Investigating the causal relationship of C-reactive protein with 32 complex somatic and psychiatric outcomes: A large scale cross-consortia Mendelian randomization study.

Supplementary Methods - 6: Consortia co-authors and collaborators

i. Schizophrenia Working Group of the Psychiatric Genomics Consortium

As published in: Nature; Article Type: Biology article DOI: 10.1038/nature13595

Authors:

Stephan Ripke[1,2], Benjamin M. Neale[1,2,3,4], Aiden Corvin[5], James T. R. Walters[6], Kai-How Farh[1], Peter A. Holmans[6,7], Phil Lee[1,2,4], Brendan Bulik-Sullivan[1,2], David A. Collier[8,9], Hailiang Huang[1,3], Tune H. Pers[3,10,11], Ingrid Agartz[12,13,14], Esben Agerbo[15,16,17], Margot Albus[18], Madeline Alexander[19], Farooq Amin[20,21], Silviu A. Bacanu[22], Martin Begemann[23], Richard A Belliveau Jr[2], Judit Bene[24,25], Sarah E. Bergen [2,26], Elizabeth Bevilacqua[2], Tim B Bigdeli [22], Donald W. Black[27], Richard Bruggeman[28], Nancy G. Buccola[29], Randy L. Buckner[30,31,32], William Byerley[33], Wiepke Cahn[34], Guiqing Cai[35,36], Murray J. Cairns[39,120,170], Dominique Campion[37], Rita M. Cantor[38], Vaughan J. Carr[39,40], Noa Carrera[6], Stanley V. Catts[39,41], Kimberly D. Chambert[2], Raymond C. K. Chan[42], Ronald Y. L. Chen[43], Eric Y. H. Chen[43,44], Wei Cheng[45], Eric F. C. Cheung[46], Siow Ann Chong[47], C. Robert Cloninger[48], David Cohen[49], Nadine Cohen[50], Paul Cormican[5], Nick Craddock[6,7], Benedicto Crespo-Facorro[210], James J. Crowley[51], David Curtis[52,53], Michael Davidson[54], Kenneth L. Davis[36], Franziska Degenhardt[55,56], Jurgen Del Favero[57], Lynn E. DeLisi[128,129] , Ditte Demontis[17,58,59], Dimitris Dikeos[60], Timothy Dinan[61], Srdjan Djurovic[14,62], Gary Donohoe[5,63], Elodie Drapeau[36], Jubao Duan[64,65], Frank Dudbridge[66], Naser Durmishi[67], Peter Eichhammer[68], Johan Eriksson[69,70,71], Valentina Escott-Price[6], Laurent Essioux[72], Ayman H. Fanous[73,74,75,76], Martilias S. Farrell[51], Josef Frank[77], Lude Franke[78], Robert Freedman[79], Nelson B. Freimer[80], Marion Friedl[81], Joseph I. Friedman[36], Menachem Fromer[1,2,4,82], Giulio Genovese[2], Lyudmila Georgieva[6], Elliot S. Gershon[209], Ina Giegling[81,83], Paola Giusti-Rodríguez[51], Stephanie Godard[84], Jacqueline I. Goldstein[1,3], Vera Golimbet[85], Srihari Gopal[86], Jacob Gratten[87], Lieuwe de Haan[88], Christian Hammer[23], Marian L. Hamshere[6], Mark Hansen[89], Thomas Hansen[17,90], Vahram Haroutunian[36,91,92], Annette M. Hartmann[81], Frans A. Henskens[39,93,94], Stefan Herms[55,56,95], Joel N. Hirschhorn[3,11,96], Per Hoffmann[55,56,95], Andrea Hofman[55,56], Mads V. Hollegaard[97], David M. Hougaard[97], Masashi Ikeda[98], Inge Joa[99], Antonio Julià[100], René S. Kahn[34], Luba Kalaydjieva[101,102], Sena Karachanak-Yankova[103], Juha Karjalainen[78], David Kavanagh[6], Matthew C. Keller[104], Brian J. Kelly[120], James L. Kennedy[105,106,107], Andrey Khrunin[108], Yunjung Kim[51], Janis Klovins[109], James A. Knowles[110], Bettina Konte[81], Vaidutis Kucinskas[111], Zita Ausrele Kucinskiene[111], Hana Kuzelova-Ptackova[112], Anna K. Kähler[26],

Claudine Laurent[19,113], Jimmy Lee Chee Keong[47,114], S. Hong Lee[87], Sophie E. Legge[6], Bernard Lerer[115], Miaoxin Li[43,44,116] Tao Li[117], Kung-Yee Liang[118], Jeffrey Lieberman[119], Svetlana Limborska[108], Carmel M. Loughland[39,120], Jan Lubinski[121], Jouko Lönnqvist[122], Milan Macek Jr[112], Patrik K. E. Magnusson[26], Brion S. Maher[123], Wolfgang Maier[124], Jacques Mallet[125], Sara Marsal[100], Manuel Mattheisen[17,58,59,126], Morten Mattingsdal[14,127], Robert W. McCarley[128,129], Colm McDonald[130], Andrew M. McIntosh[131,132], Sandra Meier[77], Carin J. Meijer[88], Bela Melegh[24,25], Ingrid Melle[14,133], Raquelle I. Mesholam-Gately[128,134], Andres Metspalu[135], Patricia T. Michie[39,136], Lili Milani[135], Vihra Milanova[137], Younes Mokrab[8], Derek W. Morris[5,63], Ole Mors[17,58,138], Kieran C. Murphy[139], Robin M. Murray[140], Inez Myin-Germeys[141], Bertram Müller-Myhsok[142,143,144], Mari Nelis[135], Igor Nenadic[145], Deborah A. Nertney[146], Gerald Nestadt[147], Kristin K. Nicodemus[148], Liene Nikitina-Zake[109], Laura Nisenbaum[149], Annelie Nordin[150], Eadbhard O'Callaghan[151], Colm O'Dushlaine[2], F. Anthony O'Neill[152], Sang-Yun Oh[153], Ann Olincy[79], Line Olsen[17,90], Jim Van Os[141,154], Psychosis Endophenotypes International Consortium[155], Christos Pantelis[39,156], George N. Papadimitriou[60], Sergi Papiol[23], Elena Parkhomenko[36], Michele T. Pato[110], Tiina Paunio[157,158], Milica Pejovic-Milovancevic[159], Diana O. Perkins[160], Olli Pietiläinen[158,161], Jonathan Pimm[53], Andrew J. Pocklington[6], John Powell[140], Alkes Price[3,162], Ann E. Pulver[147], Shaun M. Purcell[82], Digby Quested[163], Henrik B. Rasmussen[17,90], Abraham Reichenberg[36], Mark A. Reimers[164], Alexander L. Richards[6], Joshua L. Roffman[30,32], Panos Roussos[82,165], Douglas M. Ruderfer[6,82], Veikko Salomaa[71], Alan R. Sanders[64,65], Ulrich Schall[39,120], Christian R. Schubert[166], Thomas G. Schulze[77,167], Sibylle G. Schwab[168], Edward M. Scolnick[2], Rodney J. Scott[39,169,170], Larry J. Seidman[128,134], Jianxin Shi[171], Engilbert Sigurdsson[172], Teimuraz Silagadze[173], Jeremy M. Silverman[36,174], Kang Sim[47], Petr Slominsky[108], Jordan W. Smoller[2,4], Hon-Cheong So[43], Chris C. A. Spencer[175], Eli A. Stahl[3,82], Hreinn Stefansson[176], Stacy Steinberg[176], Elisabeth Stogmann[177], Richard E. Straub[178], Eric Strengman[179,34], Jana Strohmaier[77], T. Scott Stroup[119], Mythily Subramaniam[47], Jaana Suvisaari[122], Dragan M. Svrakic[48], Jin P. Szatkiewicz[51], Erik Söderman[12], Srinivas Thirumalai[180], Draga Toncheva[103], Paul A. Tooney[39,120,170] , Sarah Tosato[181], Juha Veijola[182,183], John Waddington[184], Dermot Walsh[185], Dai Wang[86], Qiang Wang[117], Bradley T. Webb[22], Mark Weiser[54], Dieter B. Wildenauer[186], Nigel M. Williams[6], Stephanie Williams[51], Stephanie H. Witt[77], Aaron R. Wolen[164], Emily H. M. Wong[43], Brandon K. Wormley[22], Jing Qin Wu[39,170], Hualin Simon Xi[187], Clement C. Zai[105,106], Xuebin Zheng[188], Fritz Zimprich[177], Naomi R. Wray[87], Kari Stefansson[176], Peter M. Visscher[87], Wellcome Trust Case-Control Consortium 2[189], Rolf Adolfsson[150], Ole A. Andreassen[14,133], Douglas H. R. Blackwood[132], Elvira Bramon[190], Joseph D. Buxbaum[35,36,91,191], Anders D. Børglum[17,58,59,138], Sven Cichon[55,56,95,192], Ariel Darvasi[193], Enrico Domenici[194], Hannelore Ehrenreich[23], Tõnu Esko[3,11,96,135], Pablo V. Gejman[64,65], Michael Gill[5], Hugh Gurling[53], Christina M. Hultman[26], Nakao Iwata[98], Assen V. Jablensky[39,102,186,195], Erik G. Jönsson[12,14], Kenneth S. Kendler[196], George Kirov[6], Jo Knight[105,106,107], Todd Lencz[197,198,199], Douglas F. Levinson[19], Qingqin S. Li[86], Jianjun Liu[188,200], Anil K. Malhotra[197,198,199], Steven A. McCarroll[2,96], Andrew McQuillin[53], Jennifer L. Moran[2], Preben B. Mortensen[15,16,17], Bryan J. Mowry[87,201], Markus M. Nöthen[55,56], Roel A. Ophoff[38,80,34], Michael J. Owen[6,7], Aarno Palotie[2,4,161], Carlos N. Pato[110], Tracey L. Petryshen[2,128,202], Danielle Posthuma[203,204,205], Marcella Rietschel[77], Brien P. Riley[196], Dan Rujescu[81,83], Pak C. Sham[43,44,116] Pamela Sklar[82,91,165], David St Clair[206], Daniel R. Weinberger[178,207], Jens R. Wendland[166], Thomas Werge[17,90,208], Mark J. Daly[1,2,3], Patrick F. Sullivan[26,51,160] & Michael C. O'Donovan[6,7]

Affiliations

[1]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [2]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA. [3]Medical and Population Genetics Program, Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA. [4]Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [5]Neuropsychiatric Genetics Research Group, Department of Psychiatry, Trinity College Dublin, Dublin 8, Ireland. [6]MRC Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, School of Medicine, Cardiff University, Cardiff, CF24 4HQ, UK. [7]National Centre for Mental Health, Cardiff University, Cardiff, CF24 4HQ, UK. [8]Eli Lilly and Company Limited, Erl Wood Manor, Sunninghill Road, Windlesham, Surrey, GU20 6PH, UK. [9]Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, SE5 8AF, UK. [10]Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, DK-2800, Denmark. [11]Division of Endocrinology and Center for Basic and Translational Obesity Research, Boston Children's Hospital, Boston, Massachusetts, 02115USA. [12]Department of Clinical Neuroscience, Psychiatry Section, Karolinska Institutet, SE-17176 Stockholm, Sweden. [13]Department of Psychiatry, Diakonhjemmet Hospital, 0319 Oslo, Norway. [14]NORMENT, KG Jebsen Centre for Psychosis Research, Institute of Clinical Medicine, University of Oslo, 0424 Oslo, Norway. [15]Centre for Integrative Register-based Research, CIRRAU, Aarhus University, DK-8210 Aarhus, Denmark. [16]National Centre for Register-based Research, Aarhus University, DK-8210 Aarhus, Denmark. [17]The Lundbeck Foundation Initiative for Integrative Psychiatric Research, iPSYCH, Denmark. [18]State Mental Hospital, 85540 Haar, Germany. [19]Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305, USA. [20]Department of Psychiatry and Behavioral Sciences, Atlanta Veterans Affairs Medical Center, Atlanta, Georgia 30033, USA. [21]Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta Georgia 30322, USA. [22]Virginia Institute for Psychiatric and Behavioral Genetics, Department of Psychiatry, Virginia Commonwealth University, Richmond, Virginia 23298, USA. [23]Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Göttingen 37075, Germany. [24]Department of Medical Genetics, University of Pécs, Pécs H-7624, Hungary. [25]Szentagothai Research Center, University of Pécs, Pécs H-7624, Hungary.
[26]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm SE-17177, Sweden.
[27]Department of Psychiatry, University of Iowa Carver College of Medicine, Iowa City, Iowa 52242, USA. [28]University Medical Center Groningen, Department of Psychiatry, University of Groningen NL-9700 RB, The Netherlands. [29]School of Nursing, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, USA. [30]Athinoula A. Martinos Center, Massachusetts General Hospital, Boston, Massachusetts 02129, USA. [31]Center for Brain Science, Harvard University, Cambridge, Massachusetts, 02138 USA. [32]Department of Psychiatry, Massachusetts General Hospital, Boston, Massachusetts, 02114 USA. [33]Department of Psychiatry, University of California at San Francisco, San Francisco, California, 94143 USA. [34]University Medical Center Utrecht, Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, 3584 Utrecht, The Netherlands. [35]Department of Human Genetics, Icahn School of Medicine at Mount Sinai, New York, New York 10029 USA. [36]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, New York 10029 USA. [37]Centre Hospitalier du Rouvray and INSERM U1079 Faculty of Medicine, 76301 Rouen, France. [38]Department of Human

Genetics, David Geffen School of Medicine, University of California, Los Angeles, California 90095, USA. [39]Schizophrenia Research Institute, Sydney NSW 2010, Australia. [40]School of Psychiatry, University of New South Wales, Sydney NSW 2031, Australia. [41]Royal Brisbane and Women's Hospital, University of Queensland, Brisbane, St Lucia QLD 4072, Australia. [42]Institute of Psychology, Chinese Academy of Science, Beijing 100101, China. [43]Department of Psychiatry, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China. [44]State Key Laboratory for Brain and Cognitive Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China. [45]Department of Computer Science, University of North Carolina, Chapel Hill, North Carolina 27514, USA. [46]Castle Peak Hospital, Hong Kong, China. [47]Institute of Mental Health, Singapore 539747, Singapore. [48]Department of Psychiatry, Washington University, St. Louis, Missouri 63110, USA. [49]Department of Child and Adolescent Psychiatry, Assistance Publique Hopitaux de Paris, Pierre and Marie Curie Faculty of Medicine and Institute for Intelligent Systems and Robotics, Paris, 75013, France. [50] Blue Note Biosciences, Princeton, New Jersey 08540, USA. [51]Department of Genetics, University of North Carolina, Chapel Hill, North Carolina 27599-7264, USA. [52]Department of Psychological Medicine, Queen Mary University of London, London E1 1BB, UK. [53]Molecular Psychiatry Laboratory, Division of Psychiatry, University College London, London WC1E 6JJ, UK. [54]Sheba Medical Center, Tel Hashomer 52621, Israel. [55]Department of Genomics, Life and Brain Center, D-53127 Bonn, Germany. [56]Institute of Human Genetics, University of Bonn, D-53127 Bonn, Germany. [57]Applied Molecular Genomics Unit, VIB Department of Molecular Genetics, University of Antwerp, B-2610 Antwerp, Belgium. [58]Centre for Integrative Sequencing, iSEQ, Aarhus University, DK-8000 Aarhus C, Denmark. [59]Department of Biomedicine, Aarhus University, DK-8000 Aarhus C, Denmark. [60]First Department of Psychiatry, University of Athens Medical School, Athens 11528, Greece. [61]Department of Psychiatry, University College Cork, Co. Cork, Ireland. [62]Department of Medical Genetics, Oslo University Hospital, 0424 Oslo, Norway. [63]Cognitive Genetics and Therapy Group, School of Psychology and Discipline of Biochemistry, National University of Ireland Galway, Co. Galway, Ireland. [64]Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, Illinois 60637, USA. [65]Department of Psychiatry and Behavioral Sciences, NorthShore University HealthSystem, Evanston, Illinois 60201, USA. [66]Department of Non-Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK. [67]Department of Child and Adolescent Psychiatry, University Clinic of Psychiatry, Skopje 1000, Republic of Macedonia. [68]Department of Psychiatry, University of Regensburg, 93053 Regensburg, Germany. [69]Department of General Practice, Helsinki University Central Hospital, University of Helsinki P.O. Box 20, Tukholmankatu 8 B, FI-00014, Helsinki, Finland. [70]Folkhälsan Research Center, Helsinki, Finland, Biomedicum Helsinki 1, Haartmaninkatu 8, FI-00290, Helsinki, Finland. [71]National Institute for Health and Welfare, P.O. BOX 30, FI-00271 Helsinki, Finland. [72]Translational Technologies and Bioinformatics, Pharma Research and Early Development, F. Hoffman-La Roche, CH-4070 Basel, Switzerland. [73]Department of Psychiatry, Georgetown University School of Medicine, Washington DC 20057, USA. [74]Department of Psychiatry, Keck School of Medicine of the University of Southern California, Los Angeles, California 90033, USA. [75]Department of Psychiatry, Virginia Commonwealth University School of Medicine, Richmond, Virginia 23298, USA. [76]Mental Health Service Line, Washington VA Medical Center, Washington DC 20422, USA. [77]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Heidelberg , D-68159 Mannheim, Germany. [78]Department of Genetics,

University of Groningen, University Medical Centre Groningen, 9700 RB Groningen, The Netherlands. [79]Department of Psychiatry, University of Colorado Denver, Aurora, Colorado 80045, USA. [80]Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, California 90095, USA. [81]Department of Psychiatry, University of Halle, 06112 Halle, Germany. [82]Division of Psychiatric Genomics, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. [83]Department of Psychiatry, University of Munich, 80336, Munich, Germany. [84]Departments of Psychiatry and Human and Molecular Genetics, INSERM, Institut de Myologie, Hôpital de la Pitiè-Salpêtrière, Paris, 75013, France. [85]Mental Health Research Centre, Russian Academy of Medical Sciences, 115522 Moscow, Russia. [86]Neuroscience Therapeutic Area, Janssen Research and Development, Raritan, New Jersey 08869, USA. [87]Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, QLD 4072, Australia. [88]Academic Medical Centre University of Amsterdam, Department of Psychiatry, 1105 AZ Amsterdam, The Netherlands. [89]Illumina, La Jolla, California, California 92122, USA. [90]Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Mental Health Services Copenhagen, DK-4000, Denmark. [91]Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. [92]J. J. Peters VA Medical Center, Bronx, New York, New York 10468, USA. [93]Priority Research Centre for Health Behaviour, University of Newcastle, Newcastle NSW 2308, Australia. [94]School of Electrical Engineering and Computer Science, University of Newcastle, Newcastle NSW 2308, Australia. [95]Division of Medical Genetics, Department of Biomedicine, University of Basel, Basel, CH-4058, Switzerland. [96]Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA. [97]Section of Neonatal Screening and Hormones, Department of Clinical Biochemistry, Immunology and Genetics, Statens Serum Institut, Copenhagen, DK-2300, Denmark. [98]Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, 470-1192, Japan. [99]Regional Centre for Clinical Research in Psychosis, Department of Psychiatry, Stavanger University Hospital, 4011 Stavanger, Norway. [100]Rheumatology Research Group, Vall d'Hebron Research Institute, Barcelona, 08035, Spain. [101]Centre for Medical Research, The University of Western Australia, Perth, WA 6009, Australia. [102]The Perkins Institute for Medical Research, The University of Western Australia, Perth, WA 6009, Australia. [103]Department of Medical Genetics, Medical University, Sofia1431, Bulgaria. [104]Department of Psychology, University of Colorado Boulder, Boulder, Colorado 80309, USA. [105]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Ontario, M5T 1R8, Canada. [106]Department of Psychiatry, University of Toronto, Toronto, Ontario, M5T 1R8, Canada. [107]Institute of Medical Science, University of Toronto, Toronto, Ontario, M5S 1A8, Canada. [108]Institute of Molecular Genetics, Russian Academy of Sciences, Moscow123182, Russia. [109]Latvian Biomedical Research and Study Centre, Riga, LV-1067, Latvia. [110]Department of Psychiatry and Zilkha Neurogenetics Institute, Keck School of Medicine at University of Southern California, Los Angeles, California 90089, USA. [111]Faculty of Medicine, Vilnius University, LT-01513 Vilnius, Lithuania. [112] Department of Biology and Medical Genetics, 2nd Faculty of Medicine and University Hospital Motol, 150 06 Prague, Czech Republic. [113] Department of Child and Adolescent Psychiatry, Pierre and Marie Curie Faculty of Medicine, Paris 75013, France. [114]Duke-NUS Graduate Medical School, Singapore 169857, Singapore. [115]Department of Psychiatry, Hadassah-Hebrew University Medical Center, Jerusalem 91120, Israel. [116]Centre for Genomic Sciences, The University of Hong Kong, Hong Kong, China. [117]Mental Health Centre and Psychiatric Laboratory, West

China Hospital, Sichuan University, Chengdu, 610041, Sichuan, China. [118]Department of Biostatistics, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland 21205, USA. [119]Department of Psychiatry, Columbia University, New York, New York 10032, USA. [120]Priority Centre for Translational Neuroscience and Mental Health, University of Newcastle, Newcastle NSW 2300, Australia. [121]Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University in Szczecin, 70-453 Szczecin, Poland. [122]Department of Mental Health and Substance Abuse Services; National Institute for Health and Welfare, P.O. BOX 30, FI-00271 Helsinki, Finland. [123]Department of Mental Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205, USA. [124]Department of Psychiatry, University of Bonn, D-53127 Bonn, Germany. [125]Centre National de la Recherche Scientifique, Laboratoire de Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératifs, Hôpital de la Pitié Salpêtrière, 75013, Paris, France. [126]Department of Genomics Mathematics, University of Bonn, D-53127 Bonn, Germany. [127]Research Unit, Sørlandet Hospital, 4604 Kristiansand, Norway. [128]Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02115, USA. [129]VA Boston Health Care System, Brockton, Massachusetts 02301, USA. [130]Department of Psychiatry, National University of Ireland Galway, Co. Galway, Ireland. [131]Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh EH16 4SB, UK. [132]Division of Psychiatry, University of Edinburgh, Edinburgh EH16 4SB, UK. [133]Division of Mental Health and Addiction, Oslo University Hospital, 0424 Oslo, Norway. [134]Massachusetts Mental Health Center Public Psychiatry Division of the Beth Israel Deaconess Medical Center, Boston, Massachusetts 02114, USA. [135]Estonian Genome Center, University of Tartu, Tartu 50090, Estonia. [136]School of Psychology, University of Newcastle, Newcastle NSW 2308, Australia. [137]First Psychiatric Clinic, Medical University, Sofia 1431, Bulgaria. [138]Department P, Aarhus University Hospital, DK-8240 Risskov, Denmark. [139]Department of Psychiatry, Royal College of Surgeons in Ireland, Dublin 2, Ireland. [140]King's College London, London SE5 8AF, UK. [141]Maastricht University Medical Centre, South Limburg Mental Health Research and Teaching Network, EURON, 6229 HX Maastricht, The Netherlands. [142]Institute of Translational Medicine, University of Liverpool, Liverpool L69 3BX, UK. [143]Max Planck Institute of Psychiatry, 80336 Munich, Germany. [144]Munich Cluster for Systems Neurology (SyNergy), 80336 Munich, Germany. [145]Department of Psychiatry and Psychotherapy, Jena University Hospital, 07743 Jena, Germany. [146]Department of Psychiatry, Queensland Brain Institute and Queensland Centre for Mental Health Research, University of Queensland, Brisbane, Queensland, St Lucia QLD 4072, Australia. [147]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA. [148]Department of Psychiatry, Trinity College Dublin, Dublin 2, Ireland. [149]Eli Lilly and Company, Lilly Corporate Center, Indianapolis, 46285 Indiana, USA. [150]Department of Clinical Sciences, Psychiatry, Umeå University, SE-901 87 Umeå, Sweden. [151]DETECT Early Intervention Service for Psychosis, Blackrock, Co. Dublin, Ireland. [152]Centre for Public Health, Institute of Clinical Sciences, Queen's University Belfast, Belfast BT12 6AB, UK. [153]Lawrence Berkeley National Laboratory, University of California at Berkeley, Berkeley, California 94720, USA. [154]Institute of Psychiatry, King's College London, London SE5 8AF, UK. [155]A list of authors and affiliations appear in the Supplementary Information. [156]Melbourne Neuropsychiatry Centre, University of Melbourne & Melbourne Health, Melbourne, Vic 3053, Australia. [157]Department of Psychiatry, University of Helsinki, P.O. Box 590, FI-00029 HUS, Helsinki, Finland. [158]Public Health Genomics Unit, National Institute for

Health and Welfare, P.O. BOX 30, FI-00271 Helsinki, Finland. [159]Medical Faculty, University of Belgrade, 11000 Belgrade, Serbia. [160]Department of Psychiatry, University of North Carolina, Chapel Hill, North Carolina 27599-7160, USA. [161]Institute for Molecular Medicine Finland, FIMM, University of Helsinki, P.O. Box 20 FI-00014, Helsinki, Finland. [162]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts 02115, USA. [163]Department of Psychiatry, University of Oxford, Oxford, OX3 7JX, UK. [164]Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, Virginia 23298, USA. [165]Institute for Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. [166]PharmaTherapeutics Clinical Research, Pfizer Worldwide Research and Development, Cambridge, Massachusetts 02139, USA. [167]Department of Psychiatry and Psychotherapy, University of Gottingen, 37073 Göttingen, Germany. [168]Psychiatry and Psychotherapy Clinic, University of Erlangen, 91054 Erlangen, Germany. [169]Hunter New England Health Service, Newcastle NSW 2308, Australia. [170]School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan NSW 2308, Australia. [171]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland 20892, USA. [172]University of Iceland, Landspitali, National University Hospital, 101 Reykjavik, Iceland. [173]Department of Psychiatry and Drug Addiction, Tbilisi State Medical University (TSMU), **N33, 0177** Tbilisi, Georgia. [174]Research and Development, Bronx Veterans Affairs Medical Center, New York, New York 10468, USA. [175]Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, UK. [176]deCODE Genetics, 101 Reykjavik, Iceland. [177]Department of Clinical Neurology, Medical University of Vienna, 1090 Wien, Austria. [178]Lieber Institute for Brain Development, Baltimore, Maryland 21205, USA. [179]Department of Medical Genetics, University Medical Centre Utrecht, Universiteitsweg 100, 3584 CG, Utrecht, The Netherlands. [180]Berkshire Healthcare NHS Foundation Trust, Bracknell RG12 1BQ, UK. [181]Section of Psychiatry, University of Verona, 37134 Verona, Italy. [182]Department of Psychiatry, University of Oulu, P.O. BOX 5000, 90014, Finland. [183]University Hospital of Oulu, P.O.BOX 20, 90029 OYS, Finland. [184]Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, Ireland. [185]Health Research Board, Dublin 2, Ireland. [186]School of Psychiatry and Clinical Neurosciences, The University of Western Australia, Perth WA6009, Australia. [187]Computational Sciences CoE, Pfizer Worldwide Research and Development, Cambridge, Massachusetts 02139, USA. [188]Human Genetics, Genome Institute of Singapore, A*STAR, Singapore 138672, Singapore. [189]A list of authors and affiliations appear in the Supplementary Information. [190]University College London, London WC1E 6BT, UK. [191]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. [192]Institute of Neuroscience and Medicine (INM-1), Research Center Juelich, 52428 Juelich, Germany. [193]Department of Genetics, The Hebrew University of Jerusalem, 91905 Jerusalem, Israel. [194]Neuroscience Discovery and Translational Area, Pharma Research and Early Development, F. Hoffman-La Roche, CH-4070 Basel, Switzerland. [195]Centre for Clinical Research in Neuropsychiatry, School of Psychiatry and Clinical Neurosciences, The University of Western Australia, Medical Research Foundation Building, Perth WA 6000, Australia. [196]Virginia Institute for Psychiatric and Behavioral Genetics, Departments of Psychiatry and Human and Molecular Genetics, Virginia Commonwealth University, Richmond, Virginia 23298, USA. [197]The Feinstein Institute for Medical Research, Manhasset, New York, 11030 USA. [198]The Hofstra NS-LIJ School of Medicine, Hempstead, New York, 11549 USA. [199]The Zucker Hillside Hospital, Glen Oaks, New York,11004 USA. [200]Saw Swee Hock School of Public Health, National University of Singapore, Singapore 117597, Singapore.

[201]Queensland Centre for Mental Health Research, University of Queensland, Brisbane 4076, Queensland, Australia. [202]Center for Human Genetic Research and Department of Psychiatry, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [203]Department of Child and Adolescent Psychiatry, Erasmus University Medical Centre, Rotterdam 3000, The Netherlands. [204]Department of Complex Trait Genetics, Neuroscience Campus Amsterdam, VU University Medical Center Amsterdam, Amsterdam 1081, The Netherlands. [205]Department of Functional Genomics, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam 1081, The Netherlands. [206]University of Aberdeen, Institute of Medical Sciences, Aberdeen, AB25 2ZD, UK. [207]Departments of Psychiatry, Neurology, Neuroscience and Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA. [208]Department of Clinical Medicine, University of Copenhagen, Copenhagen 2200, Denmark. [209]Departments of Psychiatry and Human Genetics, University of Chicago, Chicago, Illinois 60637, USA. [210]University Hospital Marqués de Valdecilla, Instituto de Formación e Investigación Marqués de Valdecilla, University of Cantabria, E-39008 Santander, Spain.

ii. The Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium [,‡]

Please address correspondence to: **pgc.autism@gmail.com**

Authors:
Richard J. L. Anney[1,2,*], Stephan Ripke[3,4,*], Verneri Anttila[3,4], Peter Holmans[2], Hailiang Huang[3,4], Lambertus Klei[5], Phil H. Lee[3,4,6], Sarah E. Medland[7], Benjamin Neale[3,4], Elise Robinson[3,4], Lauren A. Weiss[8,9], Joana Almeida[10], David Amaral[11,12,13], Evdokia Anagnostou[14], Elena Bacchelli[15], Joel S. Bader[16], Anthony J. Bailey[17,18], Gillian Baird[19], Vanessa H. Bal[8], Agatino Battaglia[20], Arthur L. Beaudet[21], Raphael Bernier[22], Catalina Betancur[23,24,25], Nadia Bolshakova[1], Sven Bölte[26,27,28], Patrick F. Bolton[29,30], Thomas Bourgeron[31,32,33,34], Sean Brennan[1], Cátia Café[10], Rita M. Cantor[35,36], Jillian Casey[37,38], Patrícia B. S. Celestino-Soper[21,39], Andreas G. Chiocchetti[26], Ines C. Conceição[40,41], Judith Conroy[37,38], Catarina T. Correia[40,41], Michael L. Cuccaro[42], Geraldine Dawson[43,44], Maretha V. De Jonge[45], Silvia De Rubeis[46,47], Richard Delorme[31,32,33,48], Eftichia Duketis[26], Frederico Duque[10,49], Sean Ennis[38,50], A. Gulhan Ercan-Sencicek[51], M. Daniele Fallin[52], Bridget Fernandez[53], Susan E. Folstein[54], Eric Fombonne[55], Christine M. Freitag[26], Louise Gallagher[1], John Gilbert[42], Christopher Gillberg[56], Arthur P. Goldberg[46,47], Jonathan M. Green[57,58], Andrew Green[38,50], Dorothy E. Grice[47], Stephen J. Guter[59], Jonathan L. Haines[60], Robert Hendren[8], Irva Hertz-Picciotto[11,61], Christina M. Hultman[62], Bozenna Iliadou[62], Suma Jacob[59,63], Sabine M. Klauck[64], Alexander Kolevzon[46,47,65,66], Christine Ladd-Acosta[52], Ann S. Le Couteur[67], Marion Leboyer[31,68,69,70], David H. Ledbetter[71], Christa Lese Martin[72], Pat Levitt[73], Catherine Lord[74], Jennifer K. Lowe[75,76,77], Elena Maestrini[15], Tiago Magalhaes[38,78], Shrikant M. Mane[79], Donna M. Martin[80], Susan G. McGrew[81], William M. McMahon[82], Alison Merikangas[1], Nancy Minshew[5], Anthony P. Monaco[83,84], Daniel Moreno-De-Luca[85], Eric M. Morrow[86], Susana Mouga[10,49], Michael T. Murtha[51], John I. Nurnberger[39,87], Guiomar Oliveira[10,49], Alistair T. Pagnamenta[83], Jeremy R. Parr[67], Andrew D. Paterson[88,89,90], Margaret A. Pericak-Vance[42], Dalila Pinto[46,47,65,66,91,92], Joseph Piven[93], Christopher S. Poultney[46,47], Fritz Poustka[26], Regina Regan[38,78], Karola Rehnström[94], Abraham Reichenberg[46,47], Jennifer Reichert[46,47], Wendy Roberts[95], Kathryn Roeder[96,97], Bernadette Rogé[98], Guy A. Rouleau[99], Stephan J. Sanders[8], Sven Sandin[62], Gerard D. Schellenberg[100], Stephen W. Scherer[88,89,101], Latha Soorya[46,47,102], Matthew W. State[8], Oscar Svantesson[62], Peter Szatmari[103], Ann P. Thompson[104], Kathryn Tsang[8,9], Herman van Engeland[45], Astrid M. Vicente[40,41], Veronica J. Vieland[105], Jacob A. S. Vorstman[45], Simon Wallace[17], Christopher A. Walsh[106,107,108,109,110], Regina Waltes[26], Thomas H. Wassink[111], Ellen M. Wijsman[112,113], A. Jeremy Willsey[8], Kerstin Wittemeyer[114], Timothy W. Yu[106], Lonnie Zwaigenbaum[115], Joseph D. Buxbaum[46,47,65,66,91,116], Aravinda Chakravarti[16], Edwin H. Cook[59], Hilary Coon[82], Daniel H. Geschwind[36,75,76,77], Michael Gill[1], Hakon Hakonarson[117,118], Joachim Hallmayer[119], Aarno Palotie[94], Susan Santangelo[120], James S. Sutcliffe[60,121], Dan Arking[16,†], Bernie Devlin[5,†], and Mark J. Daly[3,4,†]

Affiliations:
[1]Dept. of Psychiatry, Trinity College Dublin, Dublin, Ireland. [2]MRC Centre for Neuropsychiatric Genetics & Genomics, Cardiff University, Cardiff, UK. [3]Analytic and Translational Genetics Unit, Dept. of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. [4]Stanley Center for Psychiatric Research and Program in Medical and Population Genetic, Broad Institute of Harvard and MIT, Cambridge, MA, USA. [5]Dept. of Psychiatry, University of Pittsburgh School of Medicine,

Pittsburgh, PA, USA. [6]Dept. of Psychiatry, Harvard Medical School, Boston, MA, USA. [7]Queensland Institute of Medical Research, Brisbane, QLD, Australia. [8]Dept. of Psychiatry, University of California San Francisco, San Francisco, CA, USA. [9]Inst. Human Genetics, University of California San Francisco, San Francisco, CA, USA. [10]Unidade de Neurodesenvolvimento e Autismo do Serviço do Centro de Desenvolvimento da Criança and Centro de Investigação e Formação Clinica, Pediatric Hospital, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal. [11]The MIND Institute, School of Medicine, University of California Davis, Davis, CA, USA. [12]Dept. of Psychiatry, School of Medicine, University of California Davis, Davis, CA, USA. [13]Dept. of Behavioural Sciences, School of Medicine, University of California Davis, Davis, CA,USA. [14]Bloorview Research Institute, University of Toronto, Toronto, ON, Canada. [15]Dept. of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy. [16]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA. [17]Dept. of Psychiatry, University of Oxford and Warneford Hospital, Oxford, UK. [18]Mental Health and Addictions Research Unit, University of British Colombia, Vancouver, BC, Canada. [19]Paediatric Neurodisability, King's Health Partners, Kings College London, London, UK. [20]Stella Maris Institute for Child and Adolescent Neuropsychiatr, Pisa, Italy. [21]Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA. [22]Dept. of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, USA. [23]INSERM U1130, Paris, France. [24]CNRS UMR 8246, Paris, France. [25]Sorbonne Universités, UPMC Univ Paris 6, Neuroscience Paris Seine, Paris, France. [26]Dept. of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, JW Goethe University Frankfurt, Frankfurt am Main, Germany. [27]Dept. of Women's and Children's Health, Center of Neurodevelopmental Disorders, Karolinska Institutet, Stockholm, Sweden. [28]Child and Adolescent Psychiatry, Center for Psychiatry Research, Stockholm County Council, Stockholm, Sweden. [29]Institute of Psychiatry, Kings College London, London, UK. [30]South London & Maudsley Biomedical Research Centre for Mental Health, London, UK. [31]FondaMental Foundation, Créteil, France. [32]Human Genetics and Cognitive Functions Unit, Institut Pasteur, Paris, France. [33]Centre National de la Recherche Scientifique URA 2182 Institut Pasteur, Paris, France. [34]University Paris Diderot, Sorbonne Paris Cité, Paris, France. [35]Dept. of Psychiatry, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA. [36]Dept. of Human Genetics, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA. [37]Temple Street Children's University Hospital, Dublin, Ireland. [38]Academic Centre on Rare Diseases, University College Dublin, Dublin, Ireland. [39]Dept. of Medical and Molecular Genetics and Program in Medical Neuroscience, Indiana University School of Medicine, Indianapolis, IN, USA. [40]Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal. [41]Center for Biodiversity, Functional and Integrative Genomics, Campus da FCUL, Lisbon, Portugal. [42]The John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL, USA. [43]Duke Center for Autism and Brain Developments, Duke University School of Medicine, Durham, NC, USA. [44]Duke Institute for Brain Sciences, Duke University School of Medicine, Durham, NC, USA. [45]Dept. of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands. [46]Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [47]Dept. of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [48]Dept. of Child and Adolescent Psychiatry, Robert Debré Hospital, Assistance Publique – Hôpitaux de Paris, Paris, France. [49]University Clinic of Pediatrics and Institute for Biomedical Imaging and Life Science, Faculty of Medicine, University of Coimbra, Coimbra, Portugal. [50]Centre for Medical Genetics, Our Lady's Hospital Crumlin,

Dublin, Ireland. [51]Programs on Neurogenetics, Yale University School of Medicine, New Haven, CT, USA. [52]Dept. of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. [53]Memorial University of Newfoundland, St. John's, NL, Canada. [54]Division of Child and Adolescent Psychiatry, Dept.t of Psychiatry, Miller School of Medicine, University of Miami, Miami, FL, USA. [55]Dept. of Psychiatry and Institute for Development and Disability, Oregon Health & Science University, Portland, OR, USA. [56]Gillberg Neuropsychiatry Centre, University of Gothenburg, Gothenburg, Sweden. [57]Manchester Academic Health Sciences Centre, Manchester, UK. [58]Institute of Brain, Behaviour, and Mental Health, University of Manchester, Manchester, UK. [59]Institute for Juvenile Research, Dept. of Psychiatry, University of Illinois at Chicago, Chicago, IL, USA. [60]Dept. of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN, USA. [61]Dept. of Public Health Sciences, School of Medicine, University of California Davis, Davis, CA, USA. [62]Karolinska Institutet, Solna, Sweden. [63]Institute of Translational Neuroscience and Dept. of Psychiatry, University of Minnesota, Minneapolis, MN, USA. [64]Division of Molecular Genome Analysis and Working Group Cancer Genome Research, Deutsches Krebsforschungszentrum, Heidelberg, Germany. [65]Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [66]The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [67]Institutes of Neuroscience and Health and Society, Newcastle University, Newcastle Upon Tyne, UK. [68]INSERM U955, Paris, France. [69]Faculté de Médecine, Université Paris Est, Créteil, France. [70]Dept. of Psychiatry, Henri Mondor-Albert Chenevier Hospital, Assistance Publique – Hôpitaux de Paris, Créteil, France. [71]Chief Scientific Officer, Geisinger Health System, Danville, PA, USA. [72]Autism & Developmental Medicine Institute, Geisinger Health System, Danville, PA, USA. [73]Dept. of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, California, USA. [74]Dept. of Psychiatry, Weill Cornell Medical College, Cornell University, New York, NY, USA. [75]Center for Autism Research and Treatment, Semel Institute, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA. [76] Program in Neurogenetics, Dept. of Neurology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [77]Center for Neurobehavioral Genetics, Semel Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [78]National Childrens Research Centre, Our Lady's Hospital Crumlin, Dublin, Ireland. [79]Yale Center for Genomic Analysis, Yale University School of Medicine, New Haven, CT, USA. [80]Dept. of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI, USA. [81]Dept. of Pediatrics, Vanderbilt University, Nashville, TN, USA. [82]Dept. of Psychiatry, University of Utah, Salt Lake City, UT, USA. [83]Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK. [84]Tufts University, Boston, MA, USA. [85]Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT, USA. [86]Dept. of Psychiatry and Human Behaviour, Brown University, Providence, RI, USA. [87]Institute of Psychiatric Research, Dept. of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA. [88]Dept. of Molecular Genetics, University of Toronto, Toronto, ON, Canada. [89]The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada. [90]Dalla Lana School of Public Health, Toronto, ON, Canada. [91]Dept. of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [92]The Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [93]University of North Carolina, Chapel Hill, NC, USA. [94]Sanger Institute, Hinxton, UK. [95]Autism Research Unit, The Hospital for Sick Children, Toronto, ON, Canada. [96]Dept. of Computational Biology, Carnegie Mellon University, Pittsburgh, PA, USA. [97]Dept. of Statistics, Carnegie Mellon University,

Pittsburgh, PA, USA. [98]Centre d'Etudes et de Recherches en Psychopathologie, Toulouse University, Toulouse, France. [99]Montreal Neurological Institute, Dept of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada. [100]Dept. of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA. [101]McLaughlin Centre, University of Toronto, Toronto, ON, Canada. [102]Dept. of Psychiatry, Rush University Medical Center, Chicago, IL, USA. [103]Dept. of Psychiatry, University of Toronto, ON, Canada. [104]Dept. of Psychiatry and Behavioral Neurosciences, McMasters University, Hamilton, ON, Canada. [105]Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, Columbus, OH, USA. [106]Division of Genetics, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA. [107]Program in Genetics and Genomics, Harvard Medical School, Boston, MA, USA. [108]Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, USA. [109]Dept. of Pediatrics, Harvard Medical School, Boston, MA, USA. [110]Dept. of Neurology, Harvard Medical School, Boston, MA, USA. [111]Dept. of Psychiatry, Carver College of Medicine, Iowa City, IA, USA. [112]Dept. of Medicine, University of Washington, Seattle, WA, USA. [113]Dept. of Biostatistics, University of Washington, Seattle, WA, USA. [114]School of Education, University of Birmingham, Birmingham, UK. [115]Dept. of Pediatrics, University of Alberta, Edmonton, AB, Canada. [116]Dept. of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [117]The Center for Applied Genomics and Division of Human Genetics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. [118]Dept of Pediatrics, University of Pennsylvania, Philadelphia, PA.USA. [119]Dept. of Psychiatry, Stanford University, Stanford, CA, USA. [120]Maine Medical Center Research Institute, Portland, ME, USA. [121]Center for Human Genetics Research, Vanderbilt University, Nashville, TN, USA.
[*]These authors contributed equally to this work.
[†]These authors contributed equally to this work.

iii. The Genetic and Environmental Risk for Alzheimer's disease (GERAD1) Consortium

*Data used in the preparation of this article were obtained from the Genetic and Environmental Risk for Alzheimer's disease (GERAD1) Consortium. As such, the investigators within the GERAD1 consortia contributed to the design and implementation of GERAD1 and/or provided data but did not participate in analysis or writing of this report.

Authors :
Denise Harold[1], Rebecca Sims[1], Amy Gerrish[1], Jade Chapman[1], Valentina Escott-Price[1], Richard Abraham[1], Paul Hollingworth[1], Marian Hamshere[1], Jaspreet Singh Pahwa[1], Kimberley Dowzell[1], Amy Williams[1], Nicola Jones[1], Charlene Thomas[1], Alexandra Stretton[1], Angharad Morgan[1], Kate Williams[1], Sarah Taylor[1],Simon Lovestone[2], John Powell[2], Petroula Proitsi[2], Michelle K Lupton[2], Carol Brayne[3], David C. Rubinsztein[4], Michael Gill[5], Brian Lawlor[5], Aoibhinn Lynch[5], Kevin Morgan[6], Kristelle Brown[6], Peter Passmore[7], David Craig[7], Bernadette McGuinness[7], Janet A Johnston[7], Stephen Todd[7], Clive Holmes[8], David Mann[9], A. David Smith[10], Seth Love[11], Patrick G. Kehoe[11], John Hardy[12], Rita Guerreiro[13,33], Andrew Singleton[13], Simon Mead[14], Nick Fox[15], Martin Rossor[15], John Collinge[14], Wolfgang Maier[16], Frank Jessen[16], Reiner Heun[16], Britta Schürmann[16,17], Alfredo Ramirez[16], Tim Becker[34], Christine Herold[34], André Lacour[34], Dmitriy Drichel[34], Hendrik van den Bussche[18], Isabella Heuser[19], Johannes Kornhuber[20], Jens Wiltfang[21], Martin Dichgans[22,23], Lutz Frölich[24], Harald Hampel[25], Michael Hüll[26], Dan Rujescu[27], Alison Goate[28], John S.K. Kauwe[29], Carlos Cruchaga[28], Petra Nowotny[28], John C. Morris[28], Kevin Mayo[28], Gill Livingston[30], Nicholas J. Bass[30], Hugh Gurling[30], Andrew McQuillin[30], Rhian Gwilliam[31], Panagiotis Deloukas[31], Markus M. Nöthen[32], Peter Holmans[1], Michael ODonovan[1], Michael J.Owen[1], Julie Williams[1].

Affiliations
[1]Medical Research Council (MRC) Centre for Neuropsychiatric Genetics and Genomics, Neurosciences and Mental Health Research Institute, Department of Psychological Medicine and Neurology, School of Medicine, Cardiff University, Cardiff, UK. [2]Kings College London, Institute of Psychiatry, Department of Neuroscience, De Crespigny Park, Denmark Hill, London. [3]Institute of Public Health, University of Cambridge, Cambridge, UK. [4]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK. [5]Mercers Institute for Research on Aging, St. James Hospital and Trinity College, Dublin, Ireland. [6]Institute of Genetics, Queens Medical Centre, University of Nottingham, UK. [7]Ageing Group, Centre for Public Health, School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, UK. [8]Division of Clinical Neurosciences, School of Medicine, University of Southampton, Southampton, UK. [9]Clinical Neuroscience Research Group, Greater Manchester Neurosciences Centre, University of Manchester, Salford, UK. [10]Oxford Project to Investigate Memory and Ageing (OPTIMA), University of Oxford, Department of Pharmacology, Mansfield Road, Oxford, UK. [11]University of Bristol Institute of Clinical Neurosciences, School of Clinical Sciences, Frenchay Hospital, Bristol, UK. [12]Department of Molecular Neuroscience and Reta Lilla Weston Laboratories, Institute of Neurology, UCL, London, UK. [13]Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, United States of America. [14]MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK. [15]Dementia Research Centre, Department of Neurodegenerative Diseases, University College London, Institute of Neurology, London, UK. [16]Department

of Psychiatry, University of Bonn, Sigmund-Freud-Straβe 25, 53105 Bonn, Germany. [17]Institute for Molecular Psychiatry, University of Bonn, Bonn, Germany. [18]Institute of Primary Medical Care, University Medical Center Hamburg-Eppendorf, Germany. [19]Department of Psychiatry, Charité Berlin, Germany. [20]Department of Psychiatry, Friedrich-Alexander-University Erlangen-Nürnberg, Germany. [21]Department of Psychiatry and Psychotherapy, University Medical Center (UMG), Georg-August-University, Göttingen, Germany. [22]Institute for Stroke and Dementia Reserach, Klinikum der Universität München, Marchioninistr. 15, 81377, Munich, Germany. [23]Department of Neurology, Klinikum der Universität München, Marchioninistr. 15, 81377, Munich, Germany. [24]Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany. [25]Institute for Memory and Alzheimers Disease & INSERM, Sorbonne Universites, Pierre and Marie Curie University, Paris ; Institute for Brain and Spinal Cord Disorders (ICM), Department of Neurology, Hospital of Pitié-Salpétrière. [26]Centre for Geriatric Medicine and Section of Gerontopsychiatry and Neuropsychology, Medical School, University of Freiburg, Germany. [27]Department of Psychiatry, University of Halle, Halle, Germany. [28]Departments of Psychiatry, Neurology and Genetics, Washington University School of Medicine, St Louis, MO 63110, US. [29]Department of Biology, Brigham Young University, Provo, UT, 84602, USA. [30]Department of Mental Health Sciences, University College London, UK. [31]The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK. [32]Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany. [33]Department of Molecular Neuroscience, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK. [34]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Bonn.

iv. The International Consortium for Blood Pressure (ICBP)

Authors:
Georg B. Ehret1,2,3*, Patricia B. Munroe4*#, Kenneth M. Rice5*, Murielle Bochud2*, Andrew D. Johnson6,7*, Daniel I. Chasman8,9*, Albert V. Smith10,11*, Martin D. Tobin12, Germaine C. Verwoert13,14,15, Shih-Jen Hwang6,16,7, Vasyl Pihur1, Peter Vollenweider17, Paul F. O'Reilly18, Najaf Amin13, Jennifer L Bragg-Gresham19, Alexander Teumer20, Nicole L. Glazer21, Lenore Launer22, Jing Hua Zhao23, Yurii Aulchenko13, Simon Heath24, Siim Sõber25, Afshin Parsa26, Jian'an Luan23, Pankaj Arora27, Abbas Dehghan13,14,15, Feng Zhang28, Gavin Lucas29, Andrew A. Hicks30, Anne U. Jackson31, John F Peden32, Toshiko Tanaka33, Sarah H. Wild34, Igor Rudan35,36, Wilmar Igl37, Yuri Milaneschi33, Alex N. Parker38, Cristiano Fava39,40, John C. Chambers18,41, Ervin R. Fox42, Meena Kumari43, Min Jin Go44, Pim van der Harst45, Wen Hong Linda Kao46, Marketa Sjögren39, D. G. Vinay47, Myriam Alexander48, Yasuharu Tabara49, Sue Shaw-Hawkins4, Peter H. Whincup50, Yongmei Liu51, Gang Shi52, Johanna Kuusisto53, Bamidele Tayo54, Mark Seielstad55,56, Xueling Sim57, Khanh-Dung Hoang Nguyen1, Terho Lehtimäki58, Giuseppe Matullo59,60, Ying Wu61, Tom R. Gaunt62, N. Charlotte Onland-Moret63,64, Matthew N. Cooper65, Carl G.P. Platou66, Elin Org25, Rebecca Hardy67, Santosh Dahgam68, Jutta Palmen69, Veronique Vitart70, Peter S. Braund71,72, Tatiana Kuznetsova73, Cuno S.P.M. Uiterwaal63, Adebowale Adeyemo74, Walter Palmas75, Harry Campbell35, Barbara Ludwig76, Maciej Tomaszewski71,72, Ioanna Tzoulaki77,78, Nicholette D. Palmer79, CARDIoGRAM consortium80, CKDGen Consortium80, KidneyGen Consortium80, EchoGen consortium80, CHARGE-HF consortium80, Thor Aspelund10,11, Melissa Garcia22, Yen-Pei C. Chang26, Jeffrey R. O'Connell26, Nanette I. Steinle26, Diederick E. Grobbee63, Dan E. Arking1, Sharon L. Kardia81, Alanna C. Morrison82, Dena Hernandez83, Samer Najjar84,85, Wendy L. McArdle86, David Hadley50,87, Morris J. Brown88, John M. Connell89, Aroon D. Hingorani90, Ian N.M. Day62, Debbie A. Lawlor62, John P. Beilby91,92, Robert W. Lawrence65, Robert Clarke93, Rory Collins93, Jemma C Hopewell93, Halit Ongen32, Albert W. Dreisbach42, Yali Li94, J H. Young95, Joshua C. Bis21, Mika Kähönen96, Jorma Viikari97, Linda S. Adair98, Nanette R. Lee99, Ming-Huei Chen100, Matthias Olden101,102, Cristian Pattaro30, Judith A. Hoffman Bolton103, Anna Köttgen104,103, Sven Bergmann105,106, Vincent Mooser107, Nish Chaturvedi108, Timothy M. Frayling109, Muhammad Islam110, Tazeen H. Jafar110, Jeanette Erdmann111, Smita R. Kulkarni112, Stefan R. Bornstein76, Jürgen Grässler76, Leif Groop113,114, Benjamin F. Voight115, Johannes Kettunen116,126, Philip Howard117, Andrew Taylor43, Simonetta Guarrera60, Fulvio Ricceri59,60, Valur Emilsson118, Andrew Plump118, Inês Barroso119,120, Kay-Tee Khaw48, Alan B. Weder121, Steven C. Hunt122, Yan V. Sun81, Richard N. Bergman123, Francis S. Collins124, Lori L. Bonnycastle124, Laura J. Scott31, Heather M. Stringham31, Leena Peltonen119,125,126,127, Markus Perola125, Erkki Vartiainen125, Stefan-Martin Brand128,129, Jan A. Staessen73, Thomas J. Wang6,130, Paul R. Burton12,72, Maria Soler Artigas12, Yanbin Dong131, Harold Snieder132,131, Xiaoling Wang131, Haidong Zhu131, Kurt K. Lohman133, Megan E. Rudock51, Susan R Heckbert134,135, Nicholas L Smith134,136,135, Kerri L Wiggins137, Ayo Doumatey74, Daniel Shriner74, Gudrun Veldre25,138, Margus Viigimaa139,140, Sanjay Kinra141, Dorairajan Prabhakaran142, Vikal Tripathy142, Carl D. Langefeld79, Annika Rosengren143, Dag S. Thelle144, Anna Maria Corsi145, Andrew Singleton83, Terrence Forrester146, Gina Hilton1, Colin A.

McKenzie146, Tunde Salako147, Naoharu Iwai148, Yoshikuni Kita149, Toshio Ogihara150, Takayoshi Ohkubo149,151, Tomonori Okamura148, Hirotsugu Ueshima152, Satoshi Umemura153, Susana Eyheramendy154, Thomas Meitinger155,156, H.-Erich Wichmann157,158,159, Yoon Shin Cho44, Hyung-Lae Kim44, Jong-Young Lee44, James Scott160, Joban S. Sehmi160,41, Weihua Zhang18, Bo Hedblad39, Peter Nilsson39, George Davey Smith62, Andrew Wong67, Narisu Narisu124, Alena Stančáková53, Leslie J. Raffel161, Jie Yao161, Sekar Kathiresan162,27, Chris O'Donnell163,27,9, Stephen M. Schwartz134, M. Arfan Ikram13,15, W. T. Longstreth Jr.164, Thomas H. Mosley165, Sudha Seshadri166, Nick R.G. Shrine12, Louise V. Wain12, Mario A. Morken124, Amy J. Swift124, Jaana Laitinen167, Inga Prokopenko51,168, Paavo Zitting169, Jackie A. Cooper69, Steve E. Humphries69, John Danesh48, Asif Rasheed170, Anuj Goel32, Anders Hamsten171, Hugh Watkins32, Stephan J.L. Bakker172, Wiek H. van Gilst45, Charles S. Janipalli47, K. Radha Mani47, Chittaranjan S. Yajnik112, Albert Hofman13, Francesco U.S. Mattace-Raso13,14, Ben A. Oostra173, Ayse Demirkan13, Aaron Isaacs13, Fernando Rivadeneira13,14, Edward G Lakatta174, Marco Orru175,176, Angelo Scuteri174, Mika Ala-Korpela177,178,179, Antti J Kangas177, Leo-Pekka Lyytikäinen58, Pasi Soininen177,178, Taru Tukiainen180,181,177, Peter Würtz177,18,180, Rick Twee-Hee Ong56,57,182, Marcus Dörr183, Heyo K. Kroemer184, Uwe Völker20, Henry Völzke185, Pilar Galan186, Serge Hercberg186, Mark Lathrop24, Diana Zelenika24, Panos Deloukas119, Massimo Mangino28, Tim D. Spector28, Guangju Zhai28, James F. Meschia187, Michael A. Nalls83, Pankaj Sharma188, Janos Terzic189, M. J. Kranthi Kumar47, Matthew Denniff71, Ewa Zukowska-Szczechowska190, Lynne E. Wagenknecht79, F. Gerald R. Fowkes191, Fadi J. Charchar192, Peter E.H. Schwarz193, Caroline Hayward70, Xiuqing Guo161, Charles Rotimi74, Michiel L. Bots63, Eva Brand194, Nilesh J. Samani71,72, Ozren Polasek195, Philippa J. Talmud69, Fredrik Nyberg68,196, Diana Kuh67, Maris Laan25, Kristian Hveem66, Lyle J. Palmer197,198, Yvonne T. van der Schouw63, Juan P. Casas199, Karen L. Mohlke61, Paolo Vineis200,60, Olli Raitakari201, Santhi K. Ganesh202, Tien Y. Wong203,204, E Shyong Tai205,57,206, Richard S. Cooper54, Markku Laakso53, Dabeeru C. Rao207, Tamara B. Harris22, Richard W. Morris208, Anna F. Dominiczak209, Mika Kivimaki210, Michael G. Marmot210, Tetsuro Miki49, Danish Saleheen170,48, Giriraj R. Chandak47, Josef Coresh211, Gerjan Navis212, Veikko Salomaa125, Bok-Ghee Han44, Xiaofeng Zhu94, Jaspal S. Kooner160,41, Olle Melander39, Paul M Ridker8,213,9, Stefania Bandinelli214, Ulf B. Gyllensten37, Alan F. Wright70, James F. Wilson34, Luigi Ferrucci33, Martin Farrall32, Jaakko Tuomilehto215,216,217,218, Peter P. Pramstaller30,219, Roberto Elosua29,220, Nicole Soranzo119,28, Eric J.G. Sijbrands13,14, David Altshuler221,115, Ruth J.F. Loos23, Alan R. Shuldiner26,222, Christian Gieger157, Pierre Meneton223, Andre G. Uitterlinden13,14,15, Nicholas J. Wareham23, Vilmundur Gudnason10,11, Jerome I. Rotter161, Rainer Rettig224, Manuela Uda175, David P. Strachan50, Jacqueline C.M. Witteman13,15, Anna-Liisa Hartikainen225, Jacques S. Beckmann105,226, Eric Boerwinkle227, Ramachandran S. Vasan6,228, Michael Boehnke31, Martin G. Larson6,229, Marjo-Riitta Järvelin18,230,231,232,233, Bruce M. Psaty21,135*, Gonçalo R Abecasis19*, Aravinda Chakravarti1*#, Paul Elliott18,233*, Cornelia M. van Duijn13,234*, Christopher Newton-Cheh27,115*#, Daniel Levy6,16,7*#, Mark J. Caulfield4*#, Toby Johnson4*

Affiliations

1. Center for Complex Disease Genomics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA 2.

Institute of Social and Preventive Medicine (IUMSP), Centre Hospitalier Universitaire Vaudois and University of Lausanne, Bugnon 17, 1005 Lausanne, Switzerland 3. Cardiology, Department of Specialties of Internal Medicine, Geneva University Hospital, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva 14, Switzerland 4. Clinical Pharmacology and The Genome Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK 5. Department of Biostatistics, University of Washington, Seattle, WA, USA 6. Framingham Heart Study, Framingham, MA, USA 7. National Heart Lung, and Blood Institute, Bethesda, MD, USA 8. Division of Preventive Medicine, Brigham and Women's Hospital, 900 Commonwealth Avenue East, Boston MA 02215, USA 9. Harvard Medical School, Boston, MA, USA 10. Icelandic Heart Association, Kopavogur, Iceland 11. University of Iceland, Reykajvik, Iceland 12. Department of Health Sciences, University of Leicester, University Rd, Leicester LE1 7RH, UK 13. Department of Epidemiology, Erasmus Medical Center, PO Box 2040, 3000 CA, Rotterdam, The Netherlands 14. Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands 15. Netherlands Consortium for Healthy Aging (NCHA), Netherland Genome Initiative (NGI), The Netherlands 16. Center for Population Studies, National Heart Lung, and Blood Institute, Bethesda, MD, USA 17. Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland 18. Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, Norfolk Place, London W2 1PG, UK 19. Center for Statistical Genetics, Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI 48103, USA 20. Interfaculty Institute for Genetics and Functional Genomics, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany 21. Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology and Health Services, University of Washington, Seattle, WA, USA 22. Laboratory of Epidemiology, Demography, Biometry, National Institute on Aging, National Institutes of Health, Bethesda, Maryland 20892, USA 23. MRC Epidemiology Unit, Institute of Metabolic Science, Cambridge CB2 0QQ, UK 24. Centre National de Génotypage, Commissariat à L'Energie Atomique, Institut de Génomique, Evry, France 25. Institute of Molecular and Cell Biology, University of Tartu, Riia 23, Tartu 51010, Estonia 26. University of Maryland School of Medicine, Baltimore, MD, USA, 21201, USA 27. Center for Human Genetic Research, Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts, 02114, USA 28. Department of Twin Research & Genetic Epidemiology, King's College London, UK 29. Cardiovascular Epidemiology and Genetics, Institut Municipal d'Investigacio Medica, Barcelona Biomedical Research Park, 88 Doctor Aiguader, 08003 Barcelona, Spain 30. Institute of Genetic Medicine, European Academy Bozen/Bolzano (EURAC), Viale Druso 1, 39100 Bolzano, Italy - Affiliated Institute of the University of Lübeck, Germany 31. Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, 48109, USA 32. Department of Cardiovascular Medicine, The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK 33. Clinical Research Branch, National Institute on Aging, Baltimore MD 21250, USA 34. Centre for Population Health Sciences, University of Edinburgh, EH89AG, UK 35. Centre for Population Health Sciences and Institute of Genetics and Molecular Medicine, College of Medicine and Vet Medicine, University of Edinburgh, EH8 9AG, UK 36. Croatian Centre for Global Health, University of Split, Croatia. 37. Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, SE-751 85 Uppsala, Sweden 38. Amgen, 1 Kendall Square, Building 100, Cambridge, MA 02139, USA 39. Department of Clinical Sciences, Lund University,

Malmö, Sweden 40. Department of Medicine, University of Verona, Italy 41. Ealing Hospital, London, UB1 3HJ, UK 42. Department of Medicine, University of Mississippi Medical Center, USA 43. Genetic Epidemiology Group, Epidemiology and Public Health, UCL, London, WC1E 6BT, UK 44. Center for Genome Science, National Institute of Health, Seoul, Korea 45. Department of Cardiology, University Medical Center Groningen, University of Groningen, The Netherlands 46. Departments of Epidemiology and Medicine, Johns Hopkins University, Baltimore MD, USA 47. Centre for Cellular and Molecular Biology (CCMB), Council of Scientific and Industrial Research (CSIR), Uppal Road, Hyderabad 500 007, India 48. Department of Public Health and Primary Care, University of Cambridge, CB1 8RN, UK 49. Department of Basic Medical Research and Education, and Department of Geriatric Medicine, Ehime University Graduate School of Medicine, Toon, 791-0295, Japan 50. Division of Community Health Sciences, St George's University of London, London, SW17 0RE, UK 51. Epidemiology & Prevention, Division of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA 52. Division of Biostatistics and Department of Genetics, School of Medicine, Washington University in St. Louis, Saint Louis, Missouri 63110, USA 53. Department of Medicine, University of Eastern Finland and Kuopio University Hospital, 70210 Kuopio, Finland 54. Department of Preventive Medicine and Epidemiology, Loyola University Medical School, Maywood, IL, USA 55. Department of Laboratory Medicine & Institute of Human Genetics, University of California San Francisco, 513 Parnassus Ave. San Francisco CA 94143, USA 56. Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore, 138672, Singapore 57. Centre for Molecular Epidemiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 117597, Singapore 58. Department of Clinical Chemistry, University of Tampere and Tampere University Hospital, Tampere, 33521, Finland 59. Department of Genetics, Biology and Biochemistry, University of Torino, Via Santena 19, 10126, Torino, Italy 60. Human Genetics Foundation (HUGEF), Via Nizza 52, 10126, Torino, Italy 61. Department of Genetics, University of North Carolina, Chapel Hill, NC, 27599, USA 62. MRC Centre for Causal Analyses in Translational Epidemiology, School of Social & Community Medicine, University of Bristol, Bristol BS8 2BN, UK 63. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Heidelberglaan 100, 3508 GA Utrecht, The Netherlands 64. Complex Genetics Section, Department of Medical Genetics - DBG, University Medical Center Utrecht, 3508 GA Utrecht, The Netherlands 65. Centre for Genetic Epidemiology and Biostatistics, University of Western Australia, Crawley, WA, Australia 66. HUNT Research Centre, Department of Public Health and General Practice, Norwegian University of Science and Technology, 7600 Levanger, Norway 67. MRC Unit for Lifelong Health & Ageing, London, WC1B 5JU, UK 68. Occupational and Environmental Medicine, Department of Public Health and Community Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, 40530 Gothenburg, Sweden 69. Centre for Cardiovascular Genetics, University College London, London WC1E 6JF, UK 70. MRC Human Genetics Unit and Institute of Genetics and Molecular Medicine, Edinburgh, EH2, UK 71. Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, LE3 9QP, UK 72. Leicester NIHR Biomedical Research Unit in Cardiovascular Disease, Glenfield Hospital, Leicester, LE3 9QP, UK; 73. Studies Coordinating Centre, Division of Hypertension and Cardiac Rehabilitation, Department of Cardiovascular Diseases, University of Leuven, Campus Sint Rafaël, Kapucijnenvoer 35, Block D, Box 7001, 3000 Leuven, Belgium; 74. Center for Research on Genomics and Global Health, National Human Genome Research Institute, Bethesda, MD 20892, USA 75. Columbia

University, NY, USA 76. Department of Medicine III, Medical Faculty Carl Gustav Carus at the Technical University of Dresden, 01307 Dresden, Germany 77. Epidemiology and Biostatistics, School of Public Health, Imperial College, London, W2 1PG, UK 78. Clinical and Molecular Epidemiology Unit, Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece 79. Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA 80. A list of consortium members is supplied in the S Materials 81. Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI 48109, USA 82. Division of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas at Houston Health Science Center, 12 Herman Pressler, Suite 453E, Houston, TX 77030, USA 83. Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD 20892, USA 84. Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, NIH, Baltimore, Maryland, USA 85. Washington Hospital Center, Division of Cardiology, Washington DC, USA 86. ALSPAC Laboratory, University of Bristol, Bristol, BS8 2BN, UK 87. Pediatric Epidemiology Center, University of South Florida, Tampa, FL, USA 88. Clinical Pharmacology Unit, University of Cambridge, Addenbrookes Hospital, Hills Road, Cambridge CB2 2QQ, UK 89. University of Dundee, Ninewells Hospital &Medical School, Dundee, DD1 9SY, UK 90. Genetic Epidemiology Group, Department of Epidemiology and Public Health, UCL, London WC1E 6BT, UK 91. Pathology and Laboratory Medicine, University of Western Australia, Crawley, WA, Australia; 92. Molecular Genetics, PathWest Laboratory Medicine, Nedlands, WA, Australia; 93. Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Oxford, OX3 7LF, UK 94. Department of Epidemiology and Biostatistics, Case Western Reserve University, 2103 Cornell Road, Cleveland, OH 44106, USA; 95. Department of Medicine, Johns Hopkins University, Baltimore, USA; 96. Department of Clinical Physiology, University of Tampere and Tampere University Hospital, Tampere, 33521, Finland; 97. Department of Medicine, University of Turku and Turku University Hospital, Turku, 20521, Finland; 98. Department of Nutrition, University of North Carolina, Chapel Hill, NC, 27599, USA; 99. Office of Population Studies Foundation, University of San Carlos, Talamban, Cebu City 6000, Philippines; 100. Department of Neurology and Framingham Heart Study, Boston University School of Medicine, Boston, MA, 02118, USA; 101. Department of Internal Medicine II, University Medical Center Regensburg, 93053 Regensburg, Germany; 102. Department of Epidemiology and Preventive Medicine, University Medical Center Regensburg, 93053 Regensburg, Germany; 103. Department of Epidemiology, Johns Hopkins University, Baltimore MD, USA; 104. Renal Division, University Hospital Freiburg, Germany; 105. Département de Génétique Médicale, Université de Lausanne, 1015 Lausanne, Switzerland ; 106. Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland ; 107. Division of Genetics, GlaxoSmithKline, Philadelphia, Pennsylvania 19101, USA; 108. International Centre for Circulatory Health, National Heart & Lung Institute, Imperial College, London, UK; 109. Genetics of Complex Traits, Peninsula Medical School, University of Exeter, UK; 110. Department of Community Health Sciences & Department of Medicine, Aga Khan University, Karachi, Pakistan; 111. Medizinische Klinik II, Universität zu Lübeck, Lübeck, Germany; 112. Diabetes Unit, KEM Hospital and Research Centre, Rasta Peth, Pune-411011, Maharashtra, India; 113. Department of Clinical Sciences, Diabetes and Endocrinology Research Unit, University Hospital, Malmö, Sweden; 114. Lund University, Malmö 20502, Sweden; 115. Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, 02139, USA; 116. Department of Chronic Disease Prevention, National Institute for Health and Welfare,

FIN-00251 Helsinki, Finland; 117. William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK; 118. Merck Research Laboratory, 126 East Lincoln Avenue, Rahway, NJ 07065, USA; 119. Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, UK 120. University of Cambridge Metabolic Research Labs, Institute of Metabolic Science Addenbrooke's Hospital, CB2 OQQ, Cambridge, UK; 121. Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA; 122. Cardiovascular Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA; 123. Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California 90033, USA; 124. National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892,USA; 125. National Institute for Health and Welfare, 00271 Helsinki, Finland; 126. FIMM, Institute for Molecular Medicine, Finland, Biomedicum, P.O. Box 104, 00251 Helsinki, Finland; 127. Broad Institute, Cambridge, Massachusetts 02142, USA; 128. Leibniz-Institute for Arteriosclerosis Research, Department of Molecular Genetics of Cardiovascular Disease, University of Münster, Münster, Germany; 129. Medical Faculty of the Westfalian Wilhelms University Muenster, Department of Molecular Genetics of Cardiovascular Disease, University of Muenster, Muenster, Germany; 130. Division of Cardiology, Massachusetts General Hospital, Boston, MA, USA; 131. Georgia Prevention Institute, Department of Pediatrics, Medical College of Georgia, Augusta, GA, USA; 132. Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 133. Department of Biostatical Sciences, Division of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA; 134. Department of Epidemiology, University of Washington, Seattle, WA, 98195, USA; 135. Group Health Research Institute, Group Health Cooperative, Seattle, WA, USA; 136. Seattle Epidemiologic Research and Information Center, Veterans Health Administration Office of Research & Development, Seattle, WA 98108, USA; 137. Department of Medicine, University of Washington, 98195, USA; 138. Department of Cardiology, University of Tartu, L. Puusepa 8, 51014 Tartu, Estonia; 139. Tallinn University of Technology, Institute of Biomedical Engineering, Ehitajate tee 5, 19086 Tallinn, Estonia; 140. Centre of Cardiology, North Estonia Medical Centre, Sütiste tee 19, 13419 Tallinn, Estonia; 141. Division of Non-communicable disease Epidemiology, The London School of Hygiene and Tropical Medicine London, Keppel Street, London WC1E 7HT, UK; 142. South Asia Network for Chronic Disease, Public Health Foundation of India, C-1/52, SDA, New Delhi 100016, India; 143. Department of Emergency and Cardiovascular Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, 41685 Gothenburg, Sweden; 144. Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, 0317 Oslo, Norway; 145. Tuscany Regional Health Agency, Florence, Italy 146. Tropical Medicine Research Institute, University of the West Indies, Mona, Kingston, Jamaica; 147. University of Ibadan, Ibadan, Nigeria 148. Department of Genomic Medicine, and Department of Preventive Cardiology, National Cerebral and Cardiovascular Research Center, Suita, 565-8565, Japan; 149. Department of Health Science, Shiga University of Medical Science, Otsu, 520-2192, Japan; 150. Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Suita, 565-0871, Japan; 151. Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Sendai, 980-8578, Japan; 152. Lifestyle-related Disease Prevention Center, Shiga University of Medical Science, Otsu, 520-2192, Japan; 153. Department of Medical Science and Cardiorenal Medicine, Yokohama City University School of Medicine,

Yokohama, 236-0004, Japan; 154. Department of Statistics, Pontificia Universidad Catolica de Chile, Vicuña Mackena 4860, Santiago, Chile; 155. Institute of Human Genetics, Helmholtz Zentrum Munich, German Research Centre for Environmental Health, 85764 Neuherberg, Germany; 156. Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, 81675 Munich, Germany; 157. Institute of Epidemiology, Helmholtz Zentrum Munich, German Research Centre for Environmental Health, 85764 Neuherberg, Germany; 158. Chair of Epidemiology, Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-Universität, 81377 Munich, Germany; 159. Klinikum Grosshadern, 81377 Munich, Germany; 160. National Heart and Lung Institute, Imperial College London, London, UK, W12 0HS, UK; 161. Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA; 162. Medical Population Genetics, Broad Institute of Harvard and MIT, 5 Cambridge Center, Cambridge MA 02142, USA; 163. National Heart, Lung and Blood Institute and its Framingham Heart Study, 73 Mount Wayte Ave., Suite #2, Framingham, MA 01702, USA; 164. Department of Neurology and Medicine, University of Washington, Seattle, USA; 165. Department of Medicine (Geriatrics), University of Mississippi Medical Center, Jackson, MS, USA; 166. Department of Neurology, Boston University School of Medicine, USA; 167. Finnish Institute of Occupational Health, Finnish Institute of Occupational Health, Aapistie 1, 90220 Oulu, Finland; 168. Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 169. Lapland Central Hospital, Department of Physiatrics, Box 8041, 96101 Rovaniemi, Finland; 170. Center for Non-Communicable Diseases Karachi, Pakistan; 171. Atherosclerosis Research Unit, Department of Medicine, Karolinska Institute, Stockholm, Sweden; 172. Department of Internal Medicine, University Medical Center Groningen, University of Groningen, The Netherlands; 173. Department of Medical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands; 174. Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224, USA; 175. Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Cittadella Universitaria di Monserrato, Monserrato, Cagliari, Italy; 176. Unita` Operativa Semplice Cardiologia, Divisione di Medicina, Presidio Ospedaliero Santa Barbara, Iglesias, Italy; 177. Computational Medicine Research Group, Institute of Clinical Medicine, University of Oulu and Biocenter Oulu, 90014 University of Oulu, Oulu, Finland; 178. NMR Metabonomics Laboratory, Department of Biosciences, University of Eastern Finland, 70211 Kuopio, Finland; 179. Department of Internal Medicine and Biocenter Oulu, Clinical Research Center, 90014 University of Oulu, Oulu, Finland; 180. Institute for Molecular Medicine Finland FIMM, 00014 University of Helsinki, Helsinki, Finland; 181. Department of Biomedical Engineering and Computational Science, School of Science and Technology, Aalto University, 00076 Aalto, Espoo, Finland; 182. NUS Graduate School for Integrative Sciences & Engineering (NGS) Centre for Life Sciences (CeLS), Singapore, 117456, Singapore; 183. Department of Internal Medicine B, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany; 184. Institute of Pharmacology, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany; 185. Institute for Community Medicine, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany; 186. U557 Institut National de la Santé et de la Recherche Médicale, U1125 Institut National de la Recherche Agronomique, Université Paris 13, Bobigny, France ; 187. Department of Neurology, Mayo Clinic, Jacksonville, FL, USA 188. Imperial College Cerebrovascular Unit (ICCRU), Imperial College, London, W6 8RF, UK 189. Faculty of Medicine, University of Split, Croatia. 190. Department of Internal Medicine, Diabetology, and Nephrology, Medical University of Silesia, 41-800, Zabrze,

Poland 191. Public Health Sciences section, Division of Community Health Sciences, University of Edinburgh, Medical School, Teviot Place, Edinburgh, EH8 9AG, UK 192. School of Science and Engineering, University of Ballarat, 3353 Ballarat, Australia 193. Prevention and Care of Diabetes, Department of Medicine III, Medical Faculty Carl Gustav Carus at the Technical University of Dresden, 01307 Dresden, Germany 194. University Hospital Münster, Internal Medicine D, Münster, Germany 195. Department of Medical Statistics, Epidemiology and Medical Informatics, Andrija Stampar School of Public Health, University of Zagreb, Croatia 196. AstraZeneca R&D, 431 83 Mölndal, Sweden 197. Genetic Epidemiology & Biostatistics Platform, Ontario Institute for Cancer Research, Toronto 198. Samuel Lunenfeld Institute for Medical Research, University of Toronto, Canada 199. Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, UK 200. Department of Epidemiology and Public Health, Imperial College, Norfolk Place London W2 1PG, UK 201. Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and the Department of Clinical Physiology, Turku University Hospital, Turku, 20521, Finland 202. Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan Medical Center, Ann Arbor, Michigan, USA 203. Singapore Eye Research Institute, Singapore, 168751, Singapore 204. Department of Ophthalmology, National University of Singapore, Singapore, 119074, Singapore 205. Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 119074, Singapore 206. Duke-National University of Singapore Graduate Medical School, Singapore, 169857, Singapore 207. Division of Biostatistics, Washington University School of Medicine, Saint Louis, MO, 63110, USA 208. Department of Primary Care & Population Health, UCL, London, UK, NW3 2PF, UK 209. BHF Glasgow Cardiovascular Research Centre, University of Glasgow, 126 University Place, Glasgow, G12 8TA, UK 210. Epidemiology Public Health, UCL, London, UK, WC1E 6BT, UK 211. Departments of Epidemiology, Biostatistics, and Medicine, Johns Hopkins University, Baltimore MD, USA 212. Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, The Netherlands 213. Division of Cardiology, Brigham and Women's Hospital, 900 Commonwealth Avenue East, Boston MA 02215, USA 214. Geriatric Rehabilitation Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy 215. National Institute for Health and Welfare, Diabetes Prevention Unit, 00271 Helsinki, Finland 216. Hjelt Institute, Department of Public Health, University of Helsinki, 00014 Helsinki, Finland 217. South Ostrobothnia Central Hospital, 60220 Seinäjoki, Finland 218. Red RECAVA Grupo RD06/0014/0015, Hospital Universitario La Paz, 28046 Madrid, Spain 219. Department of Neurology, General Central Hospital, 39100 Bolzano, Italy 220. CIBER Epidemiología y Salud Pública, 08003 Barcelona 221. Department of Medicine and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA 222. Geriatric Research and Education Clinical Center, Veterans Administration Medical Center, Baltimore, MD, USA 223. U872 Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Paris, France 224. Institute of Physiology, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany 225. Institute of Clinical Medicine/Obstetrics and Gynecology, University of Oulu, Finland 226. Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland 227. Human Genetics Center, 1200 Hermann Pressler, Suite E447 Houston, TX 77030, USA 228. Division of Epidemiology and Prevention, Boston University School of Medicine, Boston, MA, USA 229. Department of Mathematics, Boston University, Boston, MA, USA. 230.Institute of Health Sciences, University of Oulu, BOX 5000, 90014 University of Oulu, Finland. 231.

Biocenter Oulu, University of Oulu, BOX 5000, 90014 University of Oulu, Finland 232. National Institute for Health and Welfare, Box 310, 90101 Oulu, Finland 233. MRC-HPA Centre for Environment and Health, School of Public Health, Imperial College London, Norfolk Place, London W2 1PG, UK 234. Centre of Medical Systems Biology (CMSB 1-2), NGI Erasmus Medical Center, Rotterdam, The Netherlands.

v. The Psoriasis and Psoriatic Arthritis Genetics Consortium

Name of co-author: affiliation(s):
James T Elder:
Department of Dermatology, University of Michigan Ann Arbor, MI 48109, USA;
Ann Arbor Veterans Affairs Hospital, Ann Arbor, MI 48105 USA
Philip E Stuart: Department of Dermatology, University of Michigan Ann Arbor, MI 48109, USA
Rajan P Nair: Department of Dermatology, University of Michigan Ann Arbor, MI 48109, USA
Trilokraj Tejasvi: Department of Dermatology, University of Michigan Ann Arbor, MI 48109, USA.
Johann E Gudjonsson: Department of Dermatology, University of Michigan Ann Arbor, MI 48109, USA
John J Voorhees: Department of Dermatology, University of Michigan Ann Arbor, MI 48109, USA.
Lam C Tsoi: Department of Biostatistics, Center for Statistical Genetics, University of Michigan Ann Arbor, MI 48109, USA
Jun Ding: Department of Biostatistics, Center for Statistical Genetics, University of Michigan Ann Arbor, MI 48109, USA
Yanming Li: Department of Biostatistics, Center for Statistical Genetics, University of Michigan Ann Arbor, MI 48109, USA
Hyun M Kang: Department of Biostatistics, Center for Statistical Genetics, University of Michigan Ann Arbor, MI 48109, USA;
Goncalo R Abecasis: Department of Biostatistics, Center for Statistical Genetics, University of Michigan Ann Arbor, MI 48109, USA
Andre Franke: Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany
Eva Ellinghaus: Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany
Stefan Schreiber: Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany
Ulrich Mrowietz: Department of Dermatology, University Hospital, Schleswig-Holstein, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany
Stephan Weidinger: Department of Dermatology, University Hospital, Schleswig-Holstein, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany
Michael Weichenthal ; Department of Dermatology, University Hospital, Schleswig-Holstein, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany
Sören Mucha: Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, 24105 Kiel, Germany
Dafna D Gladman: Department of Medicine, Division of Rheumatology, University of Toronto, Toronto Western Hospital, Toronto, Ontario M5T 2S8, Canada

Fawnda J Pellett: Department of Medicine, Division of Rheumatology, University of Toronto, Toronto Western Hospital, Toronto, Ontario M5T 2S8, Canada
Vinod Chandran: Department of Medicine, Division of Rheumatology, University of Toronto, Toronto Western Hospital, Toronto, Ontario M5T 2S8, Canada
Cheryl F Rosen: Department of Medicine, Division of Dermatology, University of Toronto, Toronto Western Hospital, Toronto, Ontario MST2S8
Proton Rahman: Department of Medicine, Memorial University, St. John's, Newfoundland A1C 5B8, Canada
Sulev Koks: Department of Physiology, Centre of Translational Medicine and Centre for Translational Genomics, University of Tartu, 50409 Tartu, Estonia
Külli Kingo: Department of Dermatology and Venerology, University of Tartu, 50409 Tartu, Estonia
Tonu Esko: Estonian Genome Center and Center of Translational Genomics; Estonian Biocenter; Institute of Molecular and Cell Biology, University of Tartu, 50409 Tartu, Estonia
Andres Metspalu: Estonian Genome Center and Center of Translational Genomics; Estonian Biocenter; Institute of Molecular and Cell Biology, University of Tartu, 50409 Tartu, Estonia
Peter Gregersen: Robert S. Boas Center for Genomics and Human Genetics, The Feinstein Institute for Medical Research, Manhasset, NY 11030
Andrew Henschel: National Psoriasis Foundation, Portland, OR 97223 USA
Marin Aurand National Psoriasis Foundation, Portland, OR 97223 USA
Bruce Bebo: National Psoriasis Foundation, Portland, OR 97223 USA
H. Erich Wichmann: Institute of Epidemiology I, Helmholtz Centre Munich, German Research Center for Environmental Health, 85764 Neuherberg, Germany; Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University, 81377 Munich, Germany; Klinikum Grosshadern, 81377 Munich, Germany
Christian Gieger: Institute of Genetic Epidemiology, Helmholtz Centre Munich, German Research Center for Environmental Health, 85764 Neuherberg, Germany
Thomas Illig :Research Unit Molecular Epidemiology, Helmholtz Centre Munich, German Research Center for Environmental Health, 85764 Neuherberg, Germany
Juliane Winkelmann: Department of Neurology, Technische Universität München, Munich, Germany; Institute of Human Genetics, Technische Universität München, Munich, Germany; Institute of Human Genetics, Helmholtz Zentrum Munich, German Research Center for Environmental Health, Munich, Germany
Susanne Moebus: Institute for Medical Informatics, Biometry and Epidemiology (IMIBE), University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany
Karl-Heinz Jöckel: Institute for Medical Informatics, Biometry and Epidemiology (IMIBE), University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany
Raimund Erbel: Clinic of Cardiology, West German Heart Centre, University Hospital of Essen, University Duisburg-Essen, Essen, Germany
Markus M. Nöthen: Institute of Human Genetics, University of Bonn, Bonn, Germany; Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany

Henry W Lim Henry Ford Hospital, Detroit, Michigan, 48202, USA

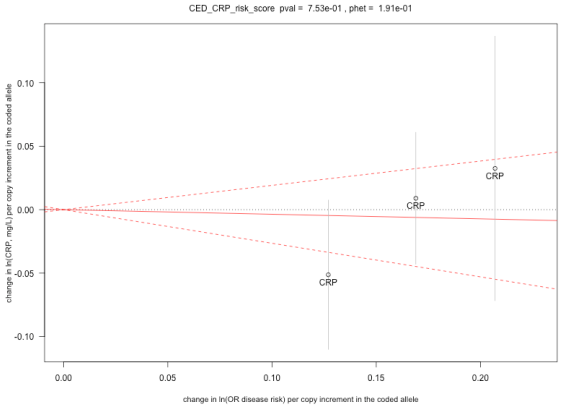
Investigating the causal relationship of C-reactive protein with 32 complex somatic and psychiatric outcomes: A large scale cross-consortia Mendelian randomization study.

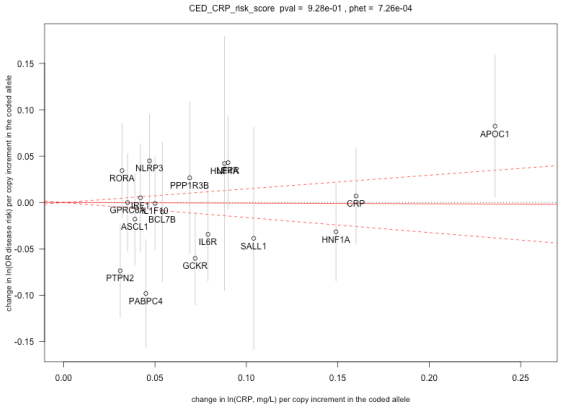
S1 Figure. GRS P Plots of CRP versus each outcome

Contents: **Page**

For each disease / trait we show the plots for two genetic risk scores ; on the left the GRS_{CRP} and on the right GRS_{GWAS}. For every graph, the estimated effects on disease risk (log odds) or trait level (vertical axis) are plotted against estimated effects on the natural log CRP levels (mg/ml) (horizontal axis), for either the GRS_{CRP} SNPs or GRS_{GWAS} SNPs that are associated with CRP levels. The grey vertical lines indicate the 95% confidence interval (CI) for each individual SNP. The effect estimate estimate of CRP levels on disease risk or trait level is represented by a red solid line with gradient α. The 95% CI of this α estimate is represented by red dashed lines.

A. Celiac disease





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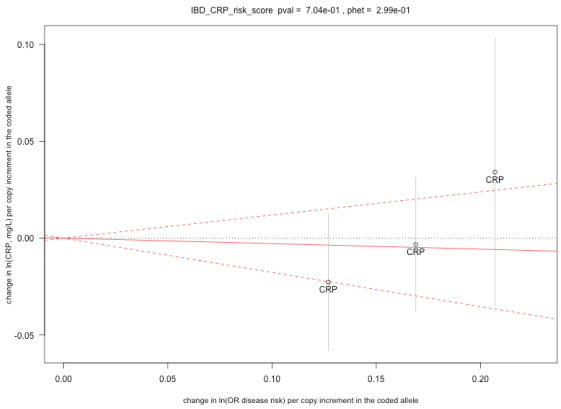
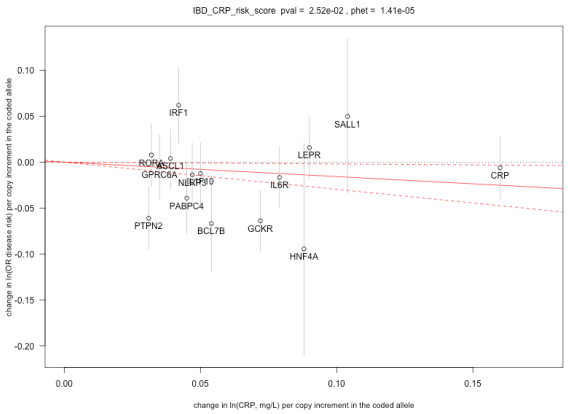
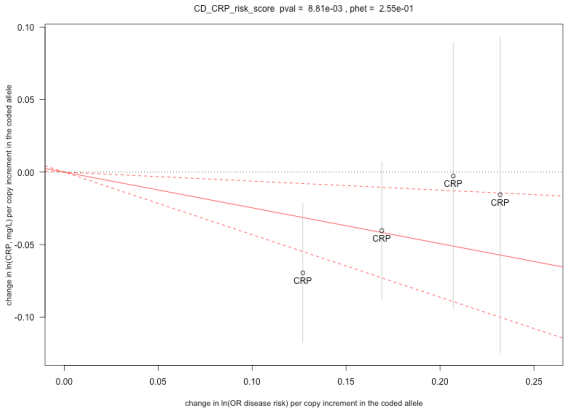
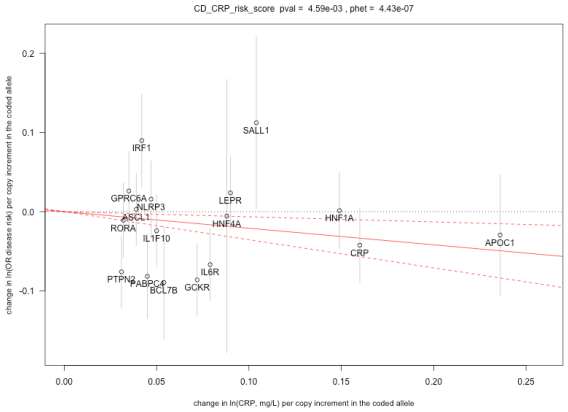




C. Crohn's Disease





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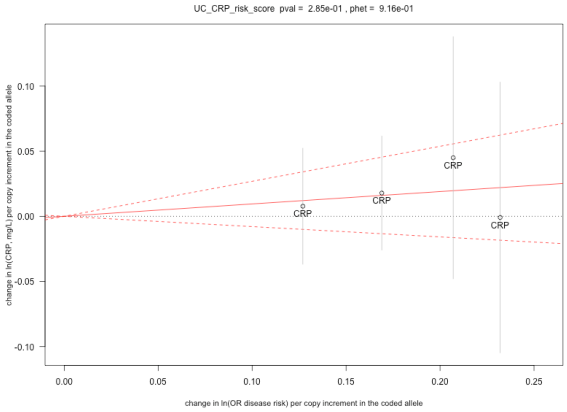

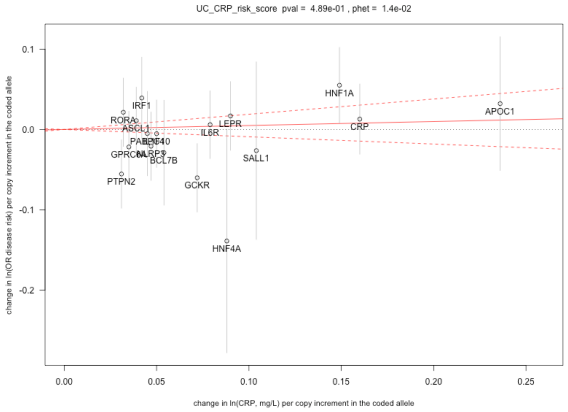

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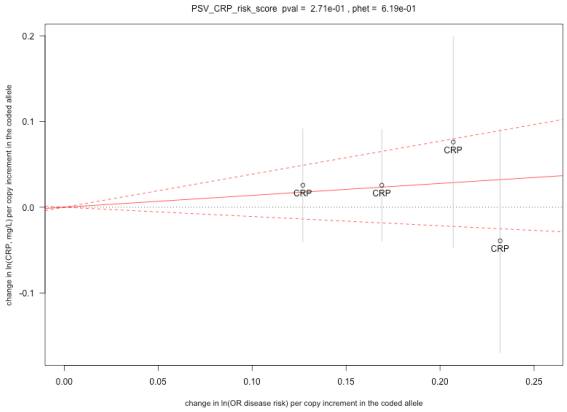

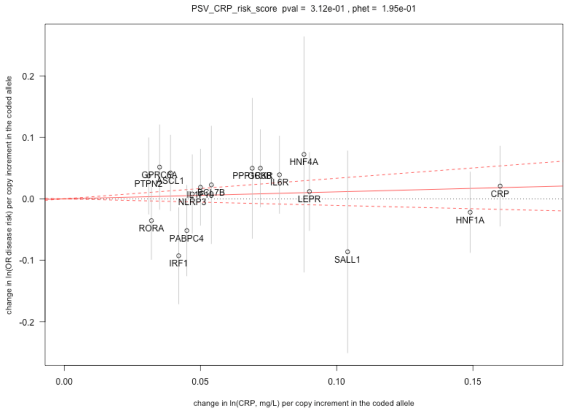

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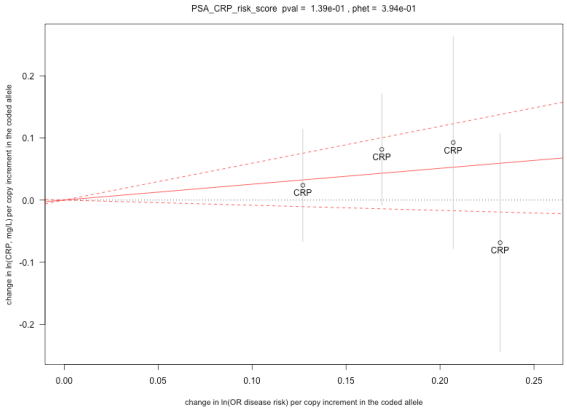
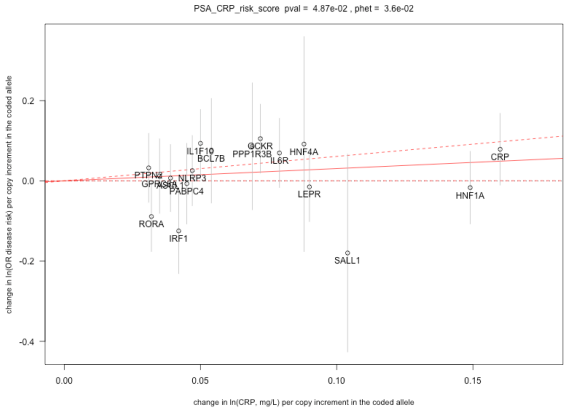
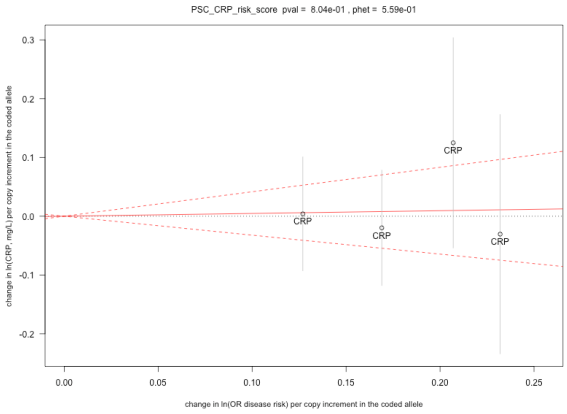
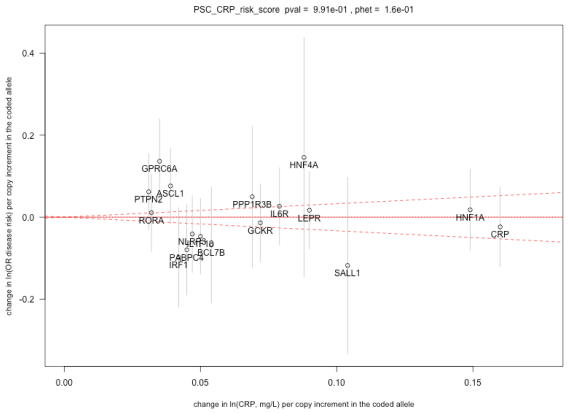




G. Psoriasis Cutaneous





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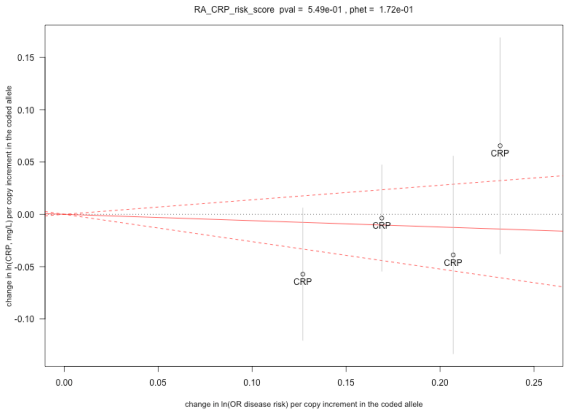
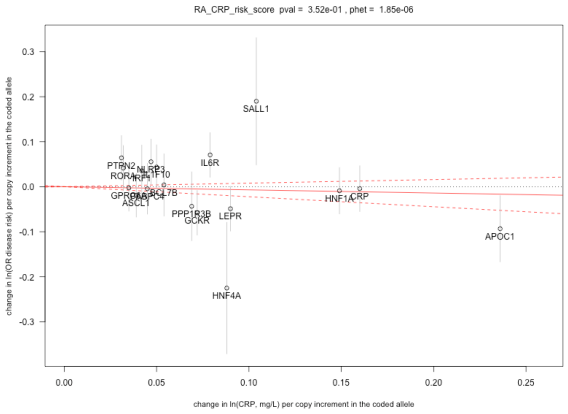
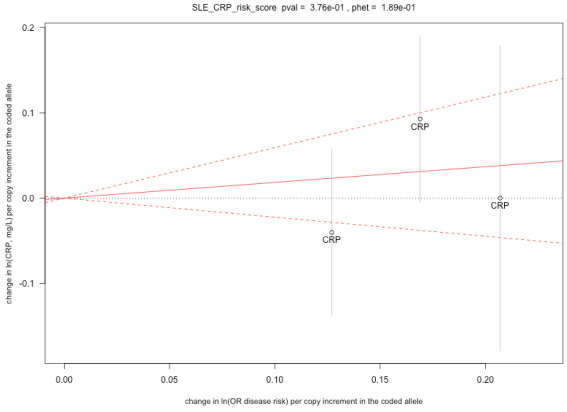
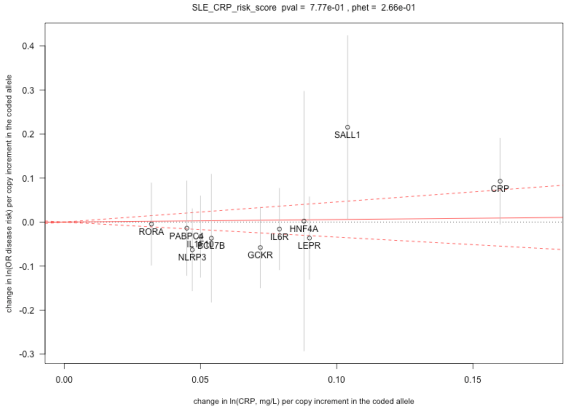


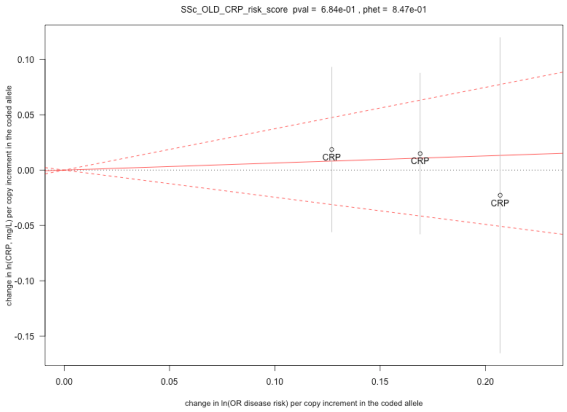


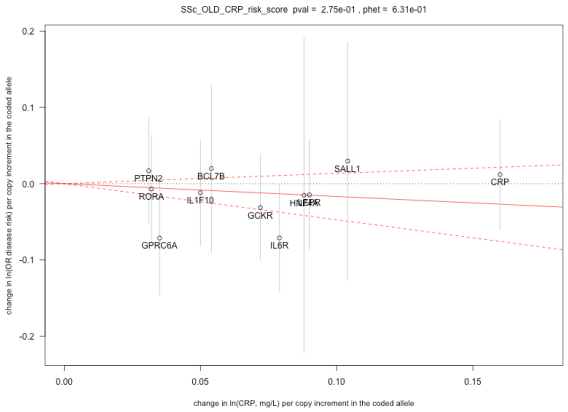
I. Systemic Lupus Erythematosus



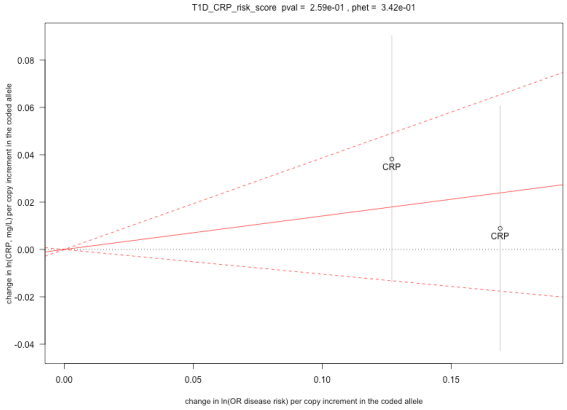


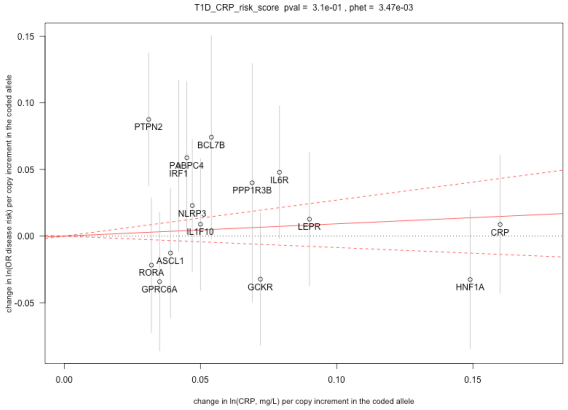
J. Systemic Sclerosis



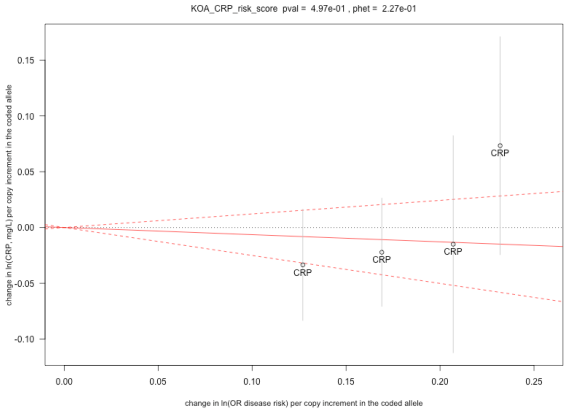


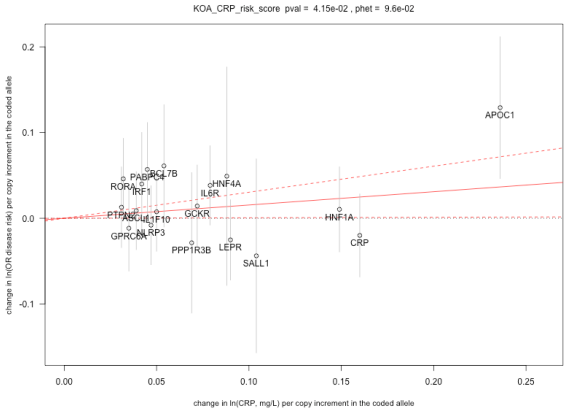
K. Type I Diabetes



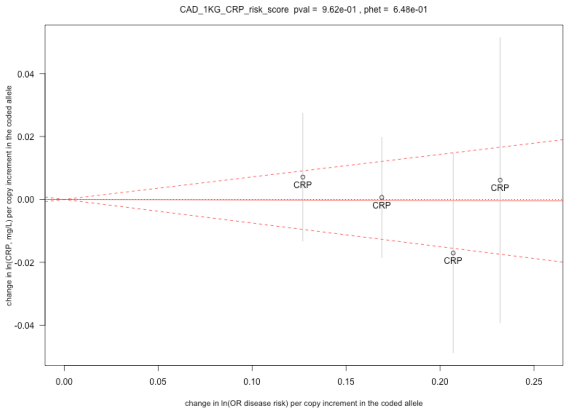


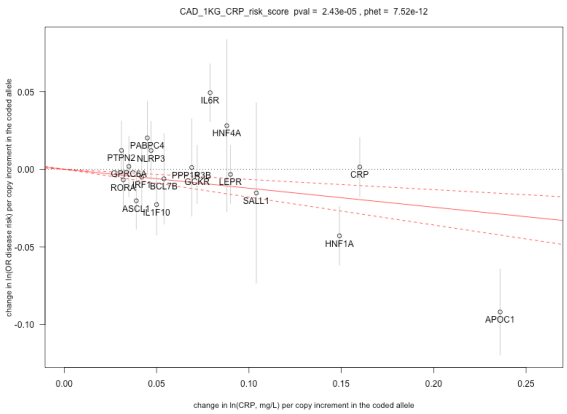
L. Knee Osteoarthritis



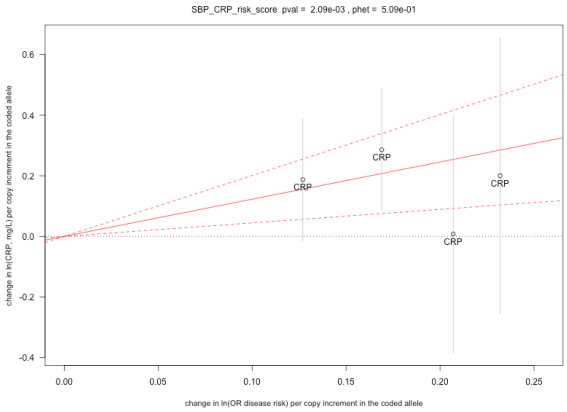


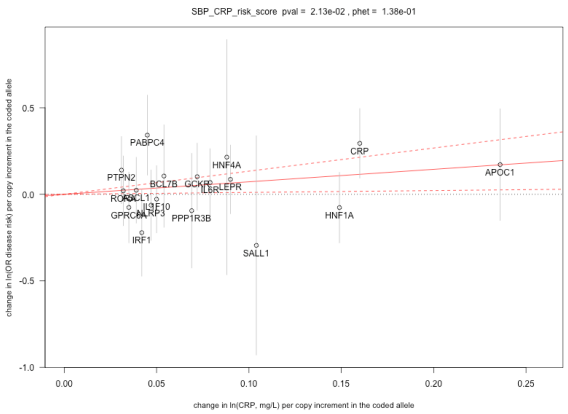
M. Coronary Artery Disease



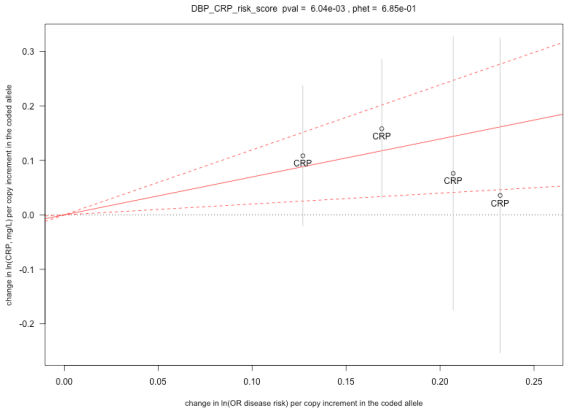


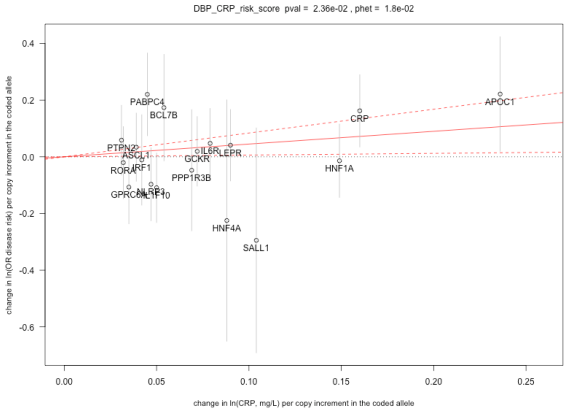
N. Systolic Blood Pressure



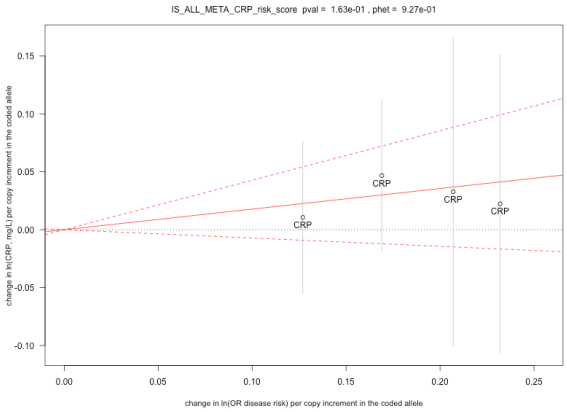


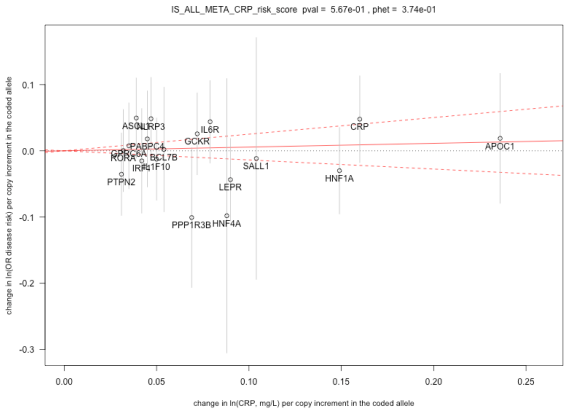
O. Diastolic Blood Pressure



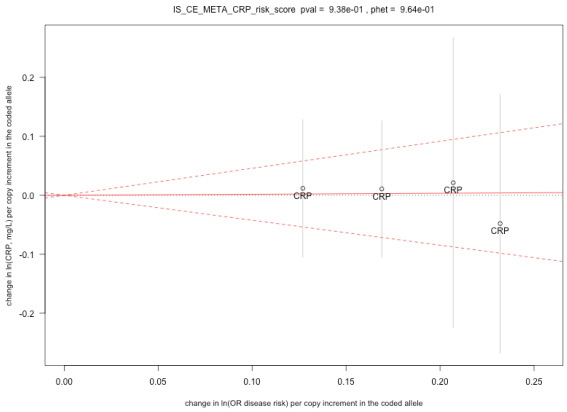


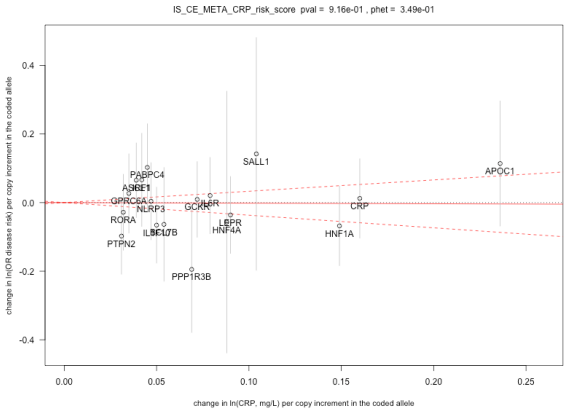
P. Ischemic Stroke (all types)



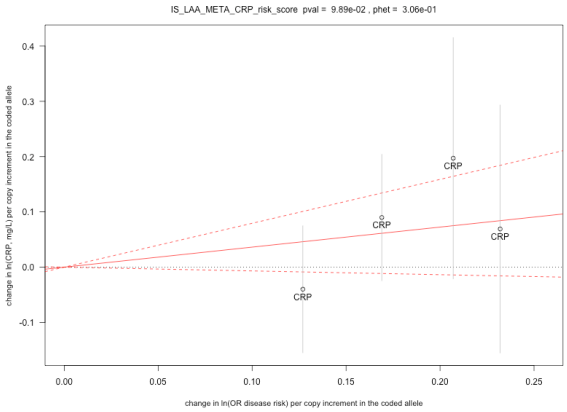


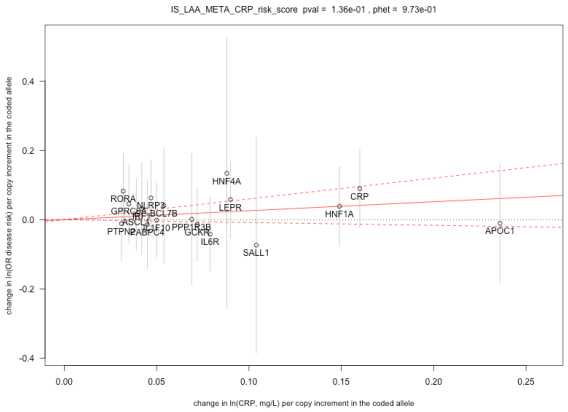
O. Ischemic Stroke (Cardioembolic Stroke)



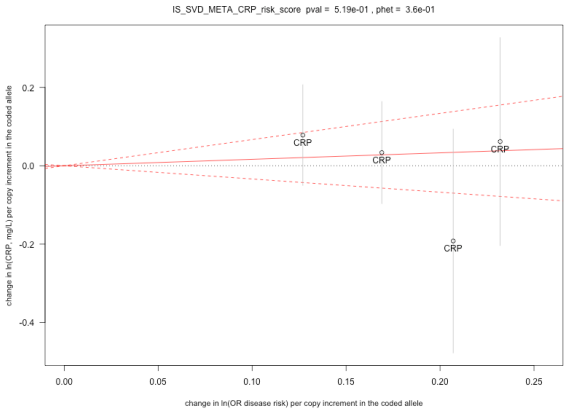


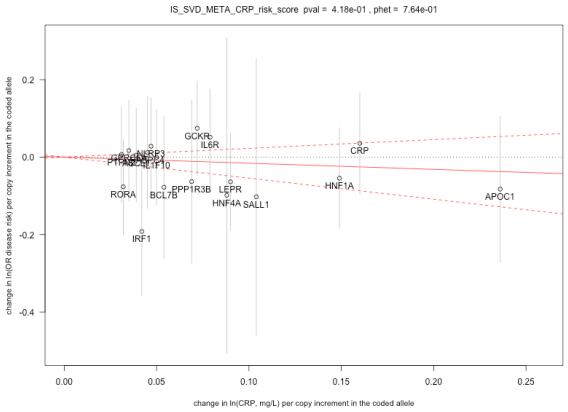
R. Ischemic Stroke (Large Vessel Disease)



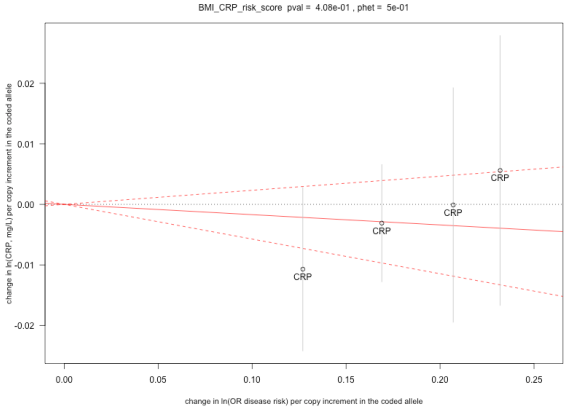


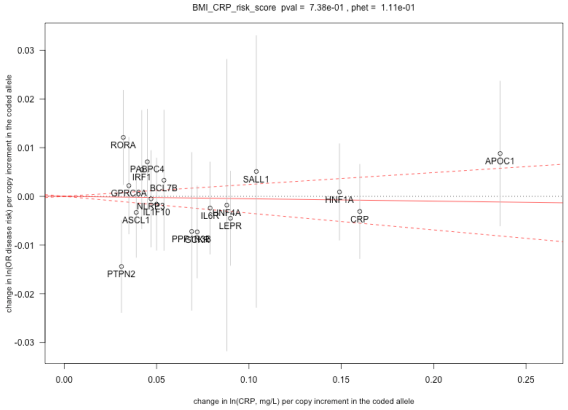
S. Ischemic Stroke (Small Vessel Disease)



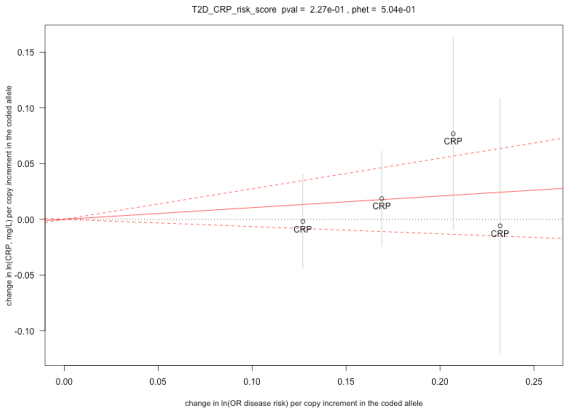


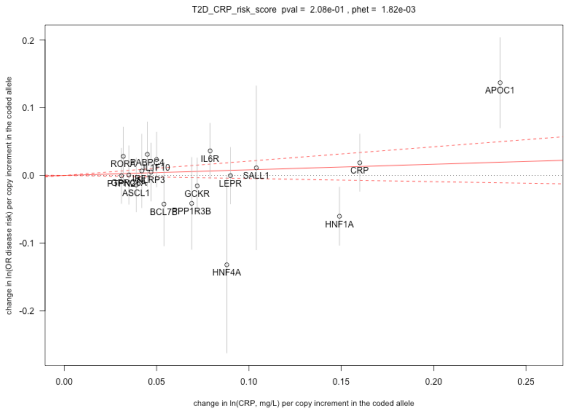
T. Bodv Mass Index



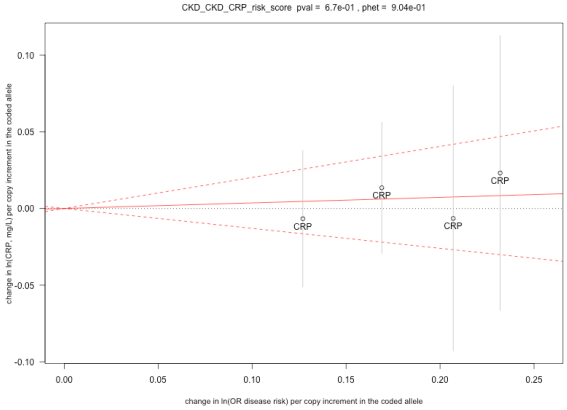


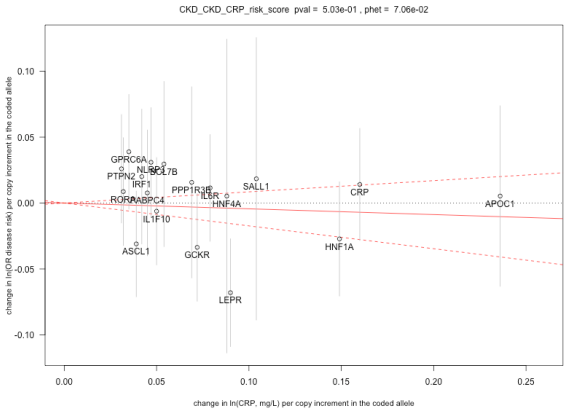
U. Type II Diabetes



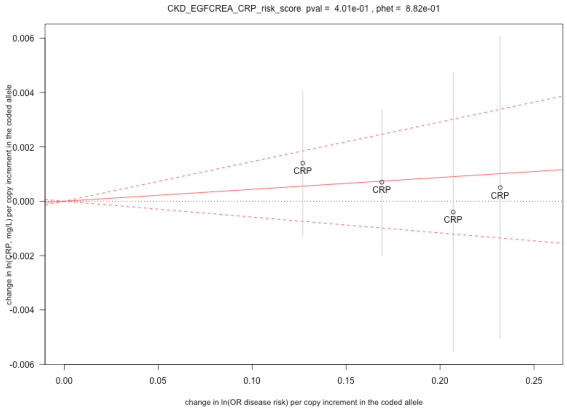


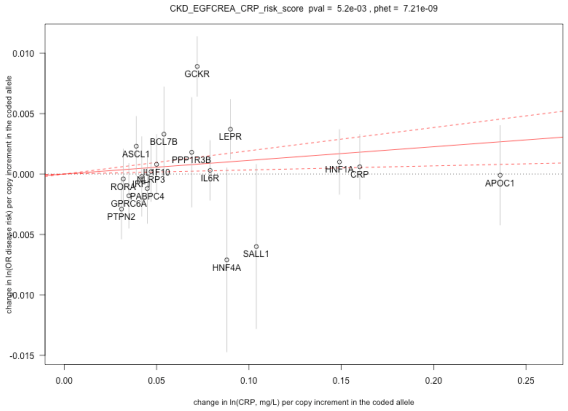
V. Chronic Kidney Disease



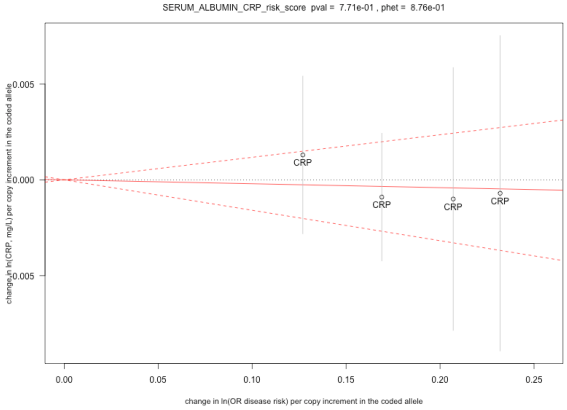


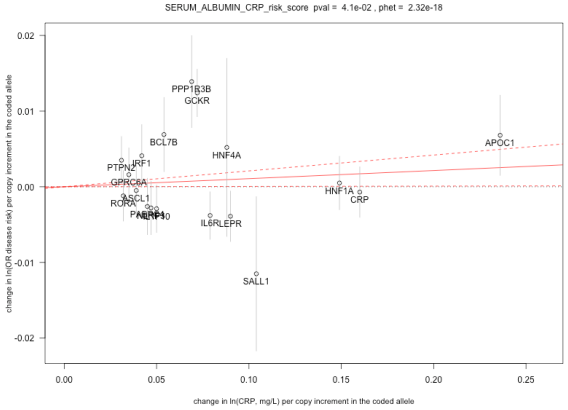
W. eGFR for Creatinine



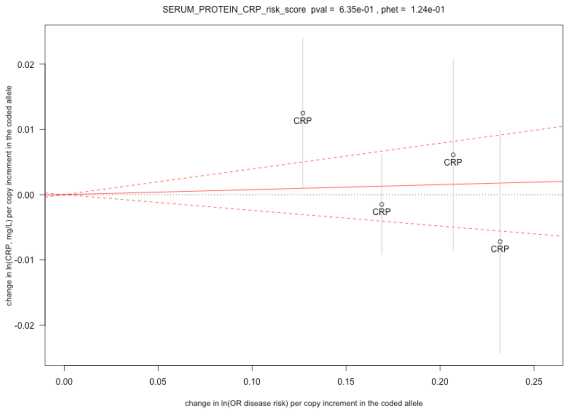


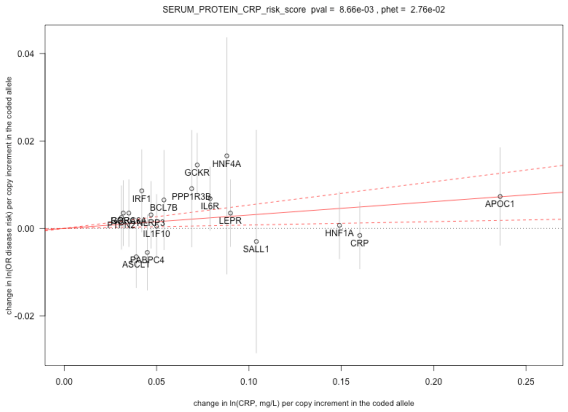
X. Serum Albumin Levels



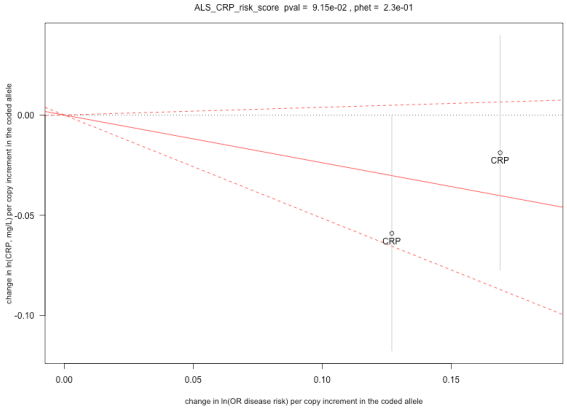


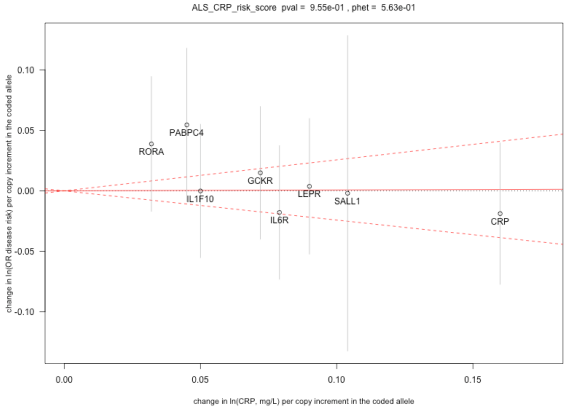
Y. Serum Protein Levels



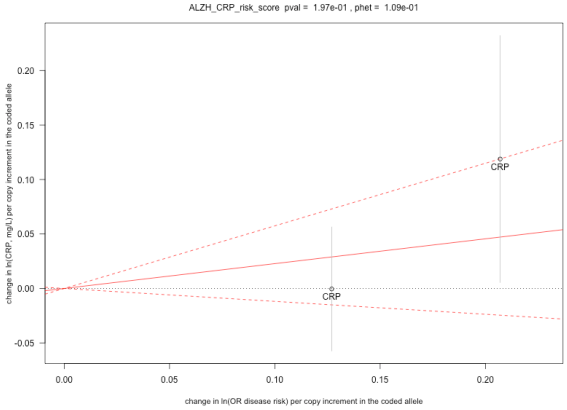


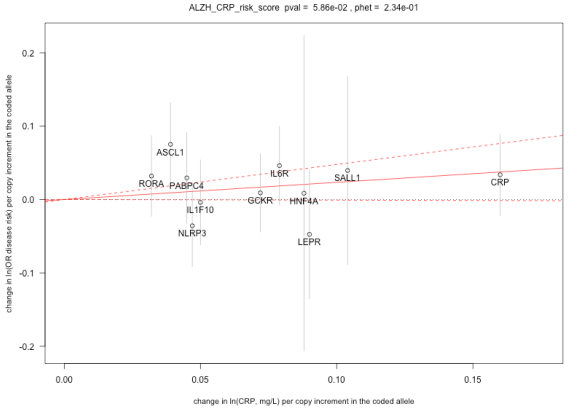
Z. Amyotrophic Lateral Sclerosis



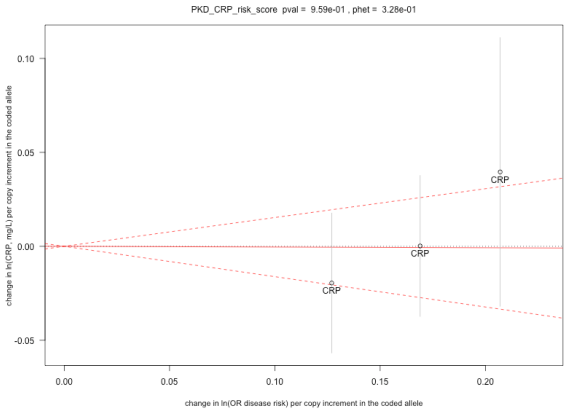


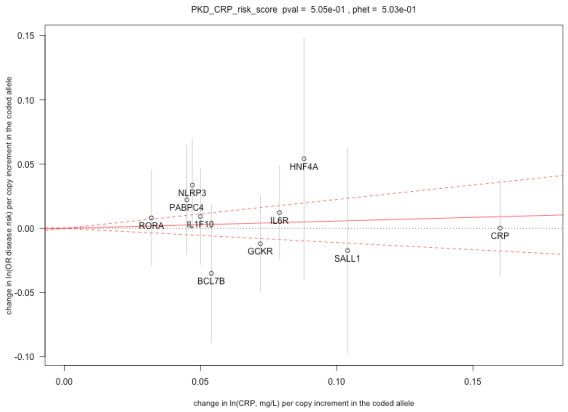
AA. Alzheimer's Disease



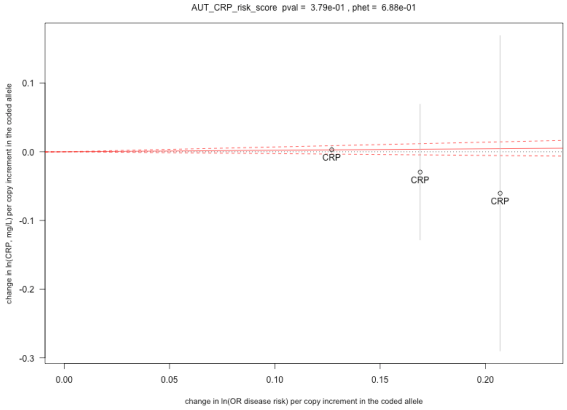


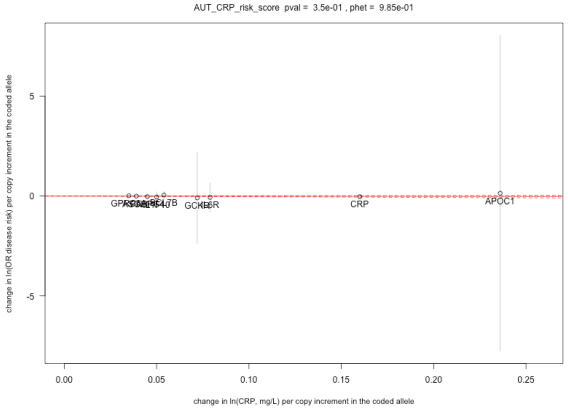
AB. Parkinsons's Disease



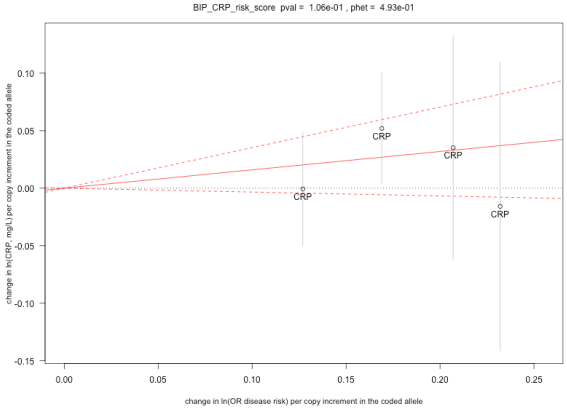


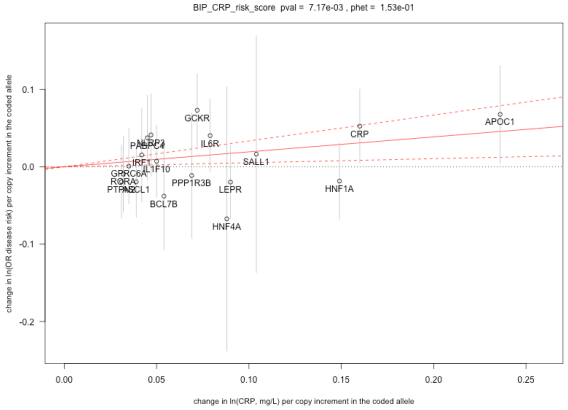
AC. Autism



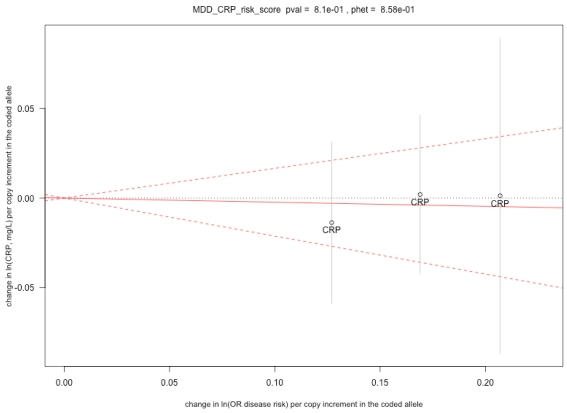


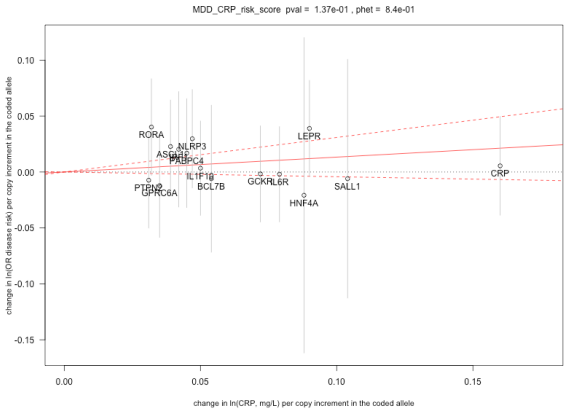
AD. Bipolar Disorder



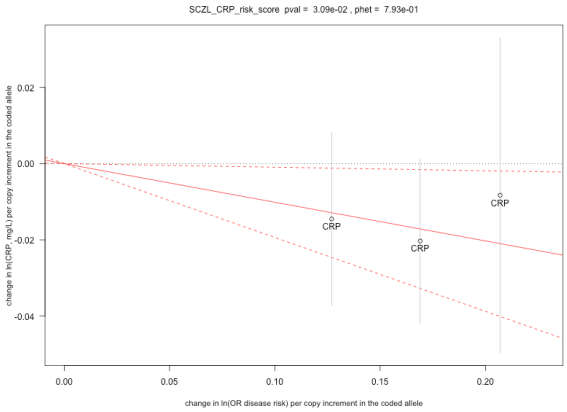


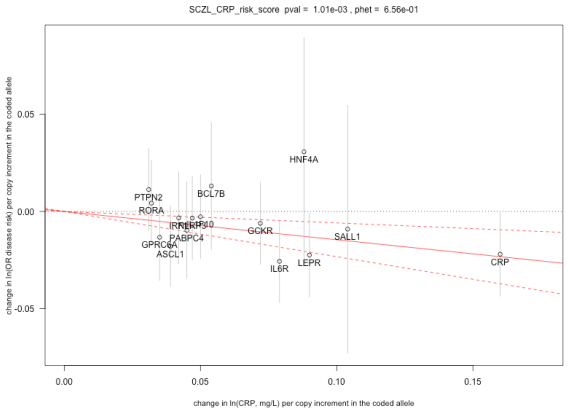
AE. Major Depressive Disorder





AF. Schizophrenia





None of the study sponsors had a role in the study design, data collection, analysis and interpretation, report writing, or the decision to submit the report for publication.

BPP was supported by a scholarship from Graduate School of Medical Sciences, UMCG, Groningen, The Netherlands, and by grants from the Economic & Social Research Council (ES/H029745/1) and the Wellcome Trust (WT098051), the United Kingdom. AV was supported by a scholarship from Isfahan University of Medical Sciences, Isfahan, Iran and a scholarship from Graduate School of Medical Sciences, UMCG. Groningen, The Netherlands. AA is supported by a Rubicon grant from the Netherlands Organization for Scientific Research (NWO project no. 825.13.004) and by Medical Research Council UK (grant reference no. MC-U106179471). H.M. is supported by an NIHR Senior Investigator award. His work is supported by a NIHR Comprehensive Biomedical Research Centre award to Cambridge University Hospitals. R.K.W. is supported by a VIDI grant (016.136.308) from the Netherlands Organization for Scientific Research (NWO). AM is a Wellcome Trust Senior Fellow in Basic Biomedical Science (grant number WT098017). PM is supported by the research program of the NIHR Barts Cardiovascular Biomedical Research Unit.

PAGE was supported by NIH Grants R01AR042742, R01AR054966, and R01AR050511. SSc consortia was supported by the grant SAF2012-34435 from the Spanish Ministry of Economy and Competitiveness and CTS-4977 from Junta de Andalucía, and is sponsored by the Orphan Disease Program grant from the European League Against Rheumatism (EULAR). The US analyses were supported by the NIH/NIAMS R01 AR055258, Two-Stage Genome Wide Association Study in Systemic Sclerosis.

T1DGC would like to state that all genotyping and other aspects of the study were funded by an Institutional Development Fund paid to the Center for Applied Genomics from the Children's Hospital of Philadelphia." are funded in part by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) award (DP3 DK085708) and SFAG and HH by a Development award from the Cotswold Foundation. T1D was also funded by the Children's Hospital of Philadelphia, Genome Canada through the Ontario Genomics Institute, and the Juvenile Diabetes Research Foundation. This research utilizes resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418., and the Wellcome Trust under award 076113.

This manuscript was not prepared in collaboration with investigators of the T1DGC study, except for those listed as authors on the current manuscript, and does not necessarily reflect the opinions or views of the T1DGC study, the NIDDK Central Repositories, or the NIDDK. The Diabetes Control and Complications Trial (DCCT) and its follow-up the

Epidemiology of Diabetes Interventions and Complications (EDIC) study were conducted by the DCCT/EDIC Research Group and supported by National Institute of Health grants and contracts and by the General Clinical Research Center Program, NCRR. This manuscript was not prepared under the auspices of the DCCT/EDIC study and does not represent analyses or conclusions of the DCCT/EDIC study group. The Genetics of Kidneys in Diabetes (GoKinD) Study was conducted by the GoKinD Investigators and supported by the Juvenile Diabetes Research Foundation, the CDC, and the Special Statutory Funding Program for Type 1 Diabetes Research administered by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). This manuscript was not prepared in collaboration with investigators of the GoKinD study and does not necessarily reflect the opinions or views of the GoKinD study, the NIDDK Central Repositories, or the NIDDK.

GD: Cardiff University was supported by the Wellcome Trust, Medical Research Council (MRC), Alzheimer's Research UK (ARUK) and the Welsh Assembly Government. Cambridge University and Kings College London acknowledge support from the MRC. ARUK supported sample collections at the South West Dementia Bank and the Universities of Nottingham, Manchester and Belfast. The Belfast group acknowledges support from the Alzheimer's Society, Ulster Garden Villages, N. Ireland R&D Office and the Royal College of Physicians/Dunhill Medical Trust. The MRC and Mercer's Institute for Research on Ageing supported the Trinity College group. The South West Dementia Brain Bank acknowledges support from Bristol Research into Alzheimer's and Care of the Elderly. The Charles Wolfson Charitable Trust supported the OPTIMA group. Washington University was funded by NIH grants, Barnes Jewish Foundation and the Charles and Joanne Knight Alzheimer's Research Initiative. Patient recruitment for the MRC Prion Unit/UCL Department of Neurodegenerative Disease collection was supported by the UCLH/UCL Biomedical Centre and NIHR Queen Square Dementia Biomedical Research Unit. LASER-AD was funded by Lundbeck SA. The Bonn group was supported by the German Federal Ministry of Education and Research (BMBF), Competence Network Dementia and Competence Network Degenerative Dementia, and by the Alfried Krupp von Bohlen und Halbach-Stiftung. GD also used samples ascertained by the NIMH AD Genetics Initiative. The Framingham Heart Study (FHS) and inflammation biomarkers collection is supported by the National Institute of Health, USA (grants numbers: R01 HL076784; R01 AG028321; and RO1 HL64753, contract numbers HHSN268201500001I & N01-HC 25195).

The ALS consortium is an EU Joint Programme - Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organisations under the aegis of JPND - www.jpnd.eu (United Kingdom, Medical Research Council and Economic and Social Research Council). AAC receives salary support from the National Institute for Health Research (NIHR) Dementia Biomedical Research Unit at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

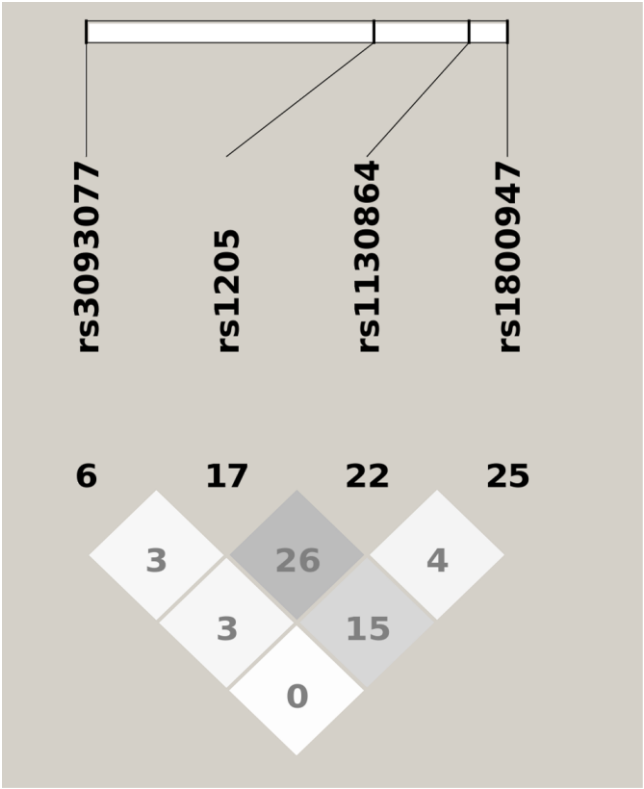
The work leading up to this publication was funded by the European Community's Health Seventh Framework Programme (FP7/2007–2013; grant agreement number 259867).

Investigating the causal relationship of C-reactive protein with 32 complex somatic and psychiatric outcomes: A large scale cross-consortia Mendelian randomization study.

Supplementary Methods 1: Linkage disequilibrium of the four GRS_{CRP} SNPs.

Methods: The figure was generated using Haploview. LD values (r^2) were calculated using Hapmap Phase II+III (r28, CEU population) data. r^2 values are shown between SNPs pairs on a scale between 0 (no LD) to 100 (full LD) in diamonds colored on a corresponding grayscale, where no LD is represented by white and full LD by black. This plot indicates low LD between each of the four SNPs spanning the CRP gene.

S2 Fig: Linkage disequilibrium (LD) heatplot for 4 SNPs as used in the GRS_{CRP} IV.



Investigating the causal relationship of C-reactive protein with 32 complex somatic and psychiatric outcomes: A large scale cross-consortia Mendelian randomization study.

Supplementary Methods - 2: CRP GRSG*WAS* in AD and BMI.

A. Calculation of the Alzheimer's Disease GWAS summary statistics.

We received summary statistics (SNP, OR, SE, P, Reference Allele, Other Allele, OR 95 L ,OR 95 U) from the Genetic and Environmental Risk in Alzheimer's Disease (GERAD) consortium for three separate Alzheimer's datasets ; from the TGEN consortium, from the ADNI consortium, and from the GERAD consortium for up to 4,663 cases and 8,357 controls. We next performed an inverse variance weighted fixed effects analysis using GWAMA[1] to calculate combined effect sizes and standard errors, which were subsequently used in our genetic risk scores.

B. Calculation of the BMI GWAS summary statistics.

We downloaded sex-stratified summary statistics for BMI from Randall et.al [2]. From https://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files. We next performed an inverse variance weighted fixed effects analysis using GWAMA[1] to calculate combined effect sizes and standard errors, which were subsequently used in our genetic risk scores.

[1] Mägi R, Morris AP. GWAMA: software for genome-wide association meta-analysis. BMC Bioinformatics. 2010 May 28;11:288.

[2] Randall JC. Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. PLoS Genet. 2013 Jun;9(6):e1003500.

Investigating the causal relationship of C-reactive protein with 32 complex somatic and psychiatric outcomes: A large scale cross-consortia Mendelian randomization study.

Supplementary Methods - 3: WEBLINKS.

Software :

Genetics ToolboX (version 0.0.8):

http://cran.r-project.org/web/packages/gtx/index.html

Functions for Medical Statistics Book with some Demographic Data

https://cran.r-project.org/web/packages/fmsb/

http://www.genemania.org

Publicly downloaded GWAS summary statistics:

1. GIANT BMI summary statistics:

www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files

2. CARDIoGRAM CAD summary statistics:

www.cardiogramplusc4d.org

3. Rheumatoid arthritis summary statistics:

www.broadinstitute.org/ftp/pub/rheumatoid arthritis/Stahl_etal_2010NG/

4. PGC consortium (psychiatric) summary statistics:

www.med.unc.edu/pgc/downloads

5. Systemic Lupus Erythematosus

Data was downloaded through dbGaP : http://www.ncbi.nlm.nih.gov/gap

Study name : Whole Genome Association Study of Systemic Lupus Erythematosus

dbGaP Study Accession: phs000122.v1.p1

Analysis Name and Accession

Name: Whole Genome Association Study of Systemic Lupus Erythematosus

Accession: pha002848.1

Investigating the causal relationship of C-reactive protein with 32 complex somatic and psychiatric outcomes: A large scale cross-consortia Mendelian randomization study.

Supplementary Methods - 4: CRP Polygenic risk score (CRP_{PRS}) in Schizophrenia.

Background: We observed a causally protective effect of CRP GWSAs against Schizophrenia. We aim to determine whether a) CRP-associated risk alleles are associated with schizophrenia by employing the use of polygenic risk scores, and b) to determine if the relationship between CRP and schizophrenia is due to genetic pleiotropy or clinical heterogeneity. Here below we explain study design and applied methods.

Sample Description: Individual-level dosage data was retrieved from the Psychiatric Genomics Consortium (PGC) Schizophrenia dataset, consisting of 36 independent cohorts with a combined 25629 cases and 30976 controls. 3 family-based samples of European ancestry (1,235 parent affected-offspring trios) were excluded from our analysis. For a more detailed treatment of the PGC schizophrenia dataset, refer to the methods section of the full paper (Ripke et al., 2014).

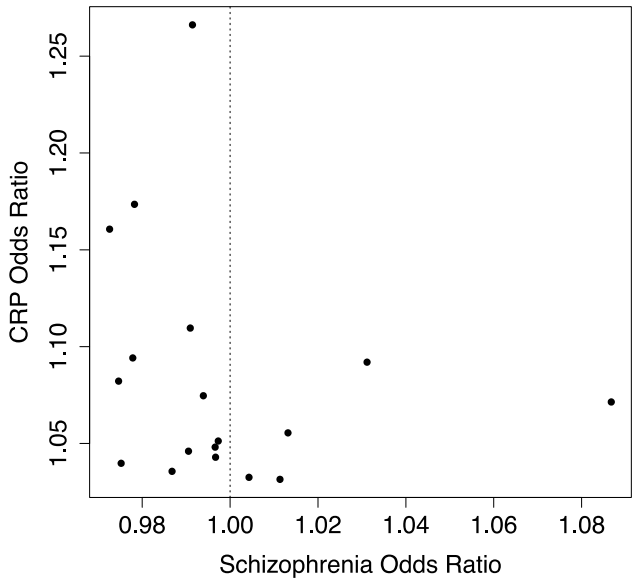
Selection of CRP-associated SNPs : Summary statistics (including SNP RSID, chromosome and position, coded allele, beta, standard error, and *p* value) were retrieved from a meta-analysis of 15 independent European population-based studies with 66,185 participants (Dehghan et al., 2011). After accounting for the replication sample, 18 SNPs reached genome-wide significance ($p < 5 \times 10^{8}$) and were thus included in the study. All non-genome-wide significant SNPs were clumped based on linkage disequilibrium estimates using plink v2 with the following flags: --clump-p1 1 –clump-p2 1 –clump-r2 0.1 –clump-kb 1000. Thus, all SNPs included in the analyses were LD-independent, as defined by SNPs with $r^2 < 0.1$ (Purcell et al., 2007). We subsequently grouped the sub-threshold CRP-associated SNPs at the following *p*-value thresholds: $1x10^{-4}$, 0.001, 0.01, 0.05, and 0.1.

Statistical Analysis: Polygenic risk scores were calculated for each individual by summing the total effect of the SNP dosages by its effect size. This was performed for each independent schizophrenia cohort using plink v2 with the –score and –qscore flags (Purcell et al., 2007). A fixed effects, inverse variance weighted meta-analysis was performed across all 36 cohorts using a custom R script. Briefly, beta estimates (log odds ratios) was weighted by the estimated standard errors. 10 PCs were regressed out of each independent cohort to account for population stratification.

Results

Examining the association between the 18 CRP-associated risk alleles in GWAS and schizophrenia

13 out of the 18 SNPs associated with elevated CRP levels were protective against schizophrenia (Fig 1, and S3 Fig). Five out of the 13 SNPs reached a significance threshold of $p < 0.05$ (S3 Fig). A sign test was performed to determine if the risk alleles for CRP are shared with schizophrenia more than expected (P<0.096).



S3 Fig: 18 risk alleles associated with elevated CRP levels that reached genome-wide significance, and their corresponding schizophrenia odds ratios.

Supplementary Methods - 5: In-silico (gene) pathway analyses highlight the role of IFN in the causal pathway between CRP and SCZ.

Background: We aimed to explore the possible underlying pathways that may underwrite the protective causal association between CRP and schizophrenia. We answered this question by performing two studies: In the first study we aimed to elucidate through which potential pathways CRP associated proteins (encoded by those genes which are tagged by CRP associated gSNPs and eSNPs potentially are linked to genes associated to SCZ, more specifically, those that are differentially expressed in schizophrenia cases versus controls

Study I : Identification of common pathways between genes / their encoded proteins involved in determination on CRP levels and proteins differentially expressed in schizophrenia cases versus controls.

The following 3 steps were taken:

1.1) We firstly retrieved a list of CRP-associated genes whom harboured by loci associated with CRP in the large GWAS meta-analysis for CRP to date [6] and genes for which their expression was associated with one or more of the above mentioned 18 loci as presented by Vaez et.al.[7]. These groups of genes and their selection processes have been discussed in greater detail in our previous publication by Vaez et.al.[7] and presented in Table 2 of the respective paper and for convenience listed again in our S4 Table (tabname "CRP genes"). We used the 40 CRP genes from Table 2 in Vaez et. al. [7] and performed enrichment analyses as explained in the same publication [7] using the well-established pathway and network analysis suite Genemania (S3 Methods Weblinks) [8], the result of which is presented in S Table 6 (tabname "S6. CRP enriched pathways"). As previously observed[7] the most enriched functions within this geneset are related to the type I interferon signaling pathway (FDR=6.08×10^{-9}).

1.2) Secondly, we retrieved a list of 144 proteins, which are significantly differentially expressed in brain tissues of schizophrenia cases compared with matched controls, hence are expected to be involved in the mechanism of schizophrenia. These proteins are presented in the study by Hwang et.al. [9] in their respective S Table 4. These proteins are listed in our S6 Table (tabname "SCZ expr genes"). We performed functional enrichment analysis for this geneset in the same fashion as described above, the results of which are presented in S7 Table (tabname "SCZ expr enriched pathways").

None of the differentially expressed proteins from the study of Hwang et.al overlap. with proteins encoded by genes identified by Vaez et.al. except the *HEYL*, which is a non-inflammatory gene. Surprisingly, we observed that the most significantly enriched function for the set of differentially expressed proteins is also the type I interferon signalling pathway (FDR=2.81×10^{-12}) .

1.3) Lastly, to confirm the type I interferon signalling pathway may be the predominant pathway that is shared between these two sets of proteins, we performed functional prediction and pathway enrichment analyses by including all the 40 CRP associated and 144 SCZ associated genes as presented S8 Table (tabname "CRP & SCZ expr gene list"), the results of which are presented in S9 Table (tabname "CRP&SCZ enriched pathways"). We observed again

that the type I interferon signalling pathway remained as the most significantly enriched pathway (FDR=8.60×10^{-22}).

Summary of study 1 : Identification of common pathways between genes / their encoded proteins involved in determination on CRP levels and proteins differentially expressed in schizophrenia cases versus controls.

Our *in-silico* functional enrichment analysis from both CRP and SCZ associated genes showed the enrichment of pathways of "response to type I interferon", "cellular response to type I interferon", "type I interferon signaling pathway".

We therefore speculate the protective causal effect of CRP might be explained by the fact that T-cell IFN cytokine release stimulates microglia to facilitate glutamate clearance in neuronal cells without evoking inflammatory mediators, and by contributing to restoration of normal homeostasis[1,2].

Study 2 : Identification of pathways involved in schizophrenia based on loci identified in the largest Genome Wide Association Study for schizophrenia and their associated eQTLs in brain and blood, excluding any known CRP associated genes and associated eQTLs.

Our second study intended to investigate through which pathways do **non-CRP** SCZ associated genes and their encoded proteins affect SCZ. We took a similar approach to our first study, where now instead we use the results from the largest meta-GWAS study for schizophrenia[10] as a basis as follows :

2.1) Firstly, we extracted the list of genes harboured by 108 SCZ associated loci at genome wide significance level from the largest meta-GWAs in SCZ published by the Schizophrenia Working Group of the Psychiatric Genomics Consortium, as listed in S Table 3 in the respective study[10] and in our S10 Table (tabname "SCZ 108 loci genes").

2.2) Next we extracted all genes tagged by expression quantitative trait loci (eQTL) from a human brain cortex eQTL study and another eQTL study performed in peripheral blood cells as listed in S Table 4 in the same publication [10], and in our S11 Table (tabname "SCZ 108 loci eQTL") and merged these with the list of genes harboured by 108 SCZ associated loci mentioned above. We removed duplicate genes and to focus on non-CRP associated pathways, we additionally removed genes overlapping with those from Vaez et. al., resulting in the removal of in total, C12orf42). The combined set of genes is presented in S12 Table (tabname "SCZ eQTL + 108 loci genes").

2.3) We performed the same functional enrichment analyses as explained earlier on this combined set of 407 genes. The (non-)results are presented in S13 Table (tabname "eQTL&SCZ loci enr pathways").

Summary of Study 2 : Identification of pathways involved in schizophrenia based on loci identified in the largest Genome Wide Association Study for schizophrenia and their associated eQTLs in brain and blood, excluding any known CRP associated genes and associated eQTLs.

Similar to the main manuscript, we failed to identify any significantly enriched pathways, in our case using Genemania, although nominally significantly enrichments were observed for several

predefined candidate pathways such as calcium channels in the original publication. In our case we included also eQTL loci whereas the original study used the meta-analysis summary statistics. We did not identify any inflammatory related pathways these analyses. The report from the Schizophrenia Working Group of the Psychiatric Genomics Consortium does however provide some evidence for a role of the immune system in schizophrenia development when searching for the most relevant cell-types based on their genomic loci and cell and tissue type specific enhancers enrichment analyses ; two peaks can be observed in Fig 2 for B-lymphocyte lineages involved in acquired immunity (CD19 and CD20 cell lines), which remained significant even after excluding the extended MHC region and regions containing brain enhancers[10]. The main original study has not report any significantly enriched pathway, and neither did our analyses led to such a conclusion based on the presented data in [10] .

Taken together, our *in-silico* analyses point to a role for pathway associated to IFN response and metabolism that may possibly underlie protective effect of CRP in schizophrenia. Specifically, we speculate that CRP-IFN pathway perhaps contributes to neuroprotection by stimulating a phenotype in neuron supporting cells such as astrocytes or microglia that facilitates glutamate reduction as observed directly for IFN by Shaked et.al [2] and Garg et.al [1] leading to the protection of neurons against oxidative stress associated with an excess of glutamate.

Associated files:

Please find lists of trait-associated genes and results of pathway enrichment analyses in S4 Table to S13 Table. The first tab in this file, named as “Contents” contains a guide to the contents of this file.

Web link: http://www.genemania.org

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