide search in stage 1, we identified 1692 significant ($P \leq 5 \times 10^{-8}$) and 2681 suggestive ($P \leq 10^{-6}$) variants associated with MAP and/or PP.

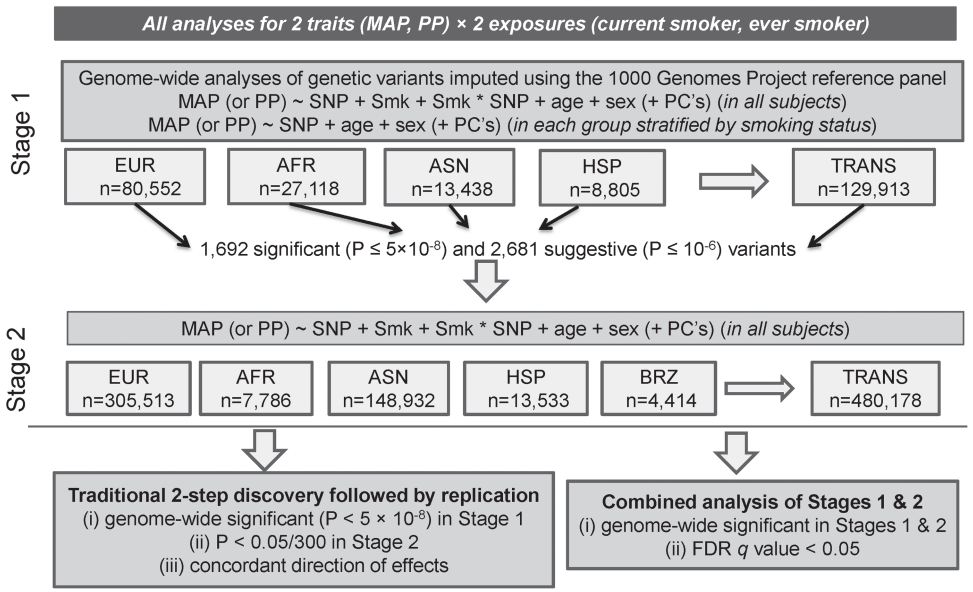

Figure 1. Study design. Summary of data included in this study. Smk: smoking status (considering either current smoking or ever smoking status separately); PC: principal component; EUR: European; AFR: African; ASN: Asian; HIS: Hispanic; BRZ: Brazilian; TRANS; *trans*-ancestry (i.e. combining all ancestry groups through meta-analysis).

Table 1. Basic characteristics of cohorts in stages 1 and 2 in each ancestry

	Current smoker		Former smoker		Never smoker		Male	HTN	HT meds	Age		MAP		PP	
	N	%	N	%	N	%	%	%	%	Mean	SD	Mean	SD	Mean	SD
Stage 1															
EUR	14 607	18.1	28 409	35.3	37 535	46.6	32.6	38.2	25.4	54.63	8	94.63	12.9	52.02	13.3
AFR	5545	21.5	7185	27.8	13 121	50.8	26.5	55.9	39.5	54.49	9.1	99.96	14.9	54.67	16.4
ASN	2465	18.3	1677	12.5	9296	69.2	51.2	46.9	27	55.42	9.7	98.70	13.4	57.86	15.8
HIS	1068	12.1	2160	24.5	5577	63.3	24.9	43.5	13.3	55.5	11	94.80	13.9	53.55	16.4
Stage 1 total	*23 685*	*18.4*	*39 431*	*30.7*	*65 529*	*50.9*	*32.8*	*43.1*	*27.7*	*54.74*	*8.6*	*96.17*	*13.4*	*53.28*	*14.4*
Stage 2															
EUR	48 198	17	89 597	31.6	145 914	51.4	47.8	44.8	25	55.91	8.6	102.17	13.5	55.29	13.9
AFR	1971	29.8	1579	23.8	3075	46.4	40.9	54.3	42.8	53.66	10.2	101.21	14.7	53.68	14.8
ASN	29 485	19.8	40 850	27.4	78 597	52.8	54.9	50.3	33.1	60.76	12.3	98.31	13.9	54.91	14.0
HIS	2739	20.3	2559	18.9	8231	60.8	41	26.9	16.3	45.86	13.8	91.36	13.7	48.99	13.3
BRZ	998	22.6	514	11.6	2902	65.8	48	15.5	6.3	27.78	3.2	89.75	12.3	45.23	9.8
Stage 2 total	*83 391*	*18.2*	*135 099*	*29.6*	*238 719*	*52.2*	*49.7*	*45.9*	*27.4*	*56.84*	*9.9*	*100.54*	*13.7*	*54.88*	*13.9*
TOTAL	**107 076**	**18.3**	**174 530**	**29.8**	**304 248**	**51.9**	**46.1**	**45.3**	**27.4**	**56.4**	**9.6**	**99.61**	**13.6**	**54.54**	**14.0**

The cell entries for the covariates and BP traits correspond to sample-size-weighted averages across all cohorts in each category. EUR: European; AFR: African; ASN: Asian; HIS: Hispanic; BRZ: Brazilian; ALL: *trans*-ancestry (i.e. combining all ancestry groups through meta-analysis); HTN: hypertension; MAP: mean arterial pressure; PP: pulse pressure.

Of these 4373 variants, 2982 variants were replicated in stage 2 with $P < 0.05/4373$ (to an aggregate replication rate of 68.2%). Of the 1692 significant variants in stage 1, a total of 1449 were replicated in stage 2 with $P < 0.05/1692$ to a replication rate of 85.6%. Among the genome-wide significant variants in stage 1, which resided in 112 loci (defined by physical distance ± 1 Mb), 53 loci were formally replicated in stage 2 using Bonferroni-adjusted significance levels ($P < 0.05/112$). Most of the remaining 59 loci were identified in African or Hispanic ancestries in stage 1, which quite plausibly failed to replicate in stage 2 due to these smaller sample sizes and hence lack of power. For 10 loci, no additional data were available in stage 2, and therefore, it was not possible to check for replication. All of these formally replicated loci had been identified previously: 44 through main effects GWAS (1–8) and 9 through gene–smoking interaction analysis we reported recently for SBP and DBP (16). For these nine formally replicated loci, estimates of the genetic main effects were all consistent between stages 1 and 2; estimates of SNP-smoking interaction effects were not statistically significant (Supplementary Material, Table S7).

We performed meta-analysis combining stages 1 and 2 (Manhattan plots, Supplementary Material, Fig. S1; quantile–quantile, QQ, plots, Supplementary Material, Fig. S2). Through this combined analysis with 610 091 individuals, we identified 136 loci that were associated with MAP and/or PP at genome-wide significance ($P \leq 5 \times 10^{-8}$). Of these, 61 loci were previously published through main effects GWAS for any BP trait (1–8), 37 loci (presented in Supplementary Material, Table S7) were recently reported by us for SBP and/or DBP through gene–smoking interaction analysis (16) and the remaining 38 loci are newly reported here (Table 2).

Among the 136 loci associated with MAP and/or PP, 38 loci are completely new and at least 1 Mb away from any of known BP loci. A total of 16 novel loci passed a more stringent threshold ($P < 6.25 \times 10^{-9}$, adjusted for two smoking exposures, two tests and two BP traits). We also identified nine additional new signals within the known BP loci but not in linkage disequilibrium (LD), $r^2 < 0.1$, with known BP loci (Table 3). Among the nine identified signals, four signals were identified in *trans*-ancestry, and the remaining five were ancestry-specific (two European, two African and one Hispanic signals). The LocusZoom plots for these completely novel 38 loci and 9 signals are shown in Supplementary Material, Figure S3. As shown in Venn diagram (Fig. 2), among 38 new loci and 9 signals, 38 were newly PP associated and 12 were newly MAP associated (with 3 common between PP and MAP). These were not associated with SBP or DBP. False discovery rate (FDR) q-values provided additional evidence for these newly identified loci (FDR < 0.01 for 43 of the 47 and FDR < 0.05 for all 47 loci or signals).

Supplementary Material, Table S8 presents more detailed results for the lead variants representing the 136 loci and the 9 signals associated with MAP and PP: ancestry-specific and *trans*-ancestry meta-analysis results within each stage (1 and 2) and ancestry-specific and *trans*-ancestry meta-analysis results combining stages 1 and 2. Scatterplots comparing ancestry-specific genetic effects at these variants are presented in Supplementary Material, Figure S4. Genetic effects between European and Hispanic ancestries had the highest correlation (0.79), whereas those between African and Hispanic ancestries had the lowest correlation (0.29).

The role of interactions

Among the 136 loci and 9 new signals associated with MAP and/or PP, variants at 8 loci showed genome-wide significant interactions (1 DF interaction $P < 5 \times 10^{-8}$) with smoking status (Fig. 3). All eight loci were identified with current smoking status; these variants have larger effects in current smokers than in non-current smokers. Of the eight loci, six loci showed increasing effects on BP in current-smokers. Five interactions were newly identified (Table 2), and the other three were previously reported for SBP or DBP (Supplementary Material, Table S7). These variants showing interaction effects were identified only in individuals of African ancestry in stage 1. These variants were not present in stage 2 because of the limited sample size (ranges from 418 to 1993) of stage 2 African ancestry cohorts, and therefore, replication of these interactions was not possible.

BP variance explained

Within each of the smoking strata, we computed the variance of MAP and PP explained by genome-wide results (25) in European

Table 2. Thirty-eight new loci associated with MAP and/or PP that are at least 1 Mb away from any known BP locus

Locus	rsID	Nearest gene	Position	EAF	Race	Trait/exposure	G effect	G StdErr	G×E effect	G×E StdErr	Interaction P	Joint P	FDR q value	N
1	rs115356163	*PADI2*	1:17466024	0.02	AFR	PP/CS	0.22	0.87	−7.70	1.53	0.04	5.17E-09*	3.63E-05	12 712
2	rs147515295	*EYA3; SESN2*	1:28389841	0.98	HIS	MAP/ES	2.94	1.04	2.80	1.52	0.10	3.47E-08	0.018721	7287
3	rs11587661	*COG2*	1:230671208	0.02	AFR	PP/CS	0.44	0.86	−7.63	1.51	1.31E-06	4.95E-08	0.010168	13 888
4	rs138318054	*KIAA1804*	1:233578559	0.02	AFR	PP/CS	−0.37	0.93	−7.58	1.66	1.40E-05	4.84E-08	0.010095	10 787
5	rs79113694	*GALNT14*	2:31253799	0.03	AFR	PP/ES	−0.60	0.58	−2.91	0.83	1.98E-04	7.65E-09	5.96E-05	25 557
6	rs183927068	*MAP2*	2:210288479	0.98	AFR	MAP/CS	−0.60	1.09	11.29	2.02	8.36E-08	2.05E-09*	0.001619	7925
7	rs75875736	*STAC*	3:36341106	0.02	AFR	PP/ES	−3.49	0.58	3.15	0.94	1.23E-03	1.41E-08	0.000108	21 985
8	rs116199364	*CLSTN2*	3:139951198	0.02	AFR	PP/CS	1.94	0.92	−10.54	1.88	2.23E-08	1.04E-07	0.000675	10 787
9	rs114619985	*BOD1L*	4:13599930	0.02	AFR	PP/ES	−2.74	0.78	−1.86	1.13	0.04	2.71E-10*	2.61E-06	18 015
10	rs201223145	*PRDM5*	4:121706475	0.97	AFR	PP/CS	2.67	0.68	2.91	1.39	0.12	5.91E-09*	0.001905	15 574
11	rs147998309	*PCDH10*	4:133596832	0.99	AFR	PP/CS	1.61	1.18	12.94	2.64	1.78E-06	2.41E-09*	1.74E-05	7925
12	rs146622638	*GPM6A*	4:176524533	0.97	AFR	PP/ES	2.76	0.65	0.16	0.98	0.95	4.55E-08	0.000334	21 332
13	rs72723039	*IRX2*	5:2664169	0.98	AFR	PP/CS	−1.69	1.10	10.76	1.88	2.39E-08	6.55E-09	0.002064	7925
14	rs79205226	*CDKAL1*	6:21103825	0.02	AFR	PP/CS	1.46	0.68	−7.94	1.30	1.60E-09	3.38E-09*	2.41E-05	15 574
15	rs200495667	*ALDH8A1*	6:135152480	0.08	ASN	PP/CS	−2.48	0.41	2.63	0.92	3.11E-03	1.50E-08	0.000378	10 110
16	rs190090939	*ACTR3B*	7:152802243	0.01	AFR	PP/CS	−0.01	1.12	−11.94	2.24	1.86E-07	5.41E-09*	3.79E-05	7925
17	rs140994551	*CSMD1*	8:4449086	0.01	AFR	PP/CS	0.43	1.07	−11.39	1.89	4.34E-09	2.07E-11*	1.93E-07	7925
18	rs7817784	*TNKS*	8:9682553	0.57	EUR	MAP/CS	−0.23	0.03	0.05	0.08	0.89	6.93E-13*	2.59E-09	364 584
19	rs12156238	*FAM167A*	8:11285135	0.19	EUR	MAP/ES	−0.30	0.06	0.10	0.08	0.29	1.03E-08	1.69E-05	349 729
20	MERGED_DEL _2_50178	*PKIA*	8:79178179	0.01	EUR	MAP/CS	1.60	1.34	−9.18	1.86	6.30E-07	1.25E-08	3.56E-05	9465
21	rs11991823	*LRRC69; SLC26A7*	8:92188440	0.37	Trans	PP/ES	−0.23	0.03	0.06	0.05	0.43	1.29E-15*	8.89E-11	552 719
22	rs7823377	*TRHR*	8:110073120	0.63	Trans	PP/CS	−0.15	0.03	0.05	0.06	0.41	3.90E-08	0.000260	583 554
23	rs76209156	*KDM4C*	9:7423109	0.99	AFR	PP/CS	0.03	1.19	10.43	2.14	1.96E-06	2.94E-08	0.000197	7925
24	rs77548020	*FLJ41200; NFIB*	9:13480744	0.98	AFR	PP/CS	0.75	0.83	7.27	1.59	1.56E-04	1.91E-08	0.00013	10 787
25	rs75872665	*LOC100128811*	10:25388468	0.99	AFR	PP/CS	0.08	1.09	8.94	1.88	3.41E-04	2.80E-08	0.000188	10 787
26	rs76497600	*BUB3*	10:125119610	0.03	AFR	PP/ES	−0.79	0.61	−2.65	0.85	0.01	2.29E-08	0.000173	21 336
27	rs148454833	*OR52A4*	11:5114798	0.98	AFR	PP/CS	0.34	0.76	7.09	1.47	8.39E-06	2.16E-08	0.000147	13 888
28	rs186331780	*FAM19A2*	12:61710810	0.02	AFR	PP/CS	−2.43	0.89	−4.88	1.66	0.02	3.15E-08	0.007099	10 787
29	rs146924684	*AVPR1A*	12:63437286	0.99	AFR	MAP/ES	4.88	0.83	−3.20	1.22	0.18	5.29E-09*	2.62E-05	18 015
30	rs117206641	*FBRSL1*	12:133086888	0.11	Trans	MAP/CS	0.32	0.05	0.03	0.13	0.70	1.14E-10*	5.71E-07	393 100
31	rs73212161	*TDRD3*	13:61261485	0.99	AFR	PP/ES	−1.39	1.40	7.80	1.77	1.50E-05	1.68E-08	0.006503	13 888
32	rs78265647	*IGF1R*	15:99247941	0.98	AFR	PP/CS	−1.71	0.72	7.64	1.28	2.02E-09*	8.86E-09	6.09E-05	15 847
33	rs145181522	*TOX3*	16:52490106	0.02	AFR	PP/CS	−0.65	0.95	−8.04	1.58	3.67E-05	3.66E-11*	3.32E-07	10 787
34	rs114511313	*NUDT7*	16:77706251	0.98	AFR	PP/CS	1.67	0.73	4.06	1.28	0.13	1.63E-08	0.000111	15 574
35	rs75129914	*RIT2*	18:40267945	0.97	AFR	PP/ES	0.32	0.61	3.42	0.85	3.81E-04	2.13E-09*	1.80E-05	21 794
36	rs115134409	*MALT1; NEDD4L*	18:56324467	0.02	AFR	PP/CS	−0.31	0.77	−6.46	1.29	3.26E-03	3.64E-10*	2.92E-06	12 890
37	rs78375085	*TNFRSF11A*	18:60032891	0.98	AFR	PP/ES	4.55	0.77	−5.57	1.21	4.71E-06	1.64E-08	0.000124	17 616
38	rs191056303	*PXMP4*	20:32306802	0.98	AFR	PP/CS	0.15	0.74	7.41	1.47	5.99E-07	1.77E-08	0.000121	13 888

A new BP locus was defined as a significantly associated variant that is at least 1 Mb away from any previously identified BP locus. Each locus is genome-wide significant ($P < 5 \times 10^{-8}$) in the combined analyses of stages 1 and 2 and had FDR q value < 0.05. Positions are based on human genome build 37. EAF: effect allele frequency; G effect: the estimate of the genetic main effect (β_G); G×E effect: the estimate of genetic–smoking interaction effect (β_{GE}); Interaction P: P-value for testing the G×E interaction effect with one DF; Joint P: P-value for jointly testing G main and G×E interaction effects with two DF; EUR: European ancestry. Trans: *trans*-ancestry (i.e. combining all ancestry groups through meta-analysis); MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking; ES: ever-smoking.

*Findings with an asterisk indicate statistical significance using a stricter P-value threshold, after Bonferroni correction for two smoking traits, two tests, and two BP traits ($5 \times 10^{-8}/8 = 6.25 \times 10^{-9}$).

Table 3. Nine new signals associated with MAP and/or PP that are near known BP loci (but not in LD, $r^2 < 0.1$)

Locus	rsID	Nearest gene	Position	EAF	Race	Trait/ exposure	G effect	G StdErr	G×E effect	G×E StdErr	Interaction P	Joint P	FDR q value
1	rs140881076	*KAZN*	1:15364113	0.01	AFR	PP/CS	0.45	1.13	−11.95	1.85	2.30E-03	3.29E-14*	4.16E-10
2	rs2071405	*AGT*	1:230850658	0.13	Trans	MAP/CS	0.28	0.04	−0.18	0.09	0.20	3.02E-12*	1.62E-08
3	rs143802076	*C3orf38*	3:88646080	0.01	AFR	PP/CS	−0.50	0.90	−8.54	1.68	8.97E-04	1.33E-09*	9.81E-06
4	rs1009382	*TNXB*	6:32026107	0.71	EUR	PP/CS	0.26	0.04	−0.16	0.08	0.15	4.84E-13*	3.30E-09
5	rs7005363	*MSRA*	8:10283748	0.54	EUR	MAP/ES	−0.34	0.04	0.15	0.06	0.02	3.13E-17*	1.59E-13
6	rs187148391	*TXN*	9:112998518	0.99	HIS	MAP/ES	0.09	0.69	4.48	1.03	1.01E-03	1.95E-08	0.013302
7	rs10894198	*ADAMTS8*	11:130285493	0.38	Trans	PP/CS	0.27	0.03	−0.12	0.07	0.33	1.38E-19*	3.19E-15
8	rs1010064	*LOC100506393 PDE3A*	12:20000315	0.75	Trans	MAP/ES	0.24	0.04	−0.12	0.06	0.03	5.91E-11*	6.64E-10
9	rs201028933	*LOC338758*	12:90111249	0.79	Trans	MAP/ES	0.32	0.08	0.16	0.11	0.28	1.73E-11*	9.75E-08

A new signal is defined as a significantly associated variant within 1 Mb of known BP loci but in weak LD $r^2 < 0.1$ with the known BP loci. LD for the *trans*-ancestry signals was based on the entire 1000 Genomes cosmopolitan data, whereas LD for ancestry-specific signals was based on ancestry-specific population (e.g. LD for European signals were based on 1000 Genomes European data). Each locus is genome-wide significant ($P < 5 \times 10^{-8}$) in the combined analyses of stages 1 and 2 and had FDR q value < 0.05. Positions are based on human genome build 37. EA: effect allele; EAF: effect allele frequency; G effect: the estimate of the genetic main effect$\left(\beta_G\right)$; G×E effect: the estimate of genetic–smoking interaction effect$\left(\beta_{GE}\right)$; Interaction P: P-value for testing the G×E interaction effect with one DF; Joint P: P-value for jointly testing G main and G×E interaction effects with two DF; EUR: European ancestry. Trans: *trans*-ancestry (i.e. combining all ancestry groups through meta-analysis); MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking; ES: ever-smoking.

*Findings with an asterisk indicate statistical significance using a stricter P-value threshold, after Bonferroni correction for two smoking traits, two tests, and two BP traits ($5 \times 10^{-8}/8 = 6.25 \times 10^{-9}$).

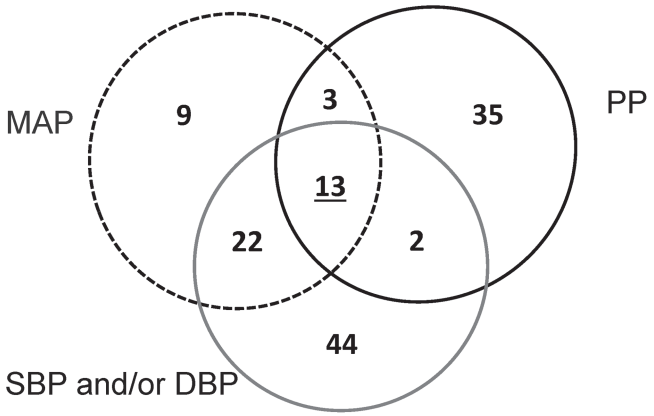

Figure 2. Venn diagram of loci/signals associated with the four BP traits. The diagram shows 133 loci and/or signals that were identified through gene–smoking interactions. In this paper, we newly identified 38 loci (Table 2) and 9 signals near known BP loci (Table 3) that are unique to MAP and/or PP (to a total of 49 new loci/signals). We had reported 81 loci associated with SBP/DBP (16), among which 37 showed association with MAP or PP. SBP: systolic blood pressure; DBP, diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure.

ancestry (Fig. 4). The independent set of variants, 38 for MAP and 12 for PP, with $P \leq 5 \times 10^{-8}$ explained 1.9% of variance in MAP and 0.5% of variance in PP. The difference in explained variance between the smokers and non-smokers was not significant, suggesting that BP variance explained by interaction effects is very small. Similar inference was observed with the results from ever-smoking status (data not shown).

Functional inferences

To obtain functional annotations from HaploReg (26), we focused on the index variants representing the 84 loci (38 novel loci, 9 new signals near known loci and 37 recently reported) that showed association with MAP and/or PP. There was one missense variant, rs1009382. Of the remaining non-coding variants (37 intronic and 51 intergenic), 15 were in promoter histone marks, 47 in enhancer histone marks, 28 in DNase I marks and 8 altered the binding sites of regulatory proteins (Supplementary Material, Table S9). Using GERP (27), five variants were identified as being conserved among vertebrates, with three variants identified as such using SiPhy (28). For 27 variants, *cis*-expression quantitative trait loci (eQTL) evidence was available with varying degrees of association with expression probes. In particular, 10 of them were identified by GTEx (29) as *cis*-eQTLs across various tissues (Supplementary Material, Table S9). In addition, we obtained information on microarray-based gene and exon expression levels in whole blood from over 5000 individuals of the Framingham Heart Study (30) (Supplementary Material, Table S10). There were 109 variant-transcript pairs (representing 26 variants) with *cis*-eQTL evidence (at $P < 8.9 \times 10^{-5}$, FDR < 0.002). Among 26 variants (Supplementary Material, Table S10), the 3 variants had the most abundant evidence of *cis*-eQTL association: rs112947839, rs1009382 and rs7753826 associated with 21, 18, and 10 transcripts, respectively.

The analyses using data-driven expression prioritized integration for complex traits (DEPICT) prioritized genes (FDR < 5%) at 40 loci, including 16 genes that did not match the nearest gene of the identified lead variant (Supplementary Material, Table S11).

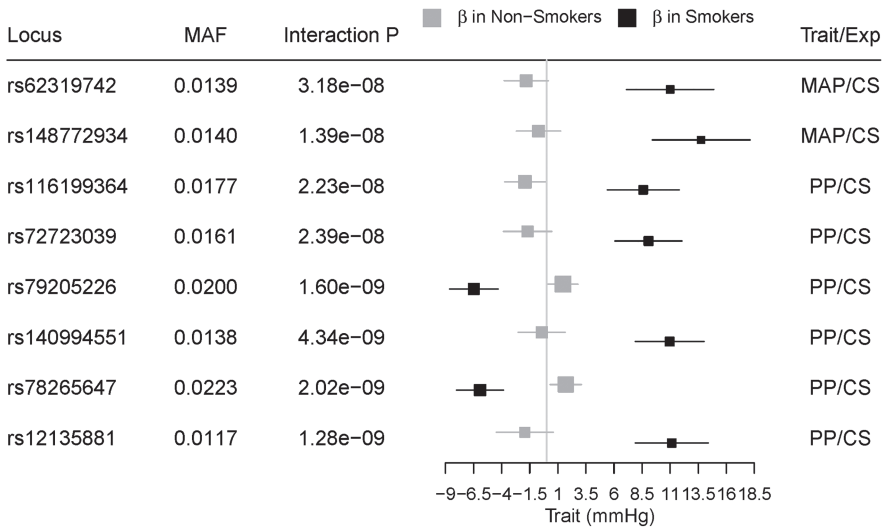

Figure 3. Smoking-specific genetic effect sizes in African ancestry for MAP or PP. Among the 138 loci significantly associated with MAP and/or PP, 8 loci show significant interactions with smoking exposure status in African ancestry. Smoking-specific effect estimates and 95% confidence intervals for variants associated with BP traits are shown as red and blue squares for current-smokers and non-current smokers, respectively. SNP effects between two strata are significantly different (one DF interaction $P < 5 \times 10^{-8}$). These results were based on African-specific results in stage 1. MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking.

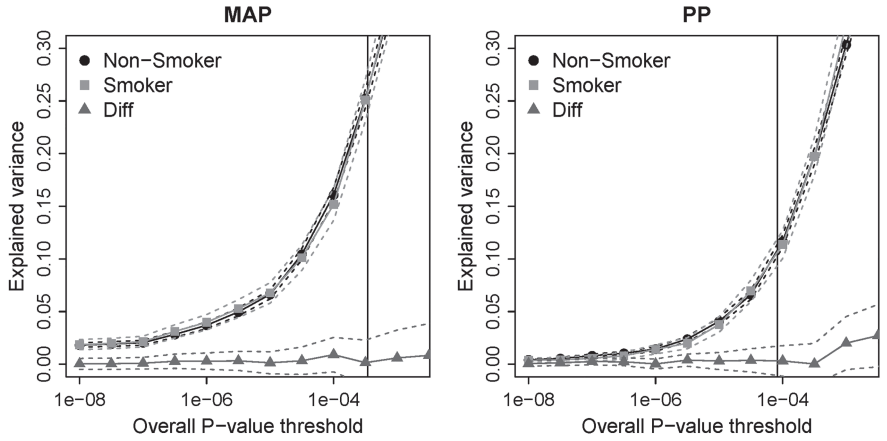

Figure 4. Smoking-specific estimates of variance explained in European ancestry. The variants with $P \leq 5 \times 10^{-8}$ explained 1.9% of variance in MAP and 0.5% of variance in PP, whereas variants with $P \leq 10^{-4}$ explained 16% of variance in MAP and 11% of variance in PP. The vertical line corresponds to FDR = 0.1.

Furthermore, the analyses highlighted 56 significantly (FDR < 5%) enriched gene sets. Many of these highlight cardiovascular mechanisms, such as 'abnormal blood vessel morphology', 'thin myocardium' or 'abnormal heart development' (Supplementary Material, Table S12). We also observed that genome-wide significant MAP and PP loci are enriched for genes expressed in the ileum (Supplementary Material, Table S13).

Associations of BP loci with cardiometabolic traits

We obtained association results of the 84 index variants associated with MAP or PP (representing 38 novel loci, 9 new signals near known loci and 37 recently reported loci) with multiple cardiometabolic traits: coronary artery disease (CAD), stroke, adiposity, diabetes and renal function (Supplementary Materials, Tables S14–S19). For 36 out of 47 scenarios (highlighted in red, Supplementary Material, Table S20), the observed number of variants with nominal evidence of association ($P < 0.05$) was higher than that expected by chance alone ($P_{Binomial} < 0.05/11$, corrected for 11 traits used in the lookups). For example, we observed 7 and 11 such associations with CAD and myocardial infarction, respectively, where the expected count is 2.2 for both traits. Corroborating evidence of the multiple cardiometabolic traits were found for the 2 of the 38 new loci: (rs146622638, *GPM6A*; rs12156238, *FAM167A*) and the 5 of the 9 new signals near known BP loci (rs2071405, *AGT*; rs1009382, *TNXB*; rs7005363, *MSRA*; rs1010064, *LOC100506393*; rs201028933, *LOC338758*). These overlapping signals support that these traits may share a common pathophysiology.

Loci overlapping with previously reported SBP or DBP loci

Among the loci that were reported by us recently as significantly associated with SBP and/or DBP based on gene-by-smoking interaction analysis (16), 37 loci were also associated with MAP

and/or PP (Supplementary Material, Table S7). Among them, nine loci were formally replicated in stage 2 and showed association with all four BP traits. Variants at these nine loci were all also genome-wide significant in the combined analysis of stages 1 and 2 in individuals of European ancestry. For variants at six of the nine loci, there was supporting evidence of association in individuals of non-European ancestry, which resulted in stronger statistical significance from *trans*-ancestry analysis. One such locus was rs351364 (in *WNT2B*), where only *trans*-ancestry analysis reached genome-wide significance in stage 1; the direction of the genetic effect was consistent across all ancestries (with 2DF $P = 2.8 \times 10^{-31}$; Supplementary Material, Table S7).

New signals near known BP loci

Nine new signals were identified near known BP loci (but not in LD, $r^2 < 0.1$). One such signal was rs140881076 (chr1:15364113, 2DF $P = 3.3 \times 10^{-14}$, Fig. 5A) in association with PP in individuals of African ancestry. This signal is 434 kb away and in complete linkage equilibrium with *CELA2A* locus (rs3820068, chr1:15798197) that was recently identified in individuals of European ancestry (7,8). Several nearby genes have been implicated in cardiovascular traits. *FHAD1* is a long non-coding RNA overexpressed in heart failure (31), *TMEM51* has been associated with contractile function in cardiomyocytes (32) and *CASP9* plays a central role in cardiomyocyte apoptosis (33). A candidate gene study identified a missense mutation in *CASP9* as associated with ischemic stroke in Koreans (34). Differential methylation patterns in *TMEM51* have also been described in peripheral blood leukocytes of smokers (35,36).

Through *trans*-ancestry analysis, we identified one locus (rs1010064) associated with both MAP and PP (2DF $P = 5.9 \times 10^{-11}$). This is located approximately 500 kb upstream of, but not in LD with, *PDE3A*, a known BP gene with a role in regulating growth in vascular smooth muscle cells (4,37). Missense mutations in *PDE3A* have been linked with autosomal dominant syndrome characterized by treatment-resistant hypertension and brachydactyly (38,39). SNPs in this locus have also shown suggestive associations with aortic root diameter (40), resistant hypertension (41) and SBP in a SNP–alcohol consumption interaction analysis (42).

Biological relevance of newly identified BP loci

Several genes near the 38 novel loci show biologic plausibility for a role in BP regulation. One such gene is *CSMD1* (rs140994551, chr8:4449086, associated with PP in individuals of African ancestry while considering interaction with current smoking status, 2DF $P = 2.1 \times 10^{-11}$, Fig. 5B). In animal models, variants in *CSMD1* were associated with both insulin resistance and BP in the spontaneously hypertensive rats (SHRs) (43). In humans, there was suggestive evidence of association with hypertension in two Korean cohorts (44), with peripheral artery disease in a Japanese population (45), with waist–hip ratio adjusted for BMI in men (46), with insulin resistance in African Americans (47) and with studies of addiction and related disorders (48). Another new locus is *LRRC69* (rs11991823, chr8:92188440, associated with PP, identified through *trans*-ancestry analysis, 2DF $P = 1.3 \times 10^{-15}$, Fig. 5C). A copy number variant in this gene has been shown to be weakly associated ($P = 0.04$) with BP in a Korean population (49). The nearby gene *SLC26A7* encodes a chloride/bicarbonate exchanger expressed specifically in the renal outer medullary collecting duct (50). Two PP loci include genes involved in the NFkB signaling pathway (*TNFRSF11A* and *NFIB*). This inflammatory pathway has been implicated in hypertension-induced renal dysfunction in murine models (51) and with endothelial dysfunction in overweight/obese and older humans (52). There was suggested evidence of association of variants in *TNFRSF11A* with BP traits in Chinese women (53).

A new locus near *AVPR1A* (rs146924684 chr12:63437286, associated with MAP, 2DF $P = 5.3 \times 10^{-9}$, Fig. 5D) also has strong biologic plausibility. Vasopressin is an antidiuretic hormone and a potent vasoconstrictor that exerts its effect through activation of a family of receptors, including the arginine vasopressin receptor subtype 1A (*AVPR1A*) that is widely expressed including in vascular smooth muscle cells, kidney, myocardium and brain (54). In glomerular macula densa cells, *AVPR1A* facilitates activation of the renin–angiotensin–aldosterone system and increases expression of the aquaporin 2 water channel (55). *AVPR1A* stimulation is also necessary for maintaining normal BP; in murine knockout models, basal BP is significantly decreased and the arterial baroreceptor reflex markedly impaired (56). Notably, there are data to support a role for vasopressin not only in the maintenance, but also in the development, of hypertension. Vasopressin receptor 1A blockade in young, still normotensive, SHR attenuates the later development of hypertension in adult SHR despite withdrawal of drug therapy (57).

We identified several loci with potential relevance to the structure and function of primary cilia, in addition to those we reported recently (16). Three PP-associated loci were near genes implicated with nephronophthisis, including those with mutations linked to Bardet–Biedl Syndrome (*BBS7* and *MYO3A*) and with Joubert Syndrome (*AHI1*). Another PP-associated locus was near *NEDD4L*, which encodes the E3 ubiquitin ligase NEDD4–2 and has been shown to regulate a renal epithelial sodium channel (ENaC/*SCNN1*) that is critical for maintenance of sodium homeostasis (58). ENaC is the channel responsible for the monogenetic disorder of BP regulation, Liddle Syndrome. Loss of NEDD4–2 in the renal tubules results in increased activity of the ENaC channel, resulting in salt-sensitive hypertension (59). Candidate gene studies identified variants in *NEDD4L* as associated with sodium lithium countertransport (60), hypertension (61), treatment response to β-blockers and diuretics in hypertensive patients (61–63).

We identified two additional loci with potential relevance to the dopaminergic system, in addition to those we reported recently (16). Dopamine signaling plays a key role in both central and peripheral BP regulation (64–66). A regulatory subunit (*PPP2R2A*) of the dopamine receptor 2R (D2R) was associated with MAP. In murine renal proximal tubule cells, inhibition of this regulatory protein leads to increased expression of markers of renal inflammation and injury (67). A newly identified MAP-associated locus *SESN2* is also related to the dopaminergic system; activation of the D2R has been shown to increase the expression of *SESN2*, which protects the kidney against renal oxidative stress (68). *SESN2* also protects endothelial cell lines against angiotensin II-induced endothelial toxicity (69). Two additional loci include genes involved in dopamine signaling: *ATP13A2* (70) and *ARPP21* (71). Activation of dopamine centers of the brain has also been implicated in drug and nicotine abuse (72).

In addition, we found a PP-associated locus near *SDHB*, which encodes the mitochondrial protein succinate dehydrogenase. Variants in this gene have been identified in individuals with carotid body tumors and pheochromocytomas/paragangliomas, endocrine tumors that secrete dopamine and/or norepinephrine and can modulate BP regulation even when tumors are not

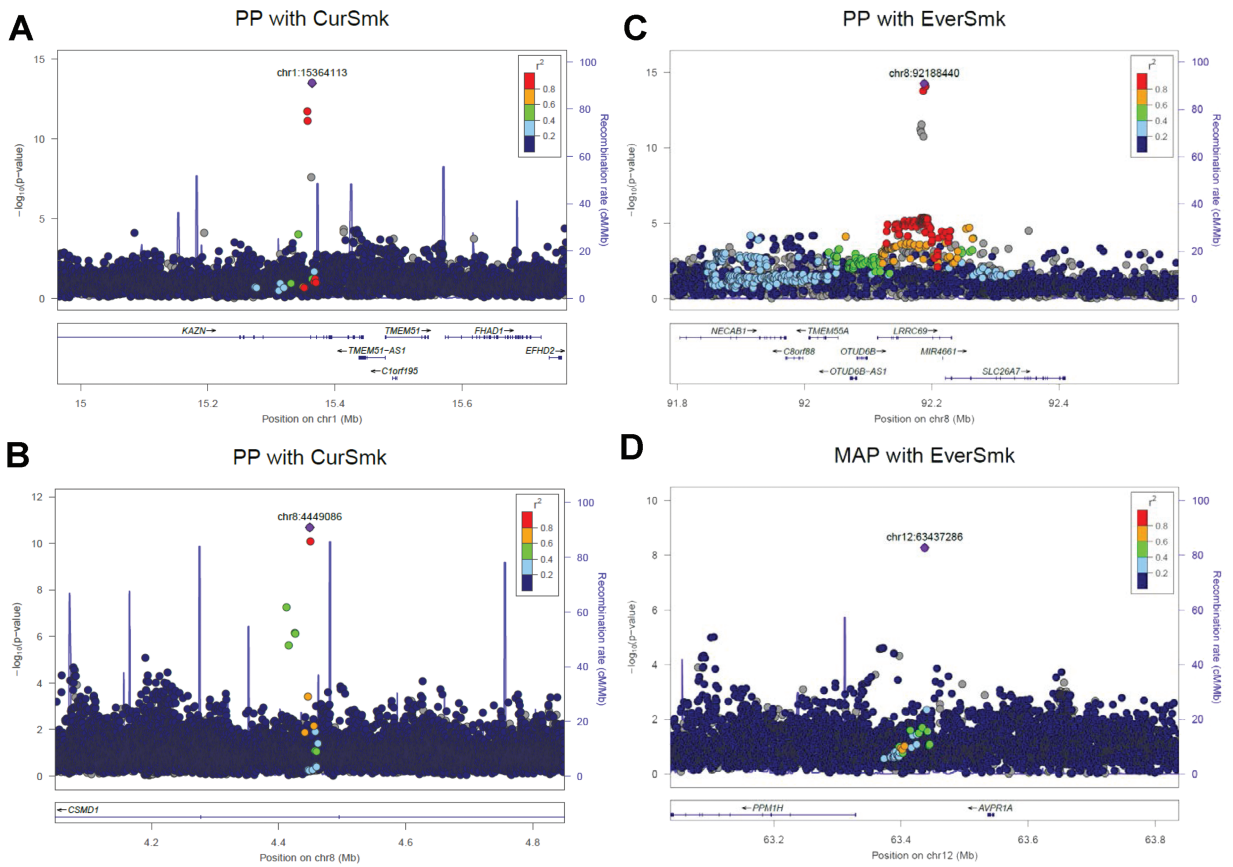

Figure 5. LocusZoom plots for four selected loci associated with MAP and/or PP. (**A**) rs140881076 (chr1:15364113) was identified in an analysis of individuals of African ancestry and is intronic to *KAZN*; neighboring genes have been implicated in cardiovascular traits. *FHAD1* is a long non-coding RNA overexpressed in heart failure, *TMEM51* has been associated with contractile function in cardiomyocytes and *CASP9* plays a central role in cardiomyocyte apoptosis. (**B**) rs140994551 (chr8:4449086), intronic to *CSMD1*, shows interaction with current smoking in individuals of African ancestry. *CSMD1* are shown to be associated with insulin resistance and BP in the spontaneously hypertensive rats. *CSMD1* is also suggestively associated with studies of addiction and related disorders. (**C**) rs11991823 (chr8:92188440) was associated with PP in *trans*-ancestry analyses and in intronic to *LRRC69*. The nearby gene *SLC26A7* encodes a chloride/bicarbonate exchanger expressed specifically in the renal outer medullary collecting duct. (**D**) rs146924684 (chr12:63437286) was associated with MAP in individuals of African ancestry. The nearby gene *AVPR1A* is widely expressed including in vascular smooth muscle cells, kidney, myocardium and brain. CurSmk: current smoking status; EverSmk: ever smoking status; MAP: mean arterial pressure; PP: pulse pressure. The plots were created using LocusZoom (http://locuszoom.sph.umich.edu/).

clinically apparent (73,74). Variants near this locus have been marginally associated with DBP in pre-pubertal European children (75). Tyrosinase (with its related protein, TYRP1) catalyzes the first rate-limiting step in pathway in the formation of L-Dopa (76). Although variants in *TYRP1* were suggestively associated with SBP by the International Consortium for Blood Pressure (77), we identified this locus as associated with PP at genome-wide significance.

Discussion

MAP measures the steady component, which is a function of the left ventricular contractility, heart rate, small-artery resistance and vascular elasticity averaged over time (17). PP measures the pulsatile component, which is a function of the left ventricular stroke volume, large-artery stiffness, early pulse wave reflectio, and heart rate (19). These BP traits not only differ in their physiologic properties but are also differently related to cardiovascular outcomes (17,19,78,79). Our genome-wide association meta-analysis incorporating gene–smoking interactions identified 136 loci significantly associated with MAP and/or PP: 61 were previously published through main-effect GWAS analysis (1–8), 37 were recently reported by us for SBP and/or DBP through gene–smoking interaction analysis (16) and 38 are newly reported here. Our analysis also identified nine new signals near known BP loci (but not in LD, $r^2 < 0.1$).

Among the loci significantly associated with MAP and/or PP, eight loci showed significant interaction with smoking status from the one DF interaction tests. At these eight loci, the joint two DF *P*-values ranged from 1×10^{-7} to 5×10^{-11}, indicating that loci were identified mostly because of their interaction with smoking status. We observed that the genetic effect at these loci is negligible in non-smokers but larger in smokers. As such, a drug that targets this locus with strong interactions may achieve a greater treatment effect among smokers than non-smokers; elevated BP may be treated in smokers using such a drug, whereas the same drug is unlikely to be effective in non-smokers. Alternatively, physicians may counsel patients on specific antihypertensive drugs that they may obtain greater treatment effect if they modify their exposure (e.g. smoking cessation). While precision medicine interventions are still emerging in cardiovascular care, a consideration of interaction effects lays an important foundation. In addition to drug targeting, a smoking interaction can also help us to identify novel biological mechanisms underlying BP traits.

One such locus showing significant interaction with smoking status is *CSMD1*. While variants of this gene were previously suggested for addiction and related disorders (48), we identified this locus at genome-wide significance (1DF $P = 4.3 \times 10^{-9}$, 2DF $P = 2.1 \times 10^{-11}$). In our study, another locus near *AHR* showed weak evidence of interaction with smoking (1DF $P = 1.6 \times 10^{-4}$, 2DF $P = 1.7 \times 10^{-9}$ associated with MAP). Variants in *AHR* are shown to interact with variants in *CYP1A1*, a detoxifying enzyme, to explain BP differences between smokers and non-smokers (80). *AHR* encodes a ligand-activated transcription factor, and *AHR* knock-out mice have increased MAP and ventricular hypertrophy/fibrosis with increased plasma levels of angiotensin II (81). Given the evidence that environmental toxins, including tobacco smoke, activate AHR, it is pertinent to note that AHR, in turn, activates tyrosinase activity, the rate limiting step for L-dopa biosynthesis (76). Activation of the AHR protein represses T-cadherin expression, which functions as a negative growth regulator in vascular smooth muscle cells (82,83). T-cadherin (encoded by *CDH13*) has been previously identified as a BP susceptibility locus (84). Notably, while the endogenous ligand for AHR remains uncertain (85), exogenous ligands include polycyclic aromatic hydrocarbons that are found in tobacco smoke and other environmental pollutants (86).

We found that most of MAP-associated loci were previously associated with SBP and/or DBP. This is not surprising given that MAP is closely related physiologically to SBP and DBP. In contrast, analysis of PP yielded a greater number of novel significant loci that are unique to PP. Loci associated with PP may be identifying different physiologic processes than loci associated with MAP, SBP and DBP. For example, the steady component of BP can be effectively targeted by β-adrenergic receptor and calcium-channel blockers that both modulate arteriolar tone. Angiotensin converting enzyme inhibitors, which favor remodeling of vascular connective tissue, may impact PP to a greater extent (87). This is a clinically important concept since hypertension is often more effectively treated by combination drug therapy to target different physiologic pathways (23).

We identified 30 loci that were statistically significant only in the meta-analyses of African ancestry individuals (forest plots in Supplementary Material, Fig. S5). Due to many prior BP GWAS discoveries, mostly based on European or Asian ancestries, identifying new BP loci in European and Asian ancestries may be challenging. There are also more opportunities to identify lower frequency variants in African ancestry individuals because there are more of these variants in this genetically more diverse population (with correspondingly smaller LD blocks, allowing closer identification of multiple underlying causal variants). The observed effect sizes (in African ancestry, Fig. 3) may be larger than their true values due to winners' curse (88). All identified loci were in low frequency [with minor allele frequency (MAF) ranging from 1.2% to 3.1%] but had good imputation quality scores ranging from 0.62 to 0.95 (presented in Supplementary Material, Fig. S5). In many of these loci, forest plots show consistent association across the contributing African cohorts. Out of 30, 23 loci were only present in African ancestry, and therefore, these associations could not be effectively evaluated in other ancestry groups as a result of their inter-ancestry differences in MAF. Because of the limited sample sizes available for African ancestry in stage 2, genome-wide significant loci in stage 1 African ancestry could not be formally replicated in stage 2; only the largest African cohort in stage 2 (Health and Retirement Study, $N = 1993$) provided association results for a subset of 23 loci (Supplementary Material, Fig. S5). For the remaining seven loci, we found evidence of association in African ancestry but not in meta-analyses in other ancestries, despite comparable or higher allele frequencies, such as those observed with rs11587661 (*COG2*) or rs72723039 (*IRX2*). We found similar smoking-specific effects on lipid traits that were unique to African ancestry (89). They may relate at least in part to inter-ancestry differences, including preference of menthol cigarettes. Therefore, African-specific loci should be treated cautiously since they require further validation.

This large-scale multi-ancestry study has some limitations. First, because most of the known BP loci were identified in European and Asian ancestries, considerable effort was made to recruit most of the available studies from the other ancestries into stage 1. Although we were able to identify several new loci in African ancestry, the relatively smaller stage 2 sample size of African ancestry ($N = 7786$) has limited our ability to replicate these new loci. Second, some of our new loci identified through the 2DF joint test may have been identified due to a main effect because of a larger sample size and more diverse ancestries, not necessarily from gene–smoking interaction. Unfortunately, we are unable to verify this because analysis of main effects alone, without regard to smoking status, was not performed. Third, conditional analysis (such as genome-wide complex trait analysis, GCTA) based on summary statistics was not performed because valid methods do not currently exist for G×E interactions. Therefore, we relied on a relatively more stringent LD threshold ($r^2 < 0.1$) for identifying additional signals within the know BP loci. Fourth, if there is a G×E correlation, a potential confounding of G×E with interaction between covariate and smoking exposure may exist. This can inflate Type I error of the G×E interaction test (90).

In summary, this study identified 38 new loci and 9 new signals near known BP loci that are uniquely associated with MAP and/or PP (and not associated with SBP or DBP), demonstrating the promise of gene–lifestyle interactions for genetic and environmental dissection of BP traits. Of our 38 loci, 10 were within 1 Mb of those recently reported by both Evangelou *et al.* (9) and Giri *et al.* (10); 6 loci were African-specific. Additional seven loci (including four African-specific loci) were within 1 Mb of those reported by Evangelou *et al.* (9). Variants in several loci were identified in individuals of African ancestry, highlighting the importance of genetic studies in diverse populations. Many of these new loci (including *CSMD1*, *TMEM51*, *SLC26A7*, *TNFRSF11A* and *AVPR1A*) show biologic plausibility for a role in BP regulation. They include additional loci of potential relevance to the structure and function of primary cilia and the dopaminergic system. Understanding underlying mechanisms for the newly identified loci and biological insights into the genetics of BP traits will require further investigation. Out of 136 significant loci, 8 showed significant interaction with smoking status. Because some interactions may be driven by other lifestyle factors that are correlated with smoking, a follow-up study such as Tyrrell and her colleague (91) that jointly examines multiple lifestyle factors can shed light on further understanding of the nature of the smoking interaction effects on BP. Our findings highlight the value of multi-ancestry investigations, particularly in studies of interaction with lifestyle factors, where genomic and lifestyle differences may contribute to novel findings.

Materials and Methods

Participating studies

Analyses included men and women between 18 and 80 years of age from European (EUR), African (AFR), Asian (ASN),

Hispanic (HIS) and Brazilian (BRZ) ancestries. A total of 48 cohorts consisting of 129 913 individuals (80 552 EUR; 27 118 AFR; 13 438 ASN; 8.805 HSP; Supplementary Material, Table S1) participated in stage 1 and performed genome-wide analyses. Studies that included data from multiple ancestries (cohorts) contributed multiple analyses, one for each ancestry/cohort. For example, multi-ethinc study of atherosclerosis has four cohorts. A total of 76 additional cohorts consisting of 480 178 individuals (305 513 EUR; 7826 AFR; 148 932 ASN; 13 533 HSP; 4414 BRZ; Supplementary Material, Table S2) participated in stage 2 and performed association analyses of 4373 variants that were identified in stage 1 as either genome-wide significant ($P < 5 \times 10^{-8}$) or suggestive ($P < 10^{-6}$). ASN participants include both south Asian and east Asians. Stage 1 ASN includes 7873 East Asians and 5566 South Asians, whereas stage 2 ASN includes 136 961 East Asians and 12 481 South Asians. All participating studies are described in the Supplementary Material. Since discoveries of BP loci to date were largely from EUR populations, considerable effort was made for recruiting most of the available non-EUR cohorts into stage 1 (which limited the availability of non-EUR cohorts in stage 2). Each study obtained informed consent from participants and approval from the appropriate institutional review boards.

Phenotypes and lifestyle variables

Resting SBP and DBP were measured using standard clinical procedures that produce comparable measurements (specific methods per study were described more in Supplementary Material). Even with some difference in measurement across studies, the measures were standardized, through previous main effect BP GWAS studies, as much as possible for BP. For individuals on any anti-hypertensive (BP lowering) medications, 15 mmHg and 10 mmHg were added to their SBP and DBP values, respectively (1). PP was computed as SBP minus DBP (PP = SBP—DBP), and MAP was computed as the sum of DBP and one-third of PP (MAP = DBP + PP/3). To reduce the influence of possible outliers, each BP value was winsorized at six standard deviations (SD) away from the mean (i.e. values greater than six SD away from the mean were set at six SD).

Obtained through interview-based or self-reported questionnaire, varying levels of smoking information were available across studies, some with a simple binary variable and others with repeated data. We considered two of the most widely available smoking variables: 'current smoking' status (CurSmk) and 'ever smoking' status (EverSmk) (Table 1). Current smoking status was defined as 1 if the individual smoked regularly in past year (and as 0 for non-current smokers, which includes both never and former smokers). Ever smoking status was defined as 1 if the individual smoked at least 100 cigarettes during his/her lifetime (and as 0 for the never smokers). Smoking status was assessed at the time of the BP measurements. Covariates include age, sex, field center (for multi-center studies) and principal components (PCs) (to account for population stratification and admixture). No additional covariates were included. Individuals with missing data for BP, the smoking variable or any covariates were excluded from analysis. Study-specific summary statistics on phenotypes are presented in Supplementary Materials, Tables S3 and S4.

Genotype data

Genotyping was obtained using Illumina (San Diego, CA, USA) or Affymetrix (Santa Clara, CA, USA) genotyping arrays. Each study performed genotype imputation at SNPs, short insertions and deletions (indels), and larger deletions that were not genotyped directly but are available from the 1000 Genomes Project (92). For imputation, most studies used the 1000 Genomes Project Phase I Integrated Release Version 3 Haplotypes (2010–11 data freeze, 2012-03-14 haplotypes), which contain haplotypes of 1092 individuals of all ancestry backgrounds. Study-specific information on genotyping and imputation is presented in Supplementary Materials,Tables S5 and S6.

Cohort-specific analysis

We identified loci through the two DF test that jointly test the genetic main effect and the gene–smoking interaction jointly. This approach has previously enabled identification of new loci associated with insulin resistance, including how the effect of variants differs with levels of BMI (11). The method is described in detail for single studies in Kraft *et al.* (93) and for implementation in meta-analyses in Manning *et al.* (24).

Participating studies performed association analyses separately within each ancestry for MAP and PP incorporating CurSmk and EverSmk. All studies performed regression analysis using a model with both genetic main and G×E interaction effects (93): $\mathbb{E}\left[Y\right] = \beta_0 + \beta_E Smk + \beta_G G + \beta_{GE} Smk * G + \boldsymbol{\beta}_C \mathbf{C}$.

Y is the medication-adjusted BP value, Smk is the smoking variable (with 0/1 coding for the absence/presence of the smoking exposure), G is the dosage of the imputed genetic variant coded additively (from 0 to 2) and **C** is the vector of all other covariates, which include age, sex, field center (for multi-center studies) and PCs (to account for population stratification and admixture). No additional cohort-specific covariates were included. From this model, the studies provided the estimated genetic main and interaction effects and a robust estimate of the corresponding covariance matrix. In addition, studies in stage 1 performed regression analyses with the genetic main-effect model, in the exposed (Smk = 1) and unexposed strata (Smk = 0) separately, and provided estimates of the stratum-specific effects and robust estimates of their standard errors (SE).

Either sandwich (94) or ProbABEL (95) packages were used to obtain robust estimates of covariance matrices and robust SEs for samples of unrelated individuals. Family studies used the generalized estimating equations approach, treating each family as a cluster, or the linear mixed effect model approach with a random polygenic component (for which the covariance matrix depends on the kinship matrix). Robust estimates of covariance matrices and SEs were used to safeguard against misspecification of the mean model and violation of the assumption of constant BP variance across smoking groups (heteroscedasticity) (96,97).

Quality control

Each study performed standard genotype quality control (QC) that includes excluding SNPs with call rate (<95% or higher) and Hardy–Weinberg equilibrium $P < 10^{-6}$. In addition, we performed extensive QC using the R package EasyQC (98) for all cohort-specific results. For GWAS results in stage 1, each cohort applied a preliminary filter on their imputed data excluding variants with MAF < 1%. Variants with imputation quality measure of < 0.5 were subsequently excluded. We performed the 'study-level' QC, which included carefully checking the observed allele frequencies against the corresponding ancestry-specific 1000 Genomes Project data and harmonizing marker names to ensure consistencies across cohorts. In addition, in stage 1, we com-

pared results from the joint and stratified models, as explained elsewhere (99). To identify cross-study issues, we then performed the 'meta-level' QC by checking result files across all cohorts for each analysis. This included visually comparing summary statistics (mean, median, inter-quartile range, etc.) on all effect estimates, SEs and *P*-values, and examining SE-N (i.e., inverse of the median standard error versus the square root of the sample size) plots and QQ plots to reveal issues with trait transformation (98) or other analytical problems. Encountered QC problems were communicated and resolved with the individual cohorts. More detailed information about QC is described elsewhere (13,16).

Meta-analyses

After selecting high-quality variants through extensive QC, ~18.8 million SNPs and small indels variants were included in the meta-analysis (the number of variants varied across the ancestry groups). To combine cohort-specific results within each ancestry, we first performed ancestry-specific meta-analyses; the results were then combined through meta-analysis to obtain evidence of 'trans-ancestry' association. Inverse-variance-weighted meta-analysis with METAL (100) was used for the one DF test of interaction effect (with H_0: $\beta_{GE} = 0$). For two DF test of both SNP main and interaction effects (with H_0: $\beta_G = \beta_{GE} = 0$), the joint meta-analysis of Manning *et al.* (24) was used. In the stratified model, we performed meta-analysis using the approach of Randall *et al.* (101) for the one DF test and the approach of Aschard *et al.* (102) for the two DF test using the R package EasyStrata (103). Additional details about the meta-analytic approach are described elsewhere (99).

In stage 1, genomic control correction (104) was applied twice, first for cohort-specific GWAS results if their genomic control lambda value was greater than 1 and again after the meta-analysis. Variants that passed QC were excluded if they were represented in fewer than 5000 samples or fewer than three cohorts. Variants that were genome-wide significant ($P < 5 \times 10^{-8}$) or suggestive ($P < 1 \times 10^{-6}$) in stage 1 were pursued in stage 2. Heterogeneity *P*-values at the selected variants were $> 1 \times 10^{-5}$, indicating limited heterogeneity (data not shown). In stage 2, genomic control correction was not applied to the replication statistics as association analysis was performed only at select variants. Meta-analysis combining results of stages 1 and 2 was also performed. In addition, genome-wide significant variants in stage 1 were tested for formal replication in stage 2 using Bonferroni-corrected significance threshold.

Genome-wide significant variants

We considered a variant with $P < 5 \times 10^{-8}$ (the standard threshold in the field) to be genome-wide significant. We also identified novel loci that pass a more stringent threshold ($P < 6.25 \times 10^{-9}$, $P < 5 \times 10^{-8}$ adjusted for two smoking exposures, two tests and two BP traits, where this correction is somewhat conservative given dependence between the various test statistics). Loci that pass the stricter *P*-value are indicated in main tables. FDR q-values were computed using the R function p.adjust using the step-up method by Benjamini and Hochberg (105). A new locus was identified if it was 1 Mb away from any previously identified BP locus. A new signal was identified if it is within 1 Mb of known BP loci but not in LD $r^2 < 0.1$ with the known BP loci. Since valid methods do not exist for conditional analysis involving interactions across multi-ancestry studies, we relied on a relatively more stringent LD threshold ($r^2 < 0.1$) for identifying additional signals. For LD reference, ancestry-specific 1000 Genomes Project data (106) were used for ancestry-specific results, and the entire cosmopolitan data set was used for *trans*-ancestry results.

BP variance explained

We computed BP variance explained by genome-wide results, based on stage 1 stratified results with current-smoking status in European ancestry (25). Within each of the smoking strata, we computed the variance of MAP and PP explained by subsets of variants selected using 15 significance thresholds ranging from 1×10^{-8} to 0.1

Functional inferences

We conducted DEPICT analyses (107) based on genome-wide significant ($P < 5 \times 10^{-8}$) variants from the combined analysis of stages 1 and 2. DEPICT performs three consecutive analyses: i) gene prioritization at the identified loci, ii) gene set enrichment analyses and iii) tissue- and cell-type-specific expression analyses. To obtain input for the analyses, DEPICT applied a combined distance and LD-based threshold (500 kb flanking regions and LD $r^2 > 0.1$) between the identified variants and the 1000 Genomes reference data (106). A further clumping (LD $r^2 > 0.5$ between the non-overlapping variants and known functional coding or *cis*-acting regulatory variants) was used to obtain a list of genes overlapping with the identified variants. The major histocompatibility complex region on chromosome 6 (25—35 Mb) was removed for further analyses.

For gene prioritization, DEPICT compared functional similarity of genes across identified loci using a gene score, which was adjusted for confounders like gene length. To obtain FDR, the scoring was repeated 50× based on 500 pre-compiled null GWAS. For gene-set enrichment analyses, DEPICT used 14 461 pre-compiled reconstituted gene sets; they include 737 Reactome pathways, 2473 phenotypic gene sets (derived from the Mouse Genetics Initiative), 184 Kyoto Encyclopedia of Genes and Genomes pathways, 5083 Gene Ontology terms and 5984 protein molecular pathways (derived from protein–protein interactions). For tissue- and cell-type enrichment analyses, DEPICT used expression data from the 209 MeSH annotations for 37 427 microarrays of the Affymetrix U133 Plus 2.0 Array platform.

Supplementary Material

Supplementary Material is available at *HMG* online.

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References

1. Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M.D., Bochud, M., Coin, L., Najjar, S.S., Zhao, J.H., Heath, S.C., Eyheramendy, S. *et al.* (2009) Genome-wide association study identifies eight loci associated with blood pressure. *Nat. Genet.*, **41**, 666–676.
2. Levy, D., Ehret, G.B., Rice, K., Verwoert, G.C., Launer, L.J., Dehghan, A., Glazer, N.L., Morrison, A.C., Johnson, A.D., Aspelund, T. *et al.* (2009) Genome-wide association study of blood pressure and hypertension. *Nat. Genet.*, **41**, 677–687.
3. Ehret, G.B., Munroe, P.B., Rice, K.M., Bochud, M., Johnson, A.D., Chasman, D.I., Smith, A.V., Tobin, M.D., Verwoert, G.C., Hwang, S.J. *et al.* (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*, **478**, 103–109.
4. Ehret, G.B., Ferreira, T., Chasman, D.I., Jackson, A.U., Schmidt, E.M., Johnson, T., Thorleifsson, G., Luan, J., Donnelly, L.A., Kanoni, S. *et al.* (2016) The genetics of blood pressure regulation and its target organs from association studies in 342 415 individuals. *Nat. Genet.*, **48**, 1171–1184.
5. Liu, C., Kraja, A.T., Smith, J.A., Brody, J.A., Franceschini, N., Bis, J.C., Rice, K., Morrison, A.C., Lu, Y., Weiss, S. *et al.* (2016) Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. *Nat. Genet.*, **48**, 1162–1170.
6. Surendran, P., Drenos, F., Young, R., Warren, H., Cook, J.P., Manning, A.K., Grarup, N., Sim, X., Barnes, D.R., Witkowska, K. *et al.* (2016) Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. *Nat. Genet.*, **48**, 1151–1161.
7. Hoffmann, T.J., Ehret, G.B., Nandakumar, P., Ranatunga, D., Schaefer, C., Kwok, P.Y., Iribarren, C., Chakravarti, A. and Risch, N. (2016) Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation. *Nat. Genet.*, **49**, 54–64.
8. Warren, H.R., Evangelou, E., Cabrera, C.P., Gao, H., Ren, M., Mifsud, B., Ntalla, I., Surendran, P., Liu, C., Cook, J.P. *et al.* (2017) Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. *Nat. Genet.*, **49**, 403–415.
9. Evangelou, E., Warren, H.R., Mosen-Ansorena, D., Mifsud, B., Pazoki, R., Gao, H., Ntritsos, G., Dimou, N., Cabrera, C.P., Karaman, I. *et al.* (2018) Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat. Genet.*, **50**, 1412–1425.
10. Giri, A., Hellwege, J.N., Keaton, J.M., Park, J., Qiu, C., Warren, H.R., Torstenson, E.S., Kovesdy, C.P., Sun, Y.V., Wilson, O.D. *et al.* (2019) Trans-ethnic association study of blood pressure determinants in over 750 000 individuals. *Nat. Genet.*, **51**, 51–62.
11. Manning, A.K., Hivert, M.F., Scott, R.A., Grimsby, J.L., Bouatia-Naji, N., Chen, H., Rybin, D., Liu, C.T., Bielak, L.F., Prokopenko, I. *et al.* (2012) A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nat. Genet.*, **44**, 659–669.
12. Kirk, E.P. (2017) Genes, environment, and the heart: putting the pieces together. *Circ. Cardiovasc. Genet.*, **10**, 1–2.
13. Rao, D.C., Sung, Y.J., Winkler, T.W., Schwander, K., Borecki, I., Cupples, L.A., Gauderman, W.J., Rice, K., Munroe, P.B. and Psaty, B. (2017) A multi-ancestry study of gene–lifestyle interactions for cardiovascular traits in 610 475 individuals from 124 cohorts: design and rationale. *Circ. Cardiovasc. Genet.*, **10**, e001649.
14. Mann, S.J., James, G.D., Wang, R.S. and Pickering, T.G. (1991) Elevation of ambulatory systolic blood pressure in hypertensive smokers. A case–control study. *JAMA*, **265**, 2226–2228.
15. Primatesta, P., Falaschetti, E., Gupta, S., Marmot, M.G. and Poulter, N.R. (2001) Association between smoking and blood pressure: evidence from the health survey for England. *Hypertension*, **37**, 187–193.
16. Sung, Y.J., Winkler, T.W., de Las Fuentes, L., Bentley, A.R., Brown, M.R., Kraja, A.T., Schwander, K., Ntalla, I., Guo, X., Franceschini, N. *et al.* (2018) A large-scale multi-ancestry genome-wide study accounting for smoking behavior identifies multiple significant loci for blood pressure. *Am. J. Hum. Genet.*, **102**, 375–400.
17. Franklin, S.S., Gustin, W.T., Wong, N.D., Larson, M.G., Weber, M.A., Kannel, W.B. and Levy, D. (1997) Hemodynamic patterns of age-related changes in blood pressure. The Framingham heart study. *Circulation*, **96**, 308–315.
18. Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R. and Prospective Studies Collaboration (2002) Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*, **360**, 1903–1913.
19. Sesso, H.D., Stampfer, M.J., Rosner, B., Hennekens, C.H., Gaziano, J.M., Manson, J.E. and Glynn, R.J. (2000) Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in men. *Hypertension*, **36**, 801–807.
20. Dart, A.M. and Kingwell, B.A. (2001) Pulse pressure—a review of mechanisms and clinical relevance. *J. Am. Coll. Cardiol.*, **37**, 975–984.
21. Franklin, S.S., Khan, S.A., Wong, N.D., Larson, M.G. and Levy, D. (1999) Is pulse pressure useful in predicting risk for coronary heart disease? The Framingham heart study. *Circulation*, **100**, 354–360.
22. Millar, J.A., Lever, A.F. and Burke, V. (1999) Pulse pressure as a risk factor for cardiovascular events in the MRC mild hypertension trial. *J. Hypertens.*, **17**, 1065–1072.

23. Whelton, P.K., Carey, R.M., Aronow, W.S., Casey, D.E., J., Collins, K.J., Dennison Himmelfarb, C., DePalma, S.M., Gidding, S., Jamerson, K.A., Jones, D.W. *et al.* (2018) 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J. Am. Coll. Cardiol.*, **71**, e127–e248.
24. Manning, A.K., LaValley, M., Liu, C.T., Rice, K., An, P., Liu, Y., Miljkovic, I., Rasmussen-Torvik, L., Harris, T.B., Province, M.A. *et al.* (2011) Meta-analysis of gene–environment interaction: joint estimation of SNP and SNP × environment regression coefficients. *Genet. Epidemiol.*, **35**, 11–18.
25. Kutalik, Z., Whittaker, J., Waterworth, D., Consortium, G., Beckmann, J.S. and Bergmann, S. (2011) Novel method to estimate the phenotypic variation explained by genome-wide association studies reveals large fraction of the missing heritability. *Genet. Epidemiol.*, **35**, 341–349.
26. Ward, L.D. and Kellis, M. (2012) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.*, **40**, D930–D934.
27. Davydov, E.V., Goode, D.L., Sirota, M., Cooper, G.M., Sidow, A. and Batzoglou, S. (2010) Identifying a high fraction of the human genome to be under selective constraint using GERP++. *PLoS Comput. Biol.*, **6**, e1001025.
28. Garber, M., Guttman, M., Clamp, M., Zody, M.C., Friedman, N. and Xie, X. (2009) Identifying novel constrained elements by exploiting biased substitution patterns. *Bioinformatics*, **25**, i54–i62.
29. Consortium, G.T. (2015) Human genomics. The genotype-tissue expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*, **348**, 648–660.
30. Joehanes, R., Zhang, X., Huan, T., Yao, C., Ying, S.X., Nguyen, Q.T., Demirkale, C.Y., Feolo, M.L., Sharopova, N.R., Sturcke, A. *et al.* (2017) Integrated genome-wide analysis of expression quantitative trait loci aids interpretation of genomic association studies. *Genome Biol.*, **18**, 16.
31. di Salvo, T.G., Yang, K.C., Brittain, E., Absi, T., Maltais, S. and Hemnes, A. (2015) Right ventricular myocardial biomarkers in human heart failure. *J. Card. Fail.*, **21**, 398–411.
32. Cingolani, O.H., Kirk, J.A., Seo, K., Koitabashi, N., Lee, D.I., Ramirez-Correa, G., Bedja, D., Barth, A.S., Moens, A.L. and Kass, D.A. (2011) Thrombospondin-4 is required for stretch-mediated contractility augmentation in cardiac muscle. *Circ. Res.*, **109**, 1410–1414.
33. Han, Y., Chen, Y.S., Liu, Z., Bodyak, N., Rigor, D., Bisping, E., Pu, W.T. and Kang, P.M. (2006) Overexpression of HAX-1 protects cardiac myocytes from apoptosis through caspase-9 inhibition. *Circ. Res.*, **99**, 415–423.
34. Lee, B.Y., Chon, J., Kim, H.S., Lee, J.H., Yun, D.H., Yoo, S.D., Kim, D.H., Lee, S.A., Han, Y.J., Lee, H. *et al.* (2017) Association between a polymorphism in CASP3 and CASP9 genes and ischemic stroke. *Ann. Rehabil. Med.*, **41**, 197–203.
35. Elliott, H.R., Tillin, T., McArdle, W.L., Ho, K., Duggirala, A., Frayling, T.M., Davey Smith, G., Hughes, A.D., Chaturvedi, N. and Relton, C.L. (2014) Differences in smoking associated DNA methylation patterns in South Asians and Europeans. *Clin. Epigenetics*, **6**, 4.
36. Markunas, C.A., Xu, Z., Harlid, S., Wade, P.A., Lie, R.T., Taylor, J.A. and Wilcox, A.J. (2014) Identification of DNA methylation changes in newborns related to maternal smoking during pregnancy. *Environ. Health Perspect.*, **122**, 1147–1153.
37. Begum, N., Hockman, S. and Manganiello, V.C. (2011) Phosphodiesterase 3A (PDE3A) deletion suppresses proliferation of cultured murine vascular smooth muscle cells (VSMCs) via inhibition of mitogen-activated protein kinase (MAPK) signaling and alterations in critical cell cycle regulatory proteins. *J. Biol. Chem.*, **286**, 26238–26249.
38. Maass, P.G., Aydin, A., Luft, F.C., Schachterle, C., Weise, A., Stricker, S., Lindschau, C., Vaegler, M., Qadri, F., Toka, H.R. *et al.* (2015) PDE3A mutations cause autosomal dominant hypertension with brachydactyly. *Nat. Genet.*, **47**, 647–653.
39. Toka, O., Tank, J., Schachterle, C., Aydin, A., Maass, P.G., Elitok, S., Bartels-Klein, E., Hollfinger, I., Lindschau, C., Mai, K. *et al.* (2015) Clinical effects of phosphodiesterase 3A mutations in inherited hypertension with brachydactyly. *Hypertension*, **66**, 800–808.
40. Vasan, R.S., Glazer, N.L., Felix, J.F., Lieb, W., Wild, P.S., Felix, S.B., Watzinger, N., Larson, M.G., Smith, N.L., Dehghan, A. *et al.* (2009) Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. *JAMA*, **302**, 168–178.
41. Fontana, V., McDonough, C.W., Gong, Y., El Rouby, N.M., Sa, A.C., Taylor, K.D., Chen, Y.D., Gums, J.G., Chapman, A.B., Turner, S.T. *et al.* (2014) Large-scale gene-centric analysis identifies polymorphisms for resistant hypertension. *J. Am. Heart Assoc.*, **3**, e001398.
42. Simino, J., Sung, Y.J., Kume, R., Schwander, K. and Rao, D.C. (2013) Gene–alcohol interactions identify several novel blood pressure loci including a promising locus near SLC16A9. *Front. Genet.*, **4**, 277.
43. Coan, P.M., Hummel, O., Garcia Diaz, A., Barrier, M., Alfazema, N., Norsworthy, P.J., Pravenec, M., Petretto, E., Hubner, N. and Aitman, T.J. (2017) Genetic, physiological and comparative genomic studies of hypertension and insulin resistance in the spontaneously hypertensive rat. *Dis. Model. Mech.*, **10**, 297–306.
44. Hong, K.W., Go, M.J., Jin, H.S., Lim, J.E., Lee, J.Y., Han, B.G., Hwang, S.Y., Lee, S.H., Park, H.K., Cho, Y.S. *et al.* (2010) Genetic variations in ATP2B1, CSK, ARSG and CSMD1 loci are related to blood pressure and/or hypertension in two Korean cohorts. *J. Hum. Hypertens.*, **24**, 367–372.
45. Koriyama, H., Nakagami, H., Katsuya, T., Sugimoto, K., Yamashita, H., Takami, Y., Maeda, S., Kubo, M., Takahashi, A., Nakamura, Y. *et al.* (2010) Identification of evidence suggestive of an association with peripheral arterial disease at the OSBPL10 locus by genome-wide investigation in the Japanese population. *J. Atheroscler. Thromb.*, **17**, 1054–1062.
46. Liu, C.T., Monda, K.L., Taylor, K.C., Lange, L., Demerath, E.W., Palmas, W., Wojczynski, M.K., Ellis, J.C., Vitolins, M.Z., Liu, S. *et al.* (2013) Genome-wide association of body fat distribution in African ancestry populations suggests new loci. *PLoS Genet.*, **9**, e1003681.
47. Irvin, M.R., Wineinger, N.E., Rice, T.K., Pajewski, N.M., Kabagambe, E.K., Gu, C.C., Pankow, J., North, K.E., Wilk, J.B., Freedman, B.I. *et al.* (2011) Genome-wide detection of allele specific copy number variation associated with insulin resistance in African Americans from the HyperGEN study. *PLoS One*, **6**, e24052.
48. Uhl, G.R., Drgon, T., Johnson, C., Li, C.Y., Contoreggi, C., Hess, J., Naiman, D. and Liu, Q.R. (2008) Molecular genetics of addiction and related heritable phenotypes: genome-wide association approaches identify 'connectivity constellation' and drug target genes with pleiotropic effects. *Ann. N. Y. Acad. Sci.*, **1141**, 318–381.

49. Moon, S.H., Kim, Y.J., Kim, Y.K., Kim, D.J., Lee, J.Y., Go, M.J., Shin, Y.A., Hong, C.B. and Kim, B.J. (2011) Genome-wide survey of copy number variants associated with blood pressure and body mass index in a Korean population. *Genomics Informatics* **9**, 152–160.
50. Petrovic, S., Barone, S., Xu, J., Conforti, L., Ma, L., Kujala, M., Kere, J. and Soleimani, M. (2004) SLC26A7: a basolateral cl-/HCO3- exchanger specific to intercalated cells of the outer medullary collecting duct. *Am. J. Physiol. Renal Physiol.*, **286**, F161–F169.
51. Henke, N., Schmidt-Ullrich, R., Dechend, R., Park, J.K., Qadri, F., Wellner, M., Obst, M., Gross, V., Dietz, R., Luft, F.C. *et al.* (2007) Vascular endothelial cell-specific NF-kappaB suppression attenuates hypertension-induced renal damage. *Circ. Res.*, **101**, 268–276.
52. Pierce, G.L., Lesniewski, L.A., Lawson, B.R., Beske, S.D. and Seals, D.R. (2009) Nuclear factor-{kappa}B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation*, **119**, 1284–1292.
53. Duan, P., Wang, Z.M., Liu, J., Wang, L.N., Yang, Z. and Tu, P. (2015) Association of gene polymorphisms in RANKL/RANK/OPG system with hypertension and blood pressure in Chinese women. *J. Hum. Hypertens.*, **29**, 749–753.
54. Woods, R.L. and Johnston, C.I. (1983) Contribution of vasopressin to the maintenance of blood pressure during dehydration. *Am. J. Physiol.*, **245**, F615–F621.
55. Aoyagi, T., Izumi, Y., Hiroyama, M., Matsuzaki, T., Yasuoka, Y., Sanbe, A., Miyazaki, H., Fujiwara, Y., Nakayama, Y., Kohda, Y. *et al.* (2008) Vasopressin regulates the renin-angiotensin-aldosterone system via V1a receptors in macula densa cells. *Am. J. Physiol. Renal Physiol.*, **295**, F100–F107.
56. Koshimizu, T.A., Nasa, Y., Tanoue, A., Oikawa, R., Kawahara, Y., Kiyono, Y., Adachi, T., Tanaka, T., Kuwaki, T., Mori, T. *et al.* (2006) V1a vasopressin receptors maintain normal blood pressure by regulating circulating blood volume and baroreflex sensitivity. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 7807–7812.
57. Burrell, L.M., Phillips, P.A., Risvanis, J., Aldred, K.L., Hutchins, A.M. and Johnston, C.I. (1995) Attenuation of genetic hypertension after short-term vasopressin V1A receptor antagonism. *Hypertension*, **26**, 828–834.
58. Ronzaud, C., Loffing-Cueni, D., Hausel, P., Debonneville, A., Malsure, S.R., Fowler-Jaeger, N., Boase, N.A., Perrier, R., Maillard, M., Yang, B. *et al.* (2013) Renal tubular NEDD4-2 deficiency causes NCC-mediated salt-dependent hypertension. *J. Clin. Invest.*, **123**, 657–665.
59. Debonneville, C., Flores, S.Y., Kamynina, E., Plant, P.J., Tauxe, C., Thomas, M.A., Munster, C., Chraibi, A., Pratt, J.H., Horisberger, J.D. *et al.* (2001) Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na(+) channel cell surface expression. *EMBO J.*, **20**, 7052–7059.
60. Zheng, X., Morrison, A.C., Feingold, E., Turner, S.T. and Ferrell, R.E. (2011) Association between NEDD4L gene and sodium lithium countertransport. *Am. J. Hypertens.*, **24**, 145–148.
61. Luo, F., Wang, Y., Wang, X., Sun, K., Zhou, X. and Hui, R. (2009) A functional variant of NEDD4L is associated with hypertension, antihypertensive response, and orthostatic hypotension. *Hypertension*, **54**, 796–801.
62. Svensson-Farbom, P., Wahlstrand, B., Almgren, P., Dahlberg, J., Fava, C., Kjeldsen, S., Hedner, T. and Melander, O. (2011) A functional variant of the NEDD4L gene is associated with beneficial treatment response with beta-blockers and diuretics in hypertensive patients. *J. Hypertens.*, **29**, 388–395.
63. McDonough, C.W., Burbage, S.E., Duarte, J.D., Gong, Y., Langaee, T.Y., Turner, S.T., Gums, J.G., Chapman, A.B., Bailey, K.R., Beitelshees, A.L. *et al.* (2013) Association of variants in NEDD4L with blood pressure response and adverse cardiovascular outcomes in hypertensive patients treated with thiazide diuretics. *J. Hypertens.*, **31**, 698–704.
64. Goldberg, L.I. (1972) Cardiovascular and renal actions of dopamine: potential clinical applications. *Pharmacol. Rev.*, **24**, 1–29.
65. Li, X.X., Bek, M., Asico, L.D., Yang, Z., Grandy, D.K., Goldstein, D.S., Rubinstein, M., Eisner, G.M. and Jose, P.A. (2001) Adrenergic and endothelin B receptor-dependent hypertension in dopamine receptor type-2 knockout mice. *Hypertension*, **38**, 303–308.
66. Armando, I., Wang, X., Villar, V.A., Jones, J.E., Asico, L.D., Escano, C. and Jose, P.A. (2007) Reactive oxygen species-dependent hypertension in dopamine D2 receptor-deficient mice. *Hypertension*, **49**, 672–678.
67. Zhang, Y., Jiang, X., Qin, C., Cuevas, S., Jose, P.A. and Armando, I. (2016) Dopamine D2 receptors' effects on renal inflammation are mediated by regulation of PP2A function. *Am. J. Physiol. Renal Physiol.*, **310**, F128–F134.
68. Yang, Y., Cuevas, S., Yang, S., Villar, V.A., Escano, C., Asico, L., Yu, P., Jiang, X., Weinman, E.J., Armando, I. *et al.* (2014) Sestrin2 decreases renal oxidative stress, lowers blood pressure, and mediates dopamine D2 receptor-induced inhibition of reactive oxygen species production. *Hypertension*, **64**, 825–832.
69. Yi, L., Li, F., Yong, Y., Jianting, D., Liting, Z., Xuansheng, H., Fei, L. and Jiewen, L. (2014) Upregulation of sestrin-2 expression protects against endothelial toxicity of angiotensin II. *Cell Biol. Toxicol.*, **30**, 147–156.
70. Paisan-Ruiz, C., Guevara, R., Federoff, M., Hanagasi, H., Sina, F., Elahi, E., Schneider, S.A., Schwingenschuh, P., Bajaj, N., Emre, M. *et al.* (2010) Early-onset L-dopa-responsive parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and spatacsin mutations. *Mov. Disord.*, **25**, 1791–1800.
71. Ouimet, C.C., Hemmings, H.C., Jr. and Greengard, P. (1989) ARPP-21, a cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. II. Immunocytochemical localization in rat brain. *J. Neurosci.*, **9**, 865–875.
72. Pierce, R.C. and Kumaresan, V. (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci. Biobehav. Rev.*, **30**, 215–238.
73. King, K.S. and Pacak, K. (2014) Familial pheochromocytomas and paragangliomas. *Mol. Cell. Endocrinol.*, **386**, 92–100.
74. Hes, F.J., Weiss, M.M., Woortman, S.A., de Miranda, N.F., van Bunderen, P.A., Bonsing, B.A., Stokkel, M.P., Morreau, H., Romijn, J.A., Jansen, J.C. *et al.* (2010) Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. *BMC Med. Genet.*, **11**, 92.
75. Parmar, P.G., Taal, H.R., Timpson, N.J., Thiering, E., Lehtimaki, T., Marinelli, M., Lind, P.A., Howe, L.D., Verwoert, G., Aalto, V. *et al.* (2016) International genome-wide association study consortium identifies novel loci associated with blood pressure in children and adolescents. *Circ. Cardiovasc. Genet.*, **9**, 266–278.
76. Luecke, S., Backlund, M., Jux, B., Esser, C., Krutmann, J. and Rannug, A. (2010) The aryl hydrocarbon receptor (AHR), a novel regulator of human melanogenesis. *Pigment Cell Melanoma Res.*, **23**, 828–833.

77. Wang, L., Chu, A., Buring, J.E., Ridker, P.M., Chasman, D.I. and Sesso, H.D. (2014) Common genetic variations in the vitamin D pathway in relation to blood pressure. *Am. J. Hypertens.*, **27**, 1387–1395.
78. Blacher, J., Staessen, J.A., Girerd, X., Gasowski, J., Thijs, L., Liu, L., Wang, J.G., Fagard, R.H. and Safar, M.E. (2000) Pulse pressure not mean pressure determines cardiovascular risk in older hypertensive patients. *Arch. Intern. Med.*, **160**, 1085–1089.
79. Gasowski, J., Fagard, R.H., Staessen, J.A., Grodzicki, T., Pocock, S., Boutitie, F., Gueyffier, F., Boissel, J.P. and Collaborators, I.P. (2002) Pulsatile blood pressure component as predictor of mortality in hypertension: a meta-analysis of clinical trial control groups. *J. Hypertens.*, **20**, 145–151.
80. Gambier, N., Marteau, J.B., Batt, A.M., Marie, B., Thompson, A., Siest, G., Foernzler, D. and Visvikis-Siest, S. (2006) Interaction between CYP1A1 T3801C and AHR G1661A polymorphisms according to smoking status on blood pressure in the Stanislas cohort. *J. Hypertens.*, **24**, 2199–2205.
81. Lund, A.K., Goens, M.B., Nunez, B.A. and Walker, M.K. (2006) Characterizing the role of endothelin-1 in the progression of cardiac hypertrophy in aryl hydrocarbon receptor (AhR) null mice. *Toxicol. Appl. Pharmacol.*, **212**, 127–135.
82. Niermann, T., Schmutz, S., Erne, P. and Resink, T. (2003) Aryl hydrocarbon receptor ligands repress T-cadherin expression in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **300**, 943–949.
83. Kuzmenko, Y.S., Kern, F., Bochkov, V.N., Tkachuk, V.A. and Resink, T.J. (1998) Density- and proliferation status-dependent expression of T-cadherin, a novel lipoprotein-binding glycoprotein: a function in negative regulation of smooth muscle cell growth? *FEBS Lett.*, **434**, 183–187.
84. Org, E., Eyheramendy, S., Juhanson, P., Gieger, C., Lichtner, P., Klopp, N., Veldre, G., Doring, A., Viigimaa, M., Sober, S. *et al.* (2009) Genome-wide scan identifies CDH13 as a novel susceptibility locus contributing to blood pressure determination in two European populations. *Hum. Mol. Genet.*, **18**, 2288–2296.
85. Nguyen, L.P. and Bradfield, C.A. (2008) The search for endogenous activators of the aryl hydrocarbon receptor. *Chem. Res. Toxicol.*, **21**, 102–116.
86. Martey, C.A., Baglole, C.J., Gasiewicz, T.A., Sime, P.J. and Phipps, R.P. (2005) The aryl hydrocarbon receptor is a regulator of cigarette smoke induction of the cyclooxygenase and prostaglandin pathways in human lung fibroblasts. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **289**, L391–L399.
87. Blacher, J. and Safar, M.E. (2005) Large-artery stiffness, hypertension and cardiovascular risk in older patients. *Nat. Clin. Pract. Cardiovasc. Med.*, **2**, 450–455.
88. Zollner, S. and Pritchard, J.K. (2007) Overcoming the winner's curse: estimating penetrance parameters from case-control data. *Am. J. Hum. Genet.*, **80**, 605–615.
89. Bentley, A.R., Sung, Y.J., Brown, M.R., Winkler, T.W., Kraja, A.T., Ntalla, I., Schwander, K., Chasman, D.I., Lim, E., Deng, E. *et al.* (2019) Multi-ancestry genome-wide gene-smoking interaction study of 387,272 individuals identifies new loci associated with serum lipids.. *Nature genetics*, **51**, 636–648.
90. Keller, M.C. (2014) Gene × environment interaction studies have not properly controlled for potential confounders: the problem and the (simple) solution. *Biol. Psychiatry*, **75**, 18–24.
91. Tyrrell, J., Wood, A.R., Ames, R.M., Yaghootkar, H., Beaumont, R.N., Jones, S.E., Tuke, M.A., Ruth, K.S., Freathy, R.M., Davey Smith, G. *et al.* (2017) Gene-obesogenic environment interactions in the UK biobank study. *Int. J. Epidemiol.*, **46**, 559–575.
92. 1000 Genomes Project Consortium, Abecasis, G.R., Auton, A., Brooks, L.D., DePristo, M.A., Durbin, R.M., Handsaker, R.E., Kang, H.M., Marth, G.T. and McVean, G.A. (2012) An integrated map of genetic variation from 1092 human genomes. *Nature*, **491**, 56–65.
93. Kraft, P., Yen, Y.C., Stram, D.O., Morrison, J. and Gauderman, W.J. (2007) Exploiting gene–environment interaction to detect genetic associations. *Hum. Hered.*, **63**, 111–119.
94. Zeileis, A. (2006) Object-oriented computation of sandwich estimators. *J. Stat. Softw.*, **16**, 1–16.
95. Aulchenko, Y.S., Struchalin, M.V. and van Duijn, C.M. (2010) ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*, **11**, 134.
96. Tchetgen Tchetgen, E.J. and Kraft, P. (2011) On the robustness of tests of genetic associations incorporating gene–environment interaction when the environmental exposure is misspecified. *Epidemiology*, **22**, 257–261.
97. Voorman, A., Lumley, T., McKnight, B. and Rice, K. (2011) Behavior of QQ-plots and genomic control in studies of gene–environment interaction. *PLoS One*, **6**, e19416.
98. Winkler, T.W., Day, F.R., Croteau-Chonka, D.C., Wood, A.R., Locke, A.E., Magi, R., Ferreira, T., Fall, T., Graff, M., Justice, A.E. *et al.* (2014) Quality control and conduct of genome-wide association meta-analyses. *Nat. Protoc.*, **9**, 1192–1212.
99. Sung, Y.J., Winkler, T.W., Manning, A.K., Aschard, H., Gudnason, V., Harris, T.B., Smith, A.V., Boerwinkle, E., Brown, M.R., Morrison, A.C. *et al.* (2016) An empirical comparison of joint and stratified frameworks for studying G × E interactions: systolic blood pressure and smoking in the CHARGE gene–lifestyle interactions working group. *Genet. Epidemiol.*, **40**, 404–415.
100. Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*, **26**, 2190–2191.
101. Randall, J.C., Winkler, T.W., Kutalik, Z., Berndt, S.I., Jackson, A.U., Monda, K.L., Kilpelainen, T.O., Esko, T., Magi, R., Li, S. *et al.* (2013) Sex-stratified genome-wide association studies including 270 000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet.*, **9**, e1003500.
102. Aschard, H., Hancock, D.B., London, S.J. and Kraft, P. (2010) Genome-wide meta-analysis of joint tests for genetic and gene–environment interaction effects. *Hum. Hered.*, **70**, 292–300.
103. Winkler, T.W., Kutalik, Z., Gorski, M., Lottaz, C., Kronenberg, F. and Heid, I.M. (2015) EasyStrata: evaluation and visualization of stratified genome-wide association meta-analysis data. *Bioinformatics*, **31**, 259–261.
104. Devlin, B. and Roeder, K. (1999) Genomic control for association studies. *Biometrics*, **55**, 997–1004.
105. Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.*, **57**, 289–300.
106. Genomes Project, C., Abecasis, G.R., Altshuler, D., Auton, A., Brooks, L.D., Durbin, R.M., Gibbs, R.A., Hurles, M.E. and McVean, G.A. (2010) A map of human genome variation from population-scale sequencing. *Nature*, **467**, 1061–1073.
107. Pers, T.H., Karjalainen, J.M., Chan, Y., Westra, H.-J., Wood, A.R., Yang, J., Lui, J.C., Vedantam, S., Gustafsson, S., Esko, T. *et al.* (2015) Biological interpretation of genome-wide association studies using predicted gene functions. *Nat. Commun.*, **6**, 5890–5890.