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Institutions: Qatar University, Broad Institute, Lund University, Boston Children's Hospital ...+79 more institutions

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Rare coding variants in 35 genes associate with circulating lipid levels – a multi-ancestry analysis of 170,000 exomes

George Hindy[1-3], Peter Dornbos[4-6], Mark D. Chaffin[2,7,8], Dajiang J. Liu[9], Minxian Wang[2,7,10], Carlos A. Aguilar-Salinas[11], Lucinda Antonacci-Fulton[12,13], Diego Ardissino[14-16], Donna K. Arnett[17], Stella Aslibekyan[18], Gil Atzmon[19,20], Christie M. Ballantyne[21,22], Francisco Barajas-Olmos[23], Nir Barzilai[19], Lewis C. Becker[24], Lawrence F. Bielak[25], Joshua C. Bis[26], John Blangero[27], Eric Boerwinkle[28,29], Lori L. Bonnycastle[30], Erwin Bottinger[31,32], Donald W. Bowden[33], Matthew J. Bown[34,35], Jennifer A. Brody[26], Jai G. Broome[36], Noël P. Burtt[4], Brian E. Cade[37,38], Federico Centeno-Cruz[23], Edmund Chan[39], Yi-Cheng Chang[40], Yii-Der I. Chen[41], Ching-Yu Cheng[42-44], Won Jung Choi[45], Rajiv Chowdhury[46,47], Cecilia Contreras-Cubas[23], Emilio J. Córdova[23], Adolfo Correa[48], L Adrienne Cupples[49,50], Joanne E. Curran[27], John Danesh[46,51], Paul S. de Vries[28], Ralph A. DeFronzo[52], Harsha Doddapaneni[29], Ravindranath Duggirala[27], Susan K. Dutcher[12,13], Patrick T. Ellinor[8,10], Leslie S. Emery[36], Jose C. Florez[2,7,53,54], Myriam Fornage[55], Barry I. Freedman[56], Valentin Fuster[57,58], Ma. Eugenia Garay-Sevilla[59], Humberto García-Ortiz[23], Soren Germer[60], Richard A. Gibbs[29,61], Christian Gieger[62-64], Benjamin Glaser[65], Clicerio Gonzalez[66], Maria Elena Gonzalez-Villalpando[67], Mariaelisa Graff[68], Sarah E Graham[69], Niels Grarup[70], Leif C. Groop[71,72], Xiuqing Guo[41], Namrata Gupta[2], Sohee Han[73], Craig L. Hanis[74], Torben Hansen[70,75], Jiang He[76,77], Nancy L. Heard-Costa[50,78], Yi-Jen Hung[79], Mi Yeong Hwang[73], Marguerite R. Irvin[80], Sergio Islas-Andrade[81], Gail P. Jarvik[82], Hyun Min Kang[83], Sharon L.R. Kardia[25], Tanika Kelly[76], Eimear E. Kenny[57,84,85], Alyna T. Khan[36], Bong-Jo Kim[73], Ryan W. Kim[45], Young Jin Kim[73], Heikki A. Koistinen[86-88], Charles Kooperberg[89], Johanna Kuusisto[90], Soo Heon Kwak[91], Markku Laakso[90], Leslie A. Lange[92], Jiwon Lee[37], Juyoung Lee[73], Seonwook Lee[45], Donna M. Lehman[52], Rozenn N. Lemaitre[26], Allan Linneberg[93,94], Jianjun Liu[39,95], Ruth J.F. Loos[96,97], Steven A. Lubitz[8,10], Valeriya Lyssenko[71,98], Ronald C.W. Ma[99-101], Lisa Warsinger Martin[102], Angélica Martínez-Hernández[23], Rasika A. Mathias[24], Stephen T. McGarvey[103], Ruth McPherson[104], James B. Meigs[54,105,106], Thomas Meitinger[107,108], Olle Melander[71,109], Elvia Mendoza-Caamal[23], Ginger A. Metcalf[29], Xuenan Mi[76], Karen L. Mohlke[110], May E. Montasser[111], Jee-Young Moon[112], Hortensia Moreno-Macías[113], Alanna C. Morrison[28], Donna M. Muzny[29], Sarah C. Nelson[36], Peter M. Nilsson[3], Jeffrey R. O'Connell[111], Marju Orho-Melander[3], Lorena Orozco[23], Colin N.A. Palmer[114], Nicholette D. Palmer[33], Cheol Joo Park[45], Kyong Soo Park[91,115,116], Oluf Pedersen[70], Juan M. Peralta[27], Patricia A. Peyser[25], Wendy S. Post[117], Michael Preuss[96], Bruce M. Psaty[26,118,119], Qibin Qi[112], DC Rao[120], Susan Redline[37,38], Alexander P. Reiner[121], Cristina Revilla-Monsalve[122], Stephen S. Rich[123], Nilesh Samani[34,35], Heribert Schunkert[124], Claudia Schurmann[31,32,96], Daekwan Seo[45], Jeong-Sun Seo[45], Xueling Sim[125], Rob Sladek[126-128], Kerrin S. Small[129], Wing Yee So[99-101], Adrienne M. Stilp[36], S. Margaret Sunitha[2,7], E Shyong Tai[39,125,130], Claudia H.T. Tam[99-101], Kent D. Taylor[41], Yik Ying Teo[125,131,132], Farook Thameem[133], Brian Tomlinson[134], Michael Y. Tsai[135], Tiinamaija Tuomi[136-138], Jaakko Tuomilehto[139-141], Teresa Tusié-Luna[11,142], Rob M. van Dam[125,143], Ramachandran S. Vasan[50,144], Karine A. Viaud Martinez[145], Fei Fei Wang[36], Xuzhi Wang[49], Hugh Watkins[146], Daniel E. Weeks[147,148], James G. Wilson[149], Daniel R. Witte[150,151], Tien-Yin Wong[42-44], Lisa R. Yanek[24], AMP-T2D-GENES, Myocardial Infarction Genetics Consortium, NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, NHLBI TOPMed Lipids Working Group, Sekar Kathiresan[2,7,54,152], Jerome I. Rotter[41], Michael Boehnke[83], Mark I. McCarthy[†153,154], Cristen J. Willer[69,155,156], Pradeep Natarajan[2,8,10,54], Jason A. Flannick[4-6], Amit V. Khera[2,7,8], Gina M. Peloso*[49]

1 Department of Population Medicine, Qatar University College of Medicine, QU Health, Doha, Qatar.

2 Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA, 02142.

3 Department of Clinical Sciences, Lund University, Malmö, Sweden.

4 Programs in Metabolism and Medical & Population Genetics, Broad Institute, Cambridge, Massachusetts, USA.

5 Department of Pediatrics, Harvard Medical School, Boston, MA, USA, 02115.

6 Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, USA, 02115.

7 Center for Genomic Medicine, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA, 02114.

8 Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA, USA, 02114.

9 Department of Public Health Sciences, Penn State College of Medicine, Hershey, PA, 17033.

10 Cardiovascular Disease Initaitive, Broad Institute of Harvard and MIT, Cambridge, MA, USA, 02142.

11 Instituto Nacional de Ciencias Medicas y Nutricion, Mexico City, Mexico.

12 Department of Genetics, Washington University in St. Louis, St. Louis, MO, 63110, USA.

13 The McDonnell Genome Institute, Washington University School of Medicine, St. Louis, MO, 63108, USA.

14 ASTC: Associazione per lo Studio Della Trombosi in Cardiologia, Pavia, Italy.

15 Azienda Ospedaliero-Universitaria di Parma, ÊParma, Italy.

16 Universitˆ degli Studi di Parma, Parma, Italy.

17 Dean's Office, College of Public Health, University of Kentucky.

18 Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL, USA.

19 Departments of Medicine and Genetics, Albert Einstein College of Medicine, New York, USA.

20 University of Haifa, Faculty of natural science, Haifa, Isarel.

21 Houston Methodist Debakey Heart and Vascular Center, Houston, TX, USA, 77030.

22 Section of Cardiovascular Research, Baylor College of Medicine, Houston, TX, USA, 77030.

23 Instituto Nacional de Medicina Genómica, Mexico City, Mexico.

24 Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA 21287.

25 Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, USA 49109.

26 Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, USA, 98101.

27 Department of Human Genetics and South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley School of Medicine, Brownsville, TX 78520, USA.

28 Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX, USA, 77030.

29 Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, 77030, USA.

30 Medical Genomics and Metabolic Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA.

31 Hasso Plattner Institute for Digital Health at Mount Sinai, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.

32 Digital Health Center, Hasso Plattner Institute, University of Potsdam, Potsdam, Germany.

33 Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem, NC, USA, 27157.

34 Department of Cardiovascular Sciences, University of Leicester, Leicester, UK.

35 NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester UK.

36 Department of Biostatistics, University of Washington, Seattle, WA, USA, 98195.

37 Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115.

38 Division of Sleep Medicine, Harvard Medical School, Boston, MA 02115.

39 Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, Singapore.

40 Institute of Biomedical Sciences, Academia Sinica, Taiwan.

41 The Institute for Translational Genomics and Population Sciences, Department of Pediatrics, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA 90502.

42 Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, , Singapore.

43 Ophthalmology & Visual Sciences Academic Clinical Program (Eye ACP), Duke-NUS Medical School, Singapore.

44 Singapore Eye Research Institute, Singapore National Eye Centre, Singapore.

45 Psomagen. Inc. (formerly Macrogen USA), 1330 Piccard Drive Ste 103, Rockville, MD 20850.

46 MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.

47 Centre for Non-Communicable disease Research (CNCR), Bangladesh.

48 Department of Medicine, University of Mississippi Medical Center, Jackson, MS, USA, 39216.

49 Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA.

50 NHLBI Framingham Heart Study, Framingham, Massachusetts, USA.

51 The National Institute for Health Research Blood and Transplant Research Unit (NIHR BTRU) in Donor Health and Genomics at the University of Cambridge, Cambridge, UK.

52 Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, USA.

53 Diabetes Research Center (Diabetes Unit), Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA.

54 Department of Medicine, Harvard Medical School, Boston, MA, USA, 02115.

55 Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 770030.

56 Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, NC, USA 27157.

57 Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029.

58 Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.

59 Department of Medical Science, Division of Health Science. University of Guanajuato.

60 New York Genome Center, New York, NY, 10013, USA.

61 Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, 77030, USA.

62 Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany.

63 Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany.

64 German Center for Diabetes Research (DZD), Neuherberg, Germany.

65 Endocrinology and Metabolism Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

66 Unidad de Diabetes y Riesgo Cardiovascular, Instituto Nacional de Salud Pœblica, Cuernavaca, Morelos, Mexico.

67 Centro de Estudios en Diabetes, Mexico City, Mexico.

68 Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA.

69 Department of Internal Medicine, University of Michigan, Ann Arbor, MI, 48109.

70 Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

71 Department of Clinical Sciences, Diabetes and Endocrinology, Lund University Diabetes Centre, Malmö, Sweden.

72 Finnish Institute for Molecular Genetics, University of Helsinki, Helsinki, Finland.

73 Division of Genome Science, Department of Precision Medicine, Chungcheongbuk-do, Republic of Korea.

74 Human Genetics Center, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas, USA.

75 Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark.

76 Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112.

77 Tulane University Translational Science Institute, New Orleans, LA 70112.

78 Department of Neurology, Boston University School of Medicine, Boston, Massachusetts, USA.

79 Division of Endocrine and Metabolism, Tri-Service General Hospital Songshan branch, Taipei, Taiwan.

80 Department of Epidemiology, School of Public Health, UAB, Birmingham AL 35294.

81 Dirección de Investigación, Hospital General de México "Dr. Eduardo Liceaga", Secretaría de Salud, Mexico City, Mexico.

82 Departments of Medicine (Medical Genetics) and Genome Sciences, University of Washington Medical Center, Seattle, WA 98195.

83 Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, 48109.

84 Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029.

85 Institute for Genomic Health, Icahn School of Medicine at Mount Sinai, New York, NY 10029.

86 Department of Public Health Solutions, Finnish Institute for Health and Welfare, Helsinki, Finland.

87 Minerva Foundation Institute for Medical Research, Helsinki, Finland.

88 University of Helsinki and Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland.

89 Division of Public Health Sciences, Fred Huchinson Cancer Research Center, Seattle WA USA, 98103.

90 Institute of Clinical Medicine, University of Eastern Finland, and Kuopio University Hospital, Kuopio, Finland.

91 Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea.

92 Department of Medicine, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA 80045.

93 Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, The Capital Region, Copenhagen, Denmark.

94 Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

95 Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore.

96 Charles R. Bronfman Institute of Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA.

97 The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029.

98 University of Bergen, Norway.

99 Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, China.

100 Hong Kong Institute of Diabetes and Obesity, The Chinese University of Hong Kong, Hong Kong, China.

101 Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China.

102 Division of Cardiology, Department of Medicine, George Washington University, Washington, D.C. USA 20037.

103 Department of Epidemiology and International Health Institute, Brown University School of Public Health, Providence, RI 02912.

104 Ruddy Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute, Ottawa, Canada.

105 General Medicine Division, Massachusetts General Hospital, Boston, Massachusetts, USA.

106 Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

107 Deutsches Forschungszentrum fŸr Herz-Kreislauferkrankungen (DZHK), Partner Site Munich Heart Alliance, Munich, Germany.

108 Institute of Human Genetics, Technische Universität München, Munich, Germany.

109 Department of Emergency and Internal Medicine, SkŒne University Hospital, Malmö, Sweden.

110 Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA.

111 University of Maryland School of Medicine, Division of Endocrinology, Diabetes and Nutrition and Program for Personalized and Genomic Medicine, Baltimore, MD, USA, 21201.

112 Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, USA 10461.

113 Universidad Autónoma Metropolitana, Mexico City, Mexico.

114 Pat Macpherson Centre for Pharmacogenetics and Pharmacogenomics, Division of Population Health and Genomics, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK.

115 Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.

116 Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea.

117 Division of Cardiology, Department of Medicine, Johns Hopkins University, Baltimore, Maryland, USA.

118 Department of Epidemiology, University of Washington, Seattle, WA, USA 98101.

119 Department of Health Services, University of Washington, Seattle, WA, USA 98101.

120 Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri, USA.

121 University of Washington, Seattle, Washington, USA.

122 Instituto Mexicano del Seguro Social SXXI, Mexico City, Mexico.

123 Center for Public Health Genomics, University of Virginia, Charlottesville, VA 22908, USA.

124 Deutsches Herzzentrum MŸnchen, Technische UniversitŠt MŸnchen, Deutsches Zentrum fŸr Herz-Kreislauf-Forschung, MŸnchen, Germany.

125 Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore.

126 Department of Human Genetics, McGill University, Montreal, Quebec, Canada.

127 Division of Endocrinology and Metabolism, Department of Medicine, McGill University, Montreal, Quebec, Canada.

128 McGill University and Génome Québec Innovation Centre, Montreal, Quebec, Canada.

129 Department of Twin Research and Genetic Epidemiology, King's College London, London, UK.

130 Duke-NUS Medical School Singapore, Singapore.

131 Department of Statistics and Applied Probability, National University of Singapore, Singapore.

132 Life Sciences Institute, National University of Singapore, Singapore.

133 Department of Biochemistry, Faculty of Medicine, Health Science Center, Kuwait University, Safat, Kuwait.

134 Faculty of Medicine, Macau University of Science & Technology, Macau, China.

135 Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, USA.

136 Department of Endocrinology, Abdominal Centre, Helsinki University Hospital, Helsinki, Finland.

137 Folkhälsan Research Centre, Helsinki, Finland.

138 Research Programs Unit, Diabetes and Obesity, University of Helsinki, Helsinki, Finland.

139 Public Health Promition Unit, Finnish Institute for Health and Welfare, Helsinki, Finland.

140 Department of Public Health, University of Helsinki, Helsinki, Finland.

141 Diabetes Research Group, King Abdulaziz University, Jeddah, Saudi Arabia.

142 Departamento de Medicina Genómica y Toxicología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México/ Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico.

143 Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, USA.

144 Departments of Medicine & Epidemiology, Boston University Schools of Medicine & Public Health, Boston, MA.

145 Illumina Laboratory Services, Illumina inc., San Diego, CA, 92122, USA.

146 Cardiovascular Medicine, Radcliffe Department of Medicine and the Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK.

147 Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, 15261.

148 Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, 15261.

149 Division of Cardiovascular Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215.

150 Department of Public Health, Aarhus University, Aarhus, Denmark.

151 Steno Diabetes Center Aarhus, Aarhus, Denmark.

152 Verve Therapeutics, Cambridge, MA, USA 02139.

153 Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

154 Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK.

155 Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, 48109.

156 Department of Human Genetics, University of Michigan, Ann Arbor, MI, 48109.

[†]CURRENT ADDRESS: Genentech, 1 DNA Way, South San Francisco, CA 94080

***Correspondence:** gpeloso@bu.edu

Summary

Large-scale gene sequencing studies for complex traits have the potential to identify causal genes with therapeutic implications. We performed gene-based association testing of blood lipid levels with rare (minor allele frequency<1%) predicted damaging coding variation using sequence data from >170,000 individuals from multiple ancestries: 97,493 European, 30,025 South Asian, 16,507 African, 16,440 Hispanic/Latino, 10,420 East Asian, and 1,182 Samoan. We identified 35 genes associated with circulating lipid levels. Ten of these: *ALB*, *SRSF2*, *JAK2, CREB3L3*, *TMEM136*, *VARS*, *NR1H3*, *PLA2G12A*, *PPARG* and *STAB1* have not been implicated for lipid levels using rare coding variation in population-based samples. We prioritize 32 genes identified in array-based genome-wide association study (GWAS) loci based on gene-based associations, of which three: *EVI5, SH2B3,* and *PLIN1*, had no prior evidence of rare coding variant associations. Most of the associated genes showed evidence of association in multiple ancestries. Also, we observed an enrichment of gene-based associations for low-density lipoprotein cholesterol drug target genes, and for genes closest to GWAS index single nucleotide polymorphisms (SNP). Our results demonstrate that gene-based associations can be beneficial for drug target development and provide evidence that the gene closest to the array-based GWAS index SNP is often the functional gene for blood lipid levels.

Introduction

Blood lipid levels are heritable complex risk factors for atherosclerotic cardiovascular diseases.[1] Array-based genome-wide association studies (GWAS) have identified >400 loci as associated with blood lipid levels, explaining 9-12% of the phenotypic variance of lipid traits.[2-8] These studies have identified mostly common (minor allele frequency (MAF)>1%) noncoding variants with modest effect and helped define the causal roles of different lipid fractions in cardiovascular disease.[9-13] Despite these advances, the mechanisms and causal genes for most of the identified variants and loci have not yet been determined.

Conventional GWAS with array-derived or imputed common variants are unlikely to directly implicate causal genes, while genetic association studies testing rare variants in coding regions have this potential. Advances in next generation sequencing over the last decade have facilitated increasingly larger studies with improved power to detect associations of rare variants with complex diseases and traits.[14,15] However, most exome sequencing studies to date have been insufficiently powered for rare variant discovery; for example, Flannick et al. estimated that it would require 75,000 to 185,000 sequenced cases of type 2 diabetes (T2D) to detect associations at known drug target genes at exome-wide significance.[15]

Identifying rare variants with impact on protein function has helped elucidate biological pathways underlying dyslipidemia and atherosclerotic diseases such as coronary artery disease (CAD).[14,16-25] Successes using this approach have led to the development of novel therapeutic targets to modify blood lipid levels and lower risk of atherosclerotic diseases.[26,27] The vast majority of participants in these studies have been of European ancestry, highlighting the need for more diverse study sample. Such diversity can identify associated variants absent or present at very low frequencies in European populations and help implicate new genes with generalizability extending to all populations.

We have assembled exome sequence data from >170,000 individuals across multiple ancestries and systematically tested the association of rare variants in each gene with six circulating lipid phenotypes: low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), non-HDL-C, total cholesterol (TC), triglycerides (TG), and the ratio of TG to HDL-C (TG:HDL). We find 35 genes associated with blood lipid levels, show evidence of gene-based signals in array-based GWAS loci, show enrichment of lipid gene-based associations in LDL-C drug targets and genes in close proximity with GWAS index variants, and test lipid genes for association with CAD, T2D, and liver enzymes.

Results

Sample and variant characteristics

Individual-level, quality-controlled data were obtained from four sequenced study sources with circulating lipid data for individuals of multiple ancestries (**Figure 1**). Characteristics of the study samples are detailed in **Supplementary Table 1.** We analyzed data on up to 172,000 individuals with LDL-C, non-HDL-C (a calculated measure of TC minus HDL-C), TC, HDL-C, TG, and TG:HDL ratio (a proxy for insulin resistance).[28,29] 56.7% (n=97,493) of the sample are of European ancestry, 17.4% (n=30,025) South Asian, 9.6% (n=16,507) African American, 9.6% (n=16,440) Hispanic, 6.1% (n=10,420) East Asian, and 0.7% (n=1,182) Samoan, based on genetically-estimated and/or self-reported ancestry.

After sequencing, we observed 15.6 M variants across all studies; 5.0 M (32.6%) we classified as transcript-altering coding variants based on an annotation of frameshift, missense, nonsense, or splice site acceptor/donor using the Variant Effect Predictor (VEP).[30] A total of 340,214 (6.7%) of the coding variants were annotated as high confidence loss-of-function (LOF) using the LOFTEE VEP plugin,[31] 238,646 (4.7%) as splice site altering identified by Splice AI,[32] 729,098 (14.3%) as damaging missense as predicted by the MetaSVM algorithm[33], and 1,106,309 (21.8%) as damaging missense as predicted by consensus in all five prediction algorithms (SIFT, PolyPhen-2 HumVar, PolyPhen-2 HumDiv, MutationTaster and LRT).[34] As expected, we observed a trend of decreasing proportions of putatively deleterious variants with increasing allele count (**Supplementary Figure 1, Supplementary Table 2**).

Single-variant association

We performed inverse-variance weighted fixed-effects meta-analyses of single-variant association results of LDL-C, non-HDL-C, TC, HDL-C, TG and TG:HDL ratio from each consortium and ancestry group. Meta-analysis results were well controlled with genomic inflation factors ranging between 1.01 and 1.04 (**Supplementary Table 3**). Single-variant results were limited to the 425,912 protein-altering coding variants with a total minor allele count (MAC) > 20 across all 172,000 individuals. We defined significant associations by a previously established exome-wide significance threshold for coding variants ($P<4.3\times10^{-7}$)[35] which was additionally corrected for testing six traits ($P=4.3\times10^{-7}$ divided by 6) within all study samples or within each of the five major ancestries (**Supplementary Tables 4-9**); this yielded in each analysis a significance threshold of $P<7.2\times10^{-8}$. A total of 104 rare coding variants in 57 genes were associated with LDL-C, 95 in 54 genes with non-HDL-C, 109 in 65 genes with TC, 92 in 56 genes with HDL-C, 61 in 36 genes with TG, and 68 in 42 genes with TG:HDL. We identified six missense variants in six genes (*TRIM5* p.Val112Phe, *ADH1B* p.His48Arg, *CHUK* p.Val268Ile, *ERLIN1* p.Ile291Val, *TMEM136* p.Gly77Asp, *PPARA* p.Val227Ala) >1Mb away from any index variant previously associated with a lipid phenotype (LDL-C, HDL-C, TC, or TG) in previous genetic discovery efforts (**Supplementary Tables 4-9**).[3,7,8] *PPARA* p.Val227Ala has previously been associated with blood lipids at a nominal significance level in East Asians ($P < 0.05$), where it is more common than in other ancestries.[36] Both *TRIM5* and *ADH1B* LDL-C increasing alleles have been associated with higher risk of CAD in a recent GWAS from CARDIOGRAM (OR: 1.08, $P=2\times10^{-9}$; OR=1.08, $P=4\times10^{-4}$).[37]

Gene-based association

Next we performed gene-based testing of transcript-altering variants in aggregated burden and sequence kernel association tests (SKAT)[38] tests in all study participants and within each of the six main ancestries for six lipid traits: LDL-C, HDL-C, non-HDL-C, TC, TG, and TG:HDL. We excluded the Samoans from the single-ancestry analysis given the small number of individuals. We limited attention to variants with MAF≤1% for each of six variant groups: 1) LOF, 2) LOF and predicted splice-site altering variants using Splice AI, 3) LOF and MetaSVM missense variants, 4) LOF, MetaSVM missense and predicted splice-site altering variants, 5) LOF and damaging 5 out 5 missense variants, and 6) LOF, damaging 5 out 5 missense and predicted splice-site altering variants. Meta-analyses results were well controlled (**Supplementary Table 10**).

We identified 35 genes reaching exome-wide significance ($P=4.3\times10^{-7}$) for at least one of the six variant groupings (**Figure 2 and Supplementary Tables 11-17**). Most of the significant results were from the multi-ancestry analysis, with multiple ancestries contributing to the top signals (**Figure 2A**) and most of the 35 genes were associated with more than one lipid phenotype (**Figure 2B**). Ten of the 35 genes did not have prior evidence of gene-based links with blood lipid phenotypes (**Table 1**), and seven genes, including *ALB*, *SRSF2*, *CREB3L3*, *NR1H3*, *PLA2G12A*, *PPARG,* and *STAB1* have evidence for a biological connection to circulating lipid levels (**Box 1**).

We also investigated which of the 35 genes were outside GWAS regions defined as those within ±200kb flanking regions of GWAS indexed Single nucleotide polymorphisms (SNPs) for TC (487 SNPs), LDL-C (531 SNPs), HDL-C, and TG (471 SNPs).[8] We identified 1,295 unique genes included in these lipid GWAS regions. Eight out of the 35 associated genes (23%) were not within a GWAS region (**Supplementary Table 11**).

To understand whether the gene-based signals were driven by variants that could be identified through single variant analyses, we looked at the proportion of the 35 genes that were associated with each trait that have at least one single contributing variant that passed the genome-wide significance threshold of 5×10^{-8}. Seventeen genes were associated with HDL-C at exome-wide significance (**Supplementary Table 11**); eight genes had at least one variant with $P<5\times10^{-8}$. Similarly, we observed 4/9 for LDL-C, 4/10 non-HDL-C, 4/14 TC, 7/18 TG, and 6/17 TG:HDL genes with at least one genome-wide significant variant.

Comparison of gene-based associations across ancestries

The gene-based associations were mostly consistent across the six ancestry groupings: European, South Asian, African, Hispanic, and East Asian. Three of the 17 HDL-C genes showed association in at least two different ancestries at exome-wide significance level ($P=4.3\times10^{-7}$). Similarly, 3/9 LDL-C, 4/10 non-HDL-C, 5/14 TC, 2/18 TG and 2/17 TG:HDL genes showed association in at least two difference ancestries at a exome-wide significance level. Using a less stringent significance level ($P<0.01$), across the six lipid traits, 59-89% of associated genes from the joint analysis were associated in at least two different ancestries.

We additionally tested the top 35 genes for heterogeneity across all 303 gene-trait-variant grouping combinations passing the exome-wide significance threshold ($P<4.3\times10^{-7}$). We observed heterogeneity in effect estimates ($P_{Het}<1.7\times10^{-4}$, accounting for 303 combinations) in 19 (6%) different gene-trait-variant grouping combinations and in six different genes: *LIPC*, *LPL*, *LCAT*, *ANGPTL3*, *APOB*, and *LDLR* (**Supplementary Table 18**). Although the LOF gene-based effect sizes were largely consistent across ancestries, there were differences in the cumulative frequencies of LOF variants for several genes including *PCSK9*, *NPC1L1*, *HBB* and *ABCG5* (**Supplementary Figures 2-4**).

We observed LOF and predicted damaging variants in the *TMEM136* gene associated with TG and TG:HDL only among individuals of South Asian ancestry ($P_{SKAT}=3\times10^{-9}$ and 2×10^{-11}, respectively) (**Table 1**, **Figure 2A**). With the same variant grouping and ancestry, we observed associations with reduced TG by burden tests (β=-15%, $P=3\times10^{-4}$) and TG:HDL (β=-20%, $P=6\times10^{-5}$) (**Supplementary Tables 16 and 17**). Additionally, a single missense variant was associated only among South Asians (rs760568794,11:120327605-G/A, p.Gly77Asp) with TG (β=-36.9%, $P=2\times10^{-8}$) (**Supplementary Table 11**). This variant was present only among South Asian (MAC=24) and Hispanics (MAC=8), but showed no association among Hispanics (P=0.86). This gene encodes a transmembrane protein of unknown function.

Gene-based associations in GWAS loci

We investigated whether genes near lipid array-based GWAS signals[8] were associated with the corresponding lipid measure using gene-based tests of rare variants with the same traits. We obtained genes from 200 Kb flanking regions on both sides of each GWAS signal; 487 annotated to LDL-C GWAS signals, 531 to HDL-C signals, and 471 to TG signals. We analyzed genes within these three sets for gene-based associations with their associated traits. A total of 13, 19, and 13 genes were associated ($P<3.4\times10^{-5}$, corrected for the number of genes tested for the three traits) with LDL-C, HDL-C or TG, with 32 unique genes identified in the GWAS loci (**Supplementary Tables 19-24)**.

Three of the 32 genes had no prior aggregate rare variant evidence of blood lipid association. Variants annotated as LOF or predicted damaging in *EVI5* were associated with LDL-C ($P_{SKAT}=2\times10^{-5}$). The burden test showed association with higher LDL-C levels (β=1.9 mg/dL, P=0.008) (**Supplementary Table 19)**. Variants annotated as LOF or predicted damaging in *SH2B3* were associated with lower HDL-C (β=-2.5 mg/dL, $P=1\times10^{-6}$) among Europeans. Variants that were annotated as LOF in *PLIN1* were associated with higher HDL-C (β=3.9 mg/dL, $P=1\times10^{-5}$) (**Supplementary Table 20**). Other genes in the regions of *EVI5*, *SH2B3*, and *PLIN1* did not show an association with the corresponding lipid traits (P>0.05) in multi-ancestry analyses. A previous report implicated two heterozygous frameshift mutations in *PLIN1* in three families with partial lipodystrophy.[39] The gene encodes perilipin, the most abundant protein that coats adipocyte lipid droplets and is critical for optimal TG storage.[40] We observed a nominal associations of *PLIN1* with TG (β=-7.0%, P=0.02). Our finding is contrary to what would be expected with hypertriglyceridemia in a lipodystrophy phenotype given the association with lower TG. This gene has an additional role where silencing in cow adipocytes has been shown to inhibit TG synthesis and promote lipolysis,[41] which may explain those contradictions.

Enrichment of Mendelian-, GWAS-, and drug targets genes

We next sought to test the utility of genes that showed some evidence for association but did not reach exome-wide significance. Within the genes that reached a sub-threshold level of significant association in this study using burden or SKAT tests ($p < 0.005$), we investigated the enrichment of i) Mendelian dyslipidemia (N=21 genes)-;[2] ii) lipid GWAS (N=487 for LDL-C, N=531 for HDL-C and N=471 for TG)[8]; and iii) drug target genes (N=53).[42] We stratified genes in GWAS loci according to coding status of the index SNP and proximity to the index SNP (nearest gene, second nearest gene, and genes further away). We tested for enrichment of gene-based signals ($P<0.005$) in the gene sets compared to matched genes (**Figure 3**). For each gene within each gene set, the most significant association in the multi-ancestry or an ancestry specific analysis was obtained and then matched to 10 genes based on sample size, total number of variants, cumulative MAC, and variant grouping. The strongest enrichment was observed for Mendelian dyslipidemia genes within the genes that reached $P < 0.005$ in our study. For example, 52% of the HDL-C Mendelian genes versus 1.4% of the matched set reached $P < 0.005$ (OR:71, 95% CI: 16-455). We also observed that 45.5% of the set of genes closest to an HDL-C protein-altering GWAS variant reached $P < 0.005$ versus 1.4% in the matched gene set (OR:57, 95% CI: 13-362). Results were significant but much less striking for genes at non-coding index variants. We observed that 8.9% of the set of genes closest to an HDL-C non-protein altering GWAS variant reached $P < 0.005$ versus 2.3% in the matched set (OR:4.1, 95% CI: 1.8-8.7). While it was 8% versus 2.6% for the set of second closest genes to an HDL-C non-protein altering GWAS variant (OR: 3, 95% CI: 1.1-8.3). There was no significant enrichment in second closest or >= third closest genes to protein altering GWAS signals and in >= third closest genes to non-protein altering GWAS signals. Drug target genes were significantly enriched in LDL-C gene-based associations (OR: 5.3, 95% CI: 1.4-17.8) but not in TG (OR: 2.2, 95% CI: 0.2-11.2) or HDL-C (OR: 1.0, 95% CI: 0.1-4.3) (**Figure 3 and Supplementary Tables 26-28**).

Association of lipid genes with CAD, T2D, glycemic traits, and liver enzymes

We tested the genes identified through our main (35 genes) and GWAS loci (32 genes) for associations with CAD or T2D in our gene-based analyses (40 genes across the two sets). The CAD analyses were restricted to a subset of the overall exome sequence data with information on CAD status which included the MIGen CAD case-control, UK Biobank (UKB) CAD nested case-control, and the UKB cohort with a total of 32,981 cases and 79,879 controls. We observed four genes significantly associated with CAD ($P_{CAD}<0.00125$, corrected for 40 genes). The four genes associated with lipids and CAD were all primarily associated with LDL-C: *LDLR* (OR: 2.97, $P=7\times10^{-24}$), *APOB* ($P_{SKAT}=4\times10^{-5}$), *PCSK9* (OR: 0.5, $P=2\times10^{-4}$) and *JAK2* ($P_{SKAT}=0.001$). Several other known CAD associated genes (*NPC1L1*, *CETP*, *APOC3*, and *LPL*) showed nominal significance for association with lipids ($P<0.05$). We observed nominal associations with CAD for two of the newly-identified lipid genes: *PLIN1* ($P_{SKAT}=0.002$) and *EVI5* (OR: 1.29, $P=0.002$; **Supplementary Table 29**). None of the 40 lipid genes reached significance for association with T2D in the latest AMP-T2D exome sequence results. We observed nominal associations of T2D

with *STAB1* (OR: 1.05, P_{T2D}=0.002) and *APOB* (OR: 1.08, P_{T2D}=0.005) (**Supplementary Table 30**).[15]

We additionally tested the 40 genes for association with six glycemic and liver biomarkers in the UKB: blood glucose, HbA1c, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and albumin. Using an exome-wide significance threshold of P=0.0012, we found associations between *PDE3B* and elevated blood glucose, *JAK2* and *SH2B3* and lower HbA1c, and *APOC3* and higher HbA1c. We found associations between *CREB3L3* and lower ALT, ALB, and higher AST, and between *A1CF* and higher GGT. *ALB* and *SRSF2* were associated with lower and higher albumin levels, respectively (**Supplementary Tables 31-36**).

Discussion

We conducted a large multi-ethnic study to identify genes in which protein-altering variants demonstrated association with blood lipid levels. First, we confirm previous associations of genes with blood lipid levels and show that we detect associations across multiple ancestries. Second, we identified gene-based associations that were not observed previously. Third, we show that along with Mendelian lipid genes, the genes closest to both protein altering and non-protein altering GWAS signals, and LDL-C drug target genes have the highest enrichment of gene-based associations. Fourth, of the new gene-based lipid associations, *PLIN1* and *EVI5* showed suggestive evidence of an association with CAD.

Our study found that evidence of gene-based associations for the same gene in multiple ancestries. The heterogeneity in genetic association with common traits and complex diseases has been discussed extensively. A recent study has shown significant heterogeneity across different ancestries in the effect sizes of multiple GWAS identified variants.[43] However, our study shows that gene-based signals are detected in multiple ancestries with limited heterogeneity in the effect sizes.

Our study highlights enrichment of gene-based associations for Mendelian dyslipidemia genes, genes with protein-altering variants identified by GWAS, and genes that are closest to non-protein altering GWAS index variants. A previous transcriptome-wide Mendelian randomization study of eQTL variants indicated that most of the genes closest to top GWAS signals (>71%) do not show significant association with the respective phenotype.[44] In contrast, our study provides evidence from sequence data that the closest gene to each top non-coding GWAS signal is most likely to be the causal one, indicating an allelic series in associated loci. This has implications for GWAS results, suggesting the prioritization of the closest genes for follow-up studies. We also observed enrichment of drug target genes only among LDL-C gene-based associations and not for HDL-C and TG gene-based associations, consistent with the fact that most approved therapeutics for cardiovascular disease targeting LDL-C

The gene-based analyses of lipid genes with CAD confirmed previously reported and known associations (*LDLR*, *APOB,* and *PCSK9).* Using a nominal P threshold of 0.05 we also confirmed associations with *NPC1L1*, *CETP*, *APOC3*, and *LPL*. Of the novel lipid genes, we observed borderline significant signals with *EVI5* and higher risk of CAD and between *PLIN1* and lower risk of CAD. The putative cardio-protective role of PLIN1 deficiency is supported by previous evidence in mice which has indicated reduced atherosclerotic lesions with Plin1 deficiency in bone marrow derived cells.[45] This suggests PLIN1 as a putative target for CAD prevention; however, replication of the CAD association would be needed to confirm those signals.

There are some limitations to our results. First, we had lower sample sizes for the non-European ancestries, limiting our power to detect ancestry-specific associations. However, we find consistency of results across ancestries, and when we relax our significance threshold, the majority of associations (59-89%) are observed in more than one ancestry. Second, it has been reported that there was an issue with the UKB functionally equivalent WES calling.[46] This mapping issue may have resulted in under-calling alternative alleles and therefore should not increase false positive findings. Third, we relied on a meta-analysis approach using summary statistics to perform our gene-based testing due to differences in sequencing

platforms and genotyping calling within the multiple consortia contributing to the results. This approach has been shown to be equivalent to a pooled approach for continuous outcomes.[47]

In summary, we demonstrated association between rare protein-altering variants with circulating lipid levels in >170,000 individuals of diverse ancestries. We identified 35 genes associated with blood lipids, including ten genes not previously shown to have gene-based signals. Our results support the hypothesis that genes closest to a GWAS index SNP are enriched for evidence of association.

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Author Contributions

Conceptualization: NB, SSR, SK, JIR, MB, MM, JAF, AVK, and GMP

Data curation: CAAS, LAF, DKA, SA, AG, NB, LFB, JCB, EB, JAB, JGB, NB, BEC, EC, CY-C, Y-DIC, RC, AC, JD, PdV, RAD, SKD, PTE, LSE, VF, GSME, BG, CG, MEGV, MG, SEG, NG, SH, TH, NLHC, HY-J, MYH, MRI, GPJ, HMK, SLRK, TNK, ATK, YJK, CK, JKU, SHK, ML, LAL, JiwonL, JuyoungL, DML, AL, SAL, RCWM, LWM, STM, OM, XM, MEM, ACM, SCN, PMN, JRO, MOM, CNAP, OP, JMP, WSP, MHP, QQ, SR, SSR, NJS, HS, CS, XS, RS, WYS, AMS, SMS, CT, KT, YYT, MT, MTTL, KAVM, FFW, DEW, CJW, DRW, TW, LRY, SK, JIR, MB, MM, and CJW

Formal analysis: GH, PD, MDC, MXW, XW, JAF, and GMP

Funding acquisition: DKA, LCB, JB, DWB, CY-C, Y-DIC, CC, JEC, RD, PTE, BIF, LG, TH, HY-J, EEK, SHK, JJ, RJFL, SAL, RCWM, RAM, STM, RM, JBM, NDP, BMP, QQ, DCR, APR, SSR, KT, FT, TT, RVD, RSV, DEW, JIR, PN, and GMP

Investigation: GH, PD, MDC, DJL, CAAS, DKA, AG, NB, JB, DWB, BEC, CY-C, Y-DIC, CC, WJC, JEC, DH, RD, BIF, SG, RAG, CG, NG, LG, TH, JH, HY-J, EEK, RK, SHK, SL, RNL, AL, JJ, RJFL, VL, RCWM, STM, RM, TM, GAM, ACM, DMM, CNAP, NDP, CJP, OP, PAP, WSP, BMP, QQ, SSR, SD, JS, KT, BT, MT, TT, JT, RVD, RSV, DEW, JGW, DRW, SK, JIR, MB, MM, CJW, PN, JAF, AVK, and GMP

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Writing - original draft: GH

Visualization: GH and GMP

Writing - review and editing: All authors read and approved the manuscript.

Declaration of Interests

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Figure titles and legends

Figure 1. Study samples and design

Flow chart of the different stages of the study. Exome sequence genotypes were derived from four major data sources: The Myocardial Infarction Genetics consortium (MIGen), the Trans-Omics from Precision Medicine (TOPMed), the UK Biobank and the Type 2 Diabetes Genetics (AMP-T2D-GENES) consortium. Single-variant association analyses were performed by ancestry and case-status in case-control studies and meta-analyzed. Single-variant summary estimates and covariance matrices were used in gene-based analyses using 6 different variant groups and in multi-ancestry and each of the five main ancestries. AFR=African ancestry, EAS=East Asian ancestry, EUR=European ancestry, HIS=Hispanic ancestry, SAM=Samoan ancestry, SAS=South Asian ancestry

Figure 2. Exome-wide significant associations with blood lipid phenotypes

A) Circular plot highlighting the evidence of association between the exome-wide significant 35 genes with any of the six different lipid traits ($P < 4.3 \times 10^{-7}$). The most significant associations from any of the six different variant groups are plotted. For almost all of the genes the most significant associations were obtained from the multi-ancestry meta-analysis. **B)** Strength of association of the 35 exome-wide significant genes based on the most significant variant grouping and ancestry across the six lipid phenotypes studied. Most of the genes indicated associations with more than one phenotype. Sign(beta)*-log10(p) displayed for associations that reached a $P < 4.3 \times 10^{-7}$. When the Sign(beta)*-log10(p) > 50, they were trimmed to 50.

Figure 3. Enrichment of Mendelian, GWAS, and drug target genes in the gene-based lipid associations

Enrichment of gene sets of Mendelian genes (n=21), GWAS loci for LDL-C (n=487), HDL-C (n=531), and triglycerides (TG) (n=471) genes and drug target genes (n=53).

Table 1. Novel Genes Associated with Blood Lipids

Gene	Name	Trait	N	cMAC	nVAR	beta	se	P	Mask	Test	Ancestry
		LDL-C	165,003	51	18	29.51	5.11	7.76E-09	LOF	Burden	Multi-ancestry
ALB	Albumin	**Non-HDL-C**	166,327	50	17	33.91	6.07	2.27E-08	LOF	Burden	Multi-ancestry
		TC	172,103	54	18	33.37	5.89	1.48E-08	LOF	Burden	Multi-ancestry
SRSF2	Serine And Arginine Rich Splicing Factor 2	**TC**	172,103	59	14	-30.59	5.49	2.46E-08	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
JAK2	Janus Kinase 2	**TC**	975,33	441	136	-7.10	1.98	1.71E-07	LOF/DAM5of5/SPLICE AI	SKAT	EUR
CREB3L3	CAMP Responsive Element Binding Protein 3 Like 3	**TG**	1702,39	874	71	0.12	0.02	2.43E-15	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
		TG/HDLC	165,380	855	69	0.14	0.02	5.76E-13	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
TMEM136	Transmembrane Protein 136	**TG**	29,571	157	24	-0.15	0.04	3.39E-09	LOF/DAM5of5/SPLICE AI	SKAT	SAS
		TG/HDLC	29,517	157	24	-0.20	0.05	1.76E-11	LOF/DAM5of5/SPLICE AI	SKAT	SAS
VARS	Valyl-TRNA Synthetase 1	**TG**	56,140	67	51	0.32	0.06	4.30E-07	LOF/MetaSVM	Burden	EUR
NR1H3	Nuclear Receptor Subfamily 1 Group H Member 3	**HDLC**	93,044	521	111	3.47	0.60	1.45E-11	LOF/MetaSVM/SPLICE AI	SKAT	EUR
		HDLC	166,441	1975	47	-2.28	0.31	8.12E-14	LOF/DAM5of5	Burden	Multi-ancestry
PLA2G12A	Phospholipase A2 Group XIIA	**TG**	170,239	2047	47	0.06	0.01	1.17E-08	LOF/DAM5of5	Burden	Multi-ancestry
		TG/HDLC	165,380	1969	46	0.11	0.01	7.56E-13	LOF/DAM5of5	Burden	Multi-ancestry
PPARG	Peroxisome Proliferator Activated Receptor Gamma	**HDLC**	166,441	147	72	-6.24	1.07	4.71E-09	LOF/DAM5of5/SPLICE AI	Burden	Multi-ancestry
STAB1	Stabilin 1	**HDLC**	166,441	6550	804	0.83	0.16	2.58E-07	LOF/MetaSVM/SPLICE AI	Burden	Multi-ancestry

cMAC=cumulative minor allele count; nVAR=number of variants in test; AFR=African ancestry, EAS=East Asian ancestry, EUR=European ancestry, HIS=Hispanic ancestry, SAS=South Asian ancestry.

Box 1. Genes with biological links to lipid metabolism

ALB	The association between mutations in the albumin gene and elevated cholesterol levels has been previously observed in rare cases of congenital analbuminemia.[48] This has been mainly suggested to result from compensatory increases in hepatic production of other non-albumin plasma proteins to maintain colloid osmotic pressure particularly apolipoprotein B-100 leading to elevations in TC and LDL-C but normal HDL-C levels – which is consistent with our findings – although the exact mechanisms remain uncertain.[49] A lipodystrophy-like phenotype has also been linked to analbuminemia which is consistent with the suggestive tendency for increased risk of T2D with LOF and predicted damaging variants in albumin in the population (OR=1.85; P=0.007) (**Supplementary Table 30**).
SRSF2	The *SRSF2* gene encodes a highly conserved serine/arginine-rich splicing factor and has previously been linked to acute liver failure in liver-specific knockout in mice with accumulation of TC in the mutant liver.[50] Thus, this gene could be linked to a non-alcoholic fatty liver phenotype with accumulation of lipids in the liver as observed with other genes as *PNPLA3* and *TM6SF2*.[7] Therefore, we looked at association with liver function markers and we found an association between *SRSF2* and higher albumin levels ($P = 1 \times 10^{-4}$) and a suggestive tendency for higher gamma glutamyl transferase (GGT) (P = 0.05), consistent with potential liver involvement (**Supplementary Table 33-36**).
CREB3L3	The association between *CREB3L3* and higher TG supports previous evidence from a single family and cohorts with severe hypertriglyceridemia but not sufficient evidence to be classified as a Mendelian lipid gene (ref).[51-53] This has been additionally supported by functional studies where *Creb3l3* knockout mice showed hypertriglyceridemia partly due to deficient expression of lipoprotein lipase coactivators (*Apoc2*, *Apoa4*, and *Apoa5*) and increased expression of activator *Apoc3*.[52]
NR1H3	The observed association of *NR1H3* with higher HDL-C and lower TG is supported by previous evidence of a role in non-alcoholic fatty liver disease in mice.[54] This gene encodes a liver X receptor alpha (LXRα) which is a nuclear receptor that acts as a cholesterol sensor and protects from cholesterol overload.[55,56] It has previously been shown that disrupting the LXRα phosphorylation at Ser196 in mice prevents non-alcoholic fatty liver disease.[54]
PLA2G12A	*PLA2G12A* is in the secretory phospholipase A2 ($sPLA_2$) family, which liberates fatty acids in the -sn2 position of phospholipids. This pattern suggests a previously unreported possible lipolytic role of this phospholipase in a manner similar to another member of the adipose-specific phospholipases, *PLA2G16*, which has been shown to have a lipolytic role in mice.[57,58] Further studies are needed to confirm whether *PLA2G12A* has a lipolytic role.
PPARG	Rare loss of function mutations in *PPARG* have been previously found to be associated with reduced adipocyte differentiation, lipodystrophy and increased risk of T2D.[59-61]
STAB1	The *STAB1* gene is a scavenger receptor that has been shown to mediate uptake of oxidized LDL-C.[62,63] There was a suggestive association between LOF variants and higher LDL-C (β = 4.3 mg/dL, $P = 2 \times 10^{-3}$) consistent with its role in LDL-C uptake.

STAR Methods

Informed consent was obtained from all subjects and committees approving the studies are available in the supplement.

Corresponding Author and Lead Contact:

Request for further information should be directed to and will be fulfilled by the Lead Contact, Gina Peloso (gpeloso@bu.edu).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

Controlled access of the individual-level data are available through dbGAP (please refer to the Supplementary Information), and the individual-level UK Biobank data are available upon application to the UK Biobank.

Study Overview

Our study samples were derived from four major data sources with exome or genome sequence data and blood lipid levels: CAD case-control studies from the Myocardial Infarction Genetics Consortium (MIGen, n = 44,208) and a UKB nested case-control study of CAD (n = 10,689); T2D cases-control studies from the AMP-T2D-GENES exomes (n = 32,486); population-based studies from the TOPMed project freeze 6a data (n = 44,101) restricted to the exome, and the UKB exome sequence data (n = 40,586) (see **Supplementary Note**). Within each data source, individuals were excluded if they failed study-specific sequencing quality metrics, lacked lipid phenotype data, or were duplicated in other sources. We additionally removed first- and second-degree relatives across data sources while we kept relatives within each data source since we were able to adjust for relatedness within each data source using kinship matrices in linear mixed models. If samples from the same study were present in different data sources, we used the samples in the data source which has the largest sample size from the study and removed the overlapping set from the other data source. For instance, samples from the Atherosclerosis Risk in Communities (ARIC) Study were removed from TOPMed and kept in MIGen which had more sequenced samples from ARIC. Similarly, samples from the Jackson Heart Study were kept in TOPMed and removed from MIGen. To obtain duplicate and kinship information across data sources we used 14,834 common (MAF>1%) and no more than weakly dependent ($r^2 < 0.2$) variants using the make-king flag in PLINK v2.0. Single-variant association analyses were performed within each data source, case-status, and ancestry combination. The data were sequenced and variant calling performed separately by data source and this allowed us to look for effects by case-status and genetically-inferred and/or self-reported

ancestry groups. We performed gene-based meta-analyses by combining single-variant summary statistics and covariance matrices generated from RVTESTS.[64] We performed ancestry-specific gene-based meta-analyses by combining single-variant summary data from five major ancestries with >10,000 across all data sources: European, South Asian, African, Hispanic, and East Asian ancestries.

Phenotypes

We studied six lipid phenotypes; total, LDL-C, HDL-C, non-HDL-C, TG and TG:HDL. TC was adjusted by dividing the value by 0.8 in individuals reporting lipid lowering medication use after 1994 or statin use at any time point. If LDL-C levels were not directly measured, then they were calculated using Friedewald equation for individuals with TG levels < 400 mg/dl using adjusted TC levels. If LDL-C levels were directly measured then, their values were divided by 0.7 in individuals reporting lipid lowering medication use after 1994 or statin use at any time point.[5] TG and TG:HDL levels were natural logarithm transformed. Non-HDL-C was obtained by subtracting HDL-C from adjusted TC levels. Residuals for each trait in each cohort, ancestry, and case status grouping were created after adjustment for age, age^2, sex, principal components, sequencing platform, and fasting status (when available) in a linear regression model. Residuals were then inverse-normal transformed and multiplied by the standard deviation of the trait to scale the effect sizes to the interpretable units.

Sequencing and Quality Control

Myocardial Infarction Genetics Consortium (MIGen)

A set of common variants was extracted for sample quality control including relative inference, principal component analysis, and estimation of heterozygosity. SNPs on autosomes and not in low complexity regions or segmental duplications were extracted. SNPs with quality of depth (QD)> 2, call rate >98%, self-reported-race-specific Hardy-Weinberg equilibrium (HWE) p-value >1×10^{-8}, Variant Quality Score Recalibration (VQSR) of PASS and MAF>1% were retained. Sample relatedness was estimated with KING and duplicate samples removed. Genetically inferred ancestry was assigned to each individual by calculating principal components jointly with 1000 Genomes phase 3 version 5 and building a 5-Nearest Neighbor classifier using the top 6 principal components. Heterozygosity was estimated within each genetic ancestry group and samples with F statistic above 0.3 were removed. Genetic sex was inferred based on high quality X-chromosome variation including variants with call rate >0.95, MAF>2%, a PASS VQSR, QD>3 if the variant is an insertion or deletion and QD>2 if it is SNP. Samples with discordant phenotypic sex and genetic sex were removed. Finally, sample quality control metrics were calculated using Hail and samples with call rate<0.9a mean depth (DP)<30 and mean genotype quality (GQ)<0.8 were excluded. A total of 44,240 samples with lipid data measurements were included after further excluding duplicates and relatives with other data sources.

Variant quality control was performed amongst remaining samples and a total of 8,716,575 autosomal variants were included after removing those that fail HWE as calculated by genetic ancestry group (p-value<1×10^{-8}), lie in low complexity regions or segmental duplications, with inbreeding coefficient< -0.3, are insertions or deletions with QD ≤ 3 or SNPs with QD ≤ 2 or variants where VQSR does not PASS

with the exception of singletons where variants with VQSRTrancheSNP99.60to99.80 were retained (**Supplementary Tables 37-38**).

Trans-Omics for Precision Medicine (TOPMed)

Whole genome sequencing at 30X mean depth was performed at one of six sequencing centers:Broad Institute of MIT and Harvard, Northwest Genomics Center, New York Genome Center, Illumina Laboratory Services, Psomagen, Inc. (formerly Macrogen USA), Baylor College of Medicine Human Genome Sequencing Center. For most studies, all individuals in the study were sequenced at the same center. Sequence reads were aligned to human genome build GRCh37 or GRCh38 at each center using similar, but not identical, processing pipelines. The resulting sequence data files were transferred from all centers to the TOPMed Informatics Research Center (IRC), where they were re-aligned to build GRCh38, using a common pipeline to produce a set of 'harmonized' .cram files. Processing was coordinated and managed by the 'GotCloud' processing pipeline. The IRC performed joint genotype calling on all samples. Quality control was performed at each stage of the process by the Sequencing Centers, the IRC, and the TOPMed Data Coordinating Center (DCC). Only samples that passed QC were included in the call set.

The two sequence quality criteria that were used to pass sequence data on for joint variant discovery and genotyping are: estimated DNA sample contamination below 3%, and fraction of the genome covered at least 10x 95% or above. DNA sample contamination was estimated from the sequencing center read mapping using software verifyBamId.[65]

The genotype used for analysis are from "freeze 6a" of the variant calling pipeline performed by the TOPMed Informatics Research Center (Center for Statistical Genetics, University of Michigan, Hyun Min Kang, Tom Blackwell and Gonçalo Abecasis). Variant detection (SNPs and indels) from each sequenced (and aligned) genome was performed by the vt discover2 software tool. The variant calling software tools are under active development; updated versions can be accessed at http://github.com/atks/vt, http://github.com/hyunminkang/apigenome, and https://github.com/statgen/topmed_variant_calling.

One individual from duplicate pairs identified by the DCC was removed, retaining the individual with lipid levels available when one did not have lipid levels. If both individuals had lipid levels, one individual was randomly selected. Individuals were excluded when their genotype determined sex did not match phenotype reported sex (n=6) and individuals <18 years old were excluded (n=865). Ancestry was defined as self-reported ancestry.

AMP-T2D-GENES

Sequencing and quality control were performed as previously described.[15] Following sequencing and variant calling, we measured samples and variants according to several sequence quality metrics and excluded those that were outliers relative to the global distribution. These exclusions produced an "analysis" dataset of 45,231 individuals and 6.33M variants. We then estimated, within each ancestry, pairwise IBD values, genetic relatedness matrices (GRMs), and PCs for use in downstream association analysis. We used the IBD values to generate lists of unrelated

individuals within each ancestry, excluding 2,157 individuals from an "unrelated analysis" set of 43,090 individuals (19,828 cases and 23,262 controls) and 6.29M non-monomorphic variants.

UK Biobank

We used two UKB datasets with exome sequence data. The first is a CAD case control study with 12,938 individuals. 29 samples were removed as they had discordant genotypes with genotyping array data, 17 showed mismatch between the reported and genetically inferred sex, 4 had excess heterozygosity and 6 had a call rate <95%. To perform the sex-mismatch analyses, variants on the X-chromosome were selected after filtering out low quality genotypes, call rate<95%, MAF<2%, low QD score (3 for INDELs and 2 for SNPs), low confidence regions and segmental duplications and those that do not have PASS VQSR. A set of high quality common autosomal variants were extracted for relative inference, principal component analysis, and estimation of heterozygosity after removing low confidence regions and segmental duplications, low quality genotypes, QD<2, call rate<98%, self-reported ancestry-specific HWE $p>1x10^{-6}$ among controls, MAF<1% and do not have PASS VQSR. Heterozygosity was estimated within each ancestry and samples with F statistic>2 were removed. Genetically inferred ancestry was obtained using the 1000 Genomes as reference. Sample QC metrics were then calculated in HAIL using autosomal variants after filtering out low-quality genotypes, variants with ancestry-specific HWE $p<1\times10^{-6}$, low confidence regions and segmental duplications, low QD score (3 for INDELs and 2 for SNPs) and those that do not have PASS VQSR. Samples with call rate below 95%, mean DP below 30 and mean GQ below 80 were removed. Variant QC was done through filtering out monomorphic variants, call rate below 95%, those with HWE ($p < 1 \times 10^{-6}$), lie in low confidence regions or segmental duplications, are insertions or deletions with QD <= 3 or SNPs with QD <= 2 or variants where VQSR does not PASS unless singleton in which case retain those with VQSRTrancheSNP99.60to99.80 (**Supplemental Table 39**). A total of 11,216 PC-identified European ancestry participants were included after additional removal of duplicates and relatives across data sources. A total of 2,734,519 variants were included.

The second UKB data set is a population-based dataset. Samples were filtered out if they showed mismatch between genetically determined and reported sex, high rates of heterozygosity or contamination (D-stat > 0.4), low sequence coverage (<85% of targeted bases achieving >20X coverage), duplicates, and exome sequence variants discordant with genotyping chip. More details are described elsewhere.[66] The "Functionally Equivalent" (FE) call set was used.[67] A total of 43,243 PC-identified European ancestry individuals were included after additional removal of duplicates and relatives across data sources.

Variant Annotation

We compiled autosomal variants with call rate>95% within each case and ancestry specific analysis dataset with MAC≥1 (across the combined data). Variants were annotated using the Ensembl Variant Effect Predictor[30] and its associated Loss-of-Function Transcript Effect Estimator (LOFTEE)[31] and the dbNSFP[34] version 3.5a plugins. We limited our annotations to the canonical transcripts. The LOFTEE plugin

assesses stop-gained, frameshift, and splice site disrupting variants. Loss-of-function variants are classified as either high confidence or low confidence. The dbNSFP is a database that provides functional prediction data and scores for non-synonymous variants using multiple algorithms.[34] This database was used to classify missense variants as damaging using two different definitions based on bioinformatic prediction algorithms. The first is based on MetaSVM[33] which is derived from 10 different component scores (SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, GERP++, MutationTaster, Mutation Assessor, FATHMM, LRT, SiPhy, PhyloP). The second is based on 5 variant prediction algorithms including SIFT, PolyPhen-2 HumVar, PolyPhen-2 HumDiv, MutationTaster and LRT score. Additionally, we ran a deep neural network analysis (Splice AI) to predict splice-site altering variants.[32] Multi-ancestry and ancestry-specific variant descriptive analyses were performed using variant-specific statistics obtained from the largest sample size out of the 6 phenotypes.

Single-Variant Association Analysis

Each data source was sub-categorized based on ancestry and CAD or T2D case status in the studies ascertained by disease status. Subgrouping data sources yielded a total of 23 distinct sample sub-categories. As relatives were kept within each sub-group, we performed generalized linear mixed models to analyze the association of single autosomal variants with standard-deviation corrected-inverse-normal transformed traits using RVTESTS.[64] RVTESTS was used to generate summary statistics and covariance matrices using 500 kilobase sliding windows. To obtain the single-variant associations, we performed a fixed-effects inverse-variance weighted meta-analysis for multi-ancestry and within each of the five major ancestries. An exome-wide significance threshold of $P<7.2\times10^{-8}$ (Bonferroni correction for six traits and using previously recommended threshold for coding variants $P<4.3\times10^{-7}$)[35] was used to determine significant coding variants.

Gene-Based Association Analysis

We used summary level score statistics and covariance matrices from autosomal single-variant association results to perform gene-based meta-analyses among all individuals and within each ancestry using RAREMETALS version 7.2.[47] Samoan individuals only contributed to the overall analysis. Gene-based association testing aggregates variants within each gene unit using burden tests and SKAT which allows variable variant effect direction and size.[68] The “rareMETALS.range.group” function was used with MAF<1%, which filters out all variants with combined MAF>1% in all meta-analytic datasets. All variants with call rates<95% and not annotated as LOF using LOFTEE, splice-site variants or damaging missense as defined by MetaSVM or by all SIFT, PolyPhen-2 HumVar, PolyPhen-2 HumDiv, MutationTaster and LRT prediction algorithms (Damaging 5 out of 5) were excluded in the gene-based meta-analyses.

We used 6 different variant groupings to determine the set of damaging variants within each gene, 1) high-confidence LOF using LOFTEE, 2) LOF and predicted splice-site altering variants, 3) LOF and MetaSVM missense variants, 4) LOF, MetaSVM missense and predicted splice-site altering variants, 5) LOF and damaging 5 out 5 missense variants, and 6) LOF, damaging 5 out 5 missense and

predicted splice-site altering variants. An exome-wide significance threshold of $P<4.3\times10^{-7}$, Bonferroni corrected for the maximum number of annotated genes (n=19,540) and six lipid traits, was used to determine significant coding variants. Two gene transcripts, *DOCK6* and *DOCK7*, that overlap with two well-studied lipid genes, *ANGPTL8* and *ANGPTL3*, respectively, met our exome-wide significance threshold. After excluding variation observed in *ANGPTL8* and *ANGPTL3*, *DOCK6* and *DOCK7,* respectively, were no longer significant and have been excluded as associated genes.

Heterogeneity of gene-based estimates in all gene-trait-variant grouping combinations passing exome-wide significant levels was assessed across the five main ancestries (European, South Asian, African, Hispanic and East Asian) using Cochran's Q.

Gene-Based Analysis of GWAS Loci and Drug Targets

We performed gene-based analysis using the six variant groups for genes in GWAS loci. A locus was defined as the region around each GWAS index variant ± 200kb. Top GWAS signals were obtained from a recent meta-analysis of >300,000 individuals in the Million Veterans Program.[8] In-silico lookup of gene-based associations for respective lipid traits were then performed for all genes within defined GWAS loci. Drug target genes were obtained from the drug bank database[42] using the following search categories: "Hypolipidemic Agents, Lipid Regulating Agents, Anticholesteremic Agents, Lipid Modifying Agents and Hypercholesterolemia". A liberal definition for drug targets was used – drugs with any number of targets and targets targeted by any number of drugs – and then in-silico lookups were performed for gene-based associations.

Gene-set Enrichment Analysis

Gene-set enrichment analyses were performed for sets of Mendelian-, protein-altering- and non-protein altering GWAS, and drug target genes with LDL-C, HDL-C and TG. 21 Mendelian genes were included based on previous literature[2]: *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *ABCG5*, *ABCG8*, *CETP*, *LIPC*, *LIPG*, *APOC3*, *ABCA1*, *APOA1*, *LCAT*, *APOA5*, *APOE*, *LPL*, *APOC2*, *GPIHBP1*, *LMF1*, *ANGPTL3*, and *ANGPTL4*. We analyzed GWAS gene sets based on their coding status and their proximity to the most significant signal in the GWAS. Coding variants were defined as missense, frameshift, or stop gained variants. Gene sets for coding or non-coding variants were then stratified into three categories based on proximity to the most significant variant within each locus – closest-, second closest- and greater than second closest gene. For each gene within each set, we obtained the most significant association in the multi-ancestry or ancestry specific meta-analysis set using any of the six different variant groups. Then each gene within each gene set was matched to 10 other genes based on sample size, total number of variants, cumulative MAC, and variant grouping nearest neighbors using the matchit R function. Then we compared the proportions using Fisher's exact test between the main and matched gene sets by applying different P-value thresholds.

Association of Lipid Genes with CAD and T2D data and liver fat/markers

We determined the associations of 40 genes identified in the main and GWAS loci analyses with CAD, T2D, and glycemic and liver enzyme blood measurements. The association with T2D was obtained from the latest gene-based exome association data from the AMP-T2D-GENES consortium.[15] The reported associations were obtained from different variant groups based on their previous analyses. We additionally performed gene-based association analyses with CAD using the MIGen case-control, UKB case-control, and UKB cohort samples using the variant groups described above. Further, six traits including fasting plasma glucose, HbA1c, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and albumin were analyzed in the UKB dataset. Single variant association analyses were performed with RVTESTS. Linear mixed models incorporating kinship matrices were used to adjust for relatedness within each study. Covariance matrices were generated using 500 kilobase sliding windows. RAREMETALS was used to assess associations between aggregated variants (MAF<1%) in burden and SKAT tests with CAD and each of the six quantitative traits. We used 6 different variant groupings to determine the set of damaging variants within each gene, 1) high-confidence LOF using LOFTEE, 2) LOF and predicted splice-site altering variants, 3) LOF and MetaSVM missense variants, 4) LOF, MetaSVM missense and predicted splice-site altering variants, 5) LOF and damaging 5 out 5 missense variants, and 6) LOF, damaging 5 out 5 missense and predicted splice-site altering variants.

Figure 1. Study samples and design

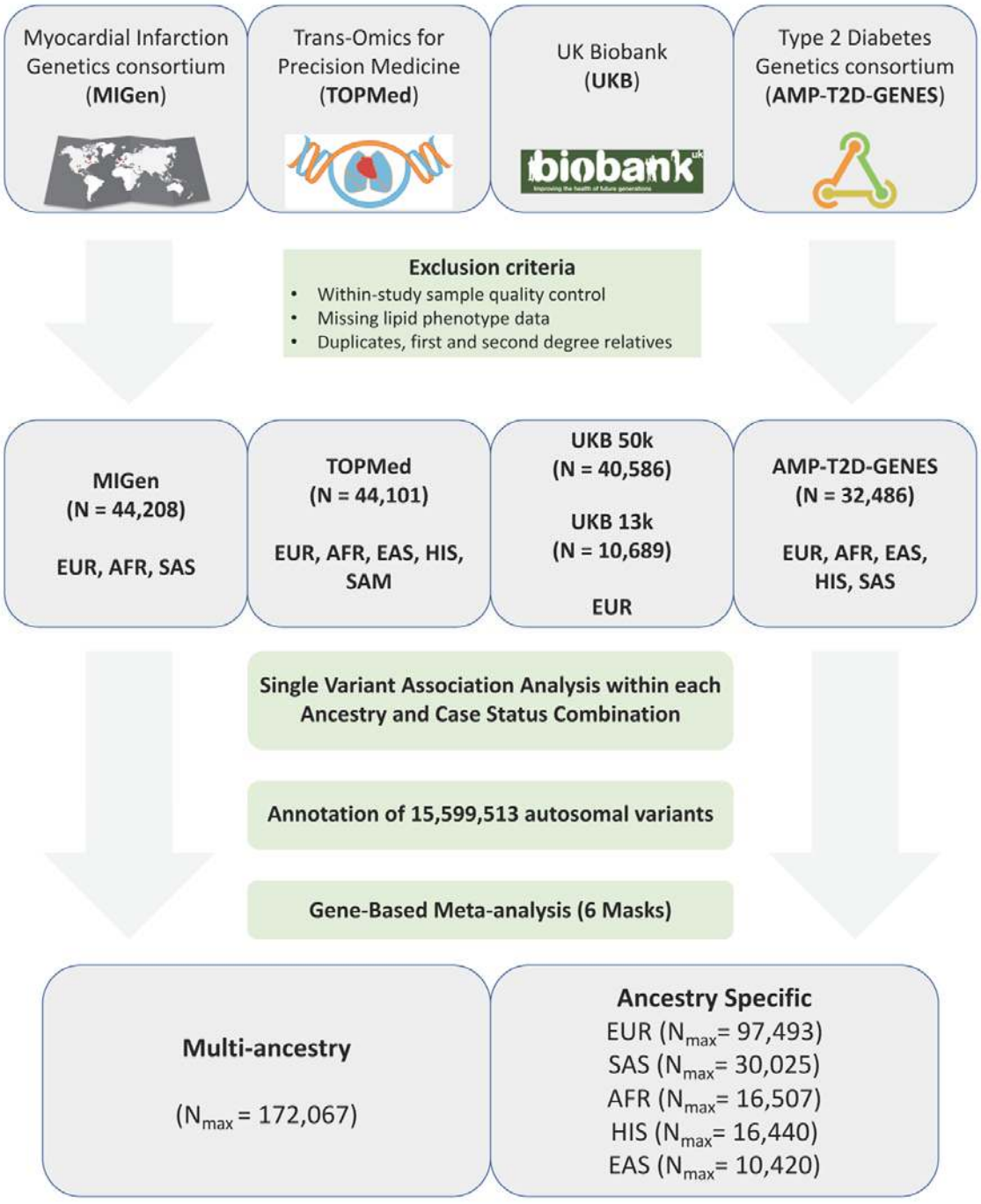
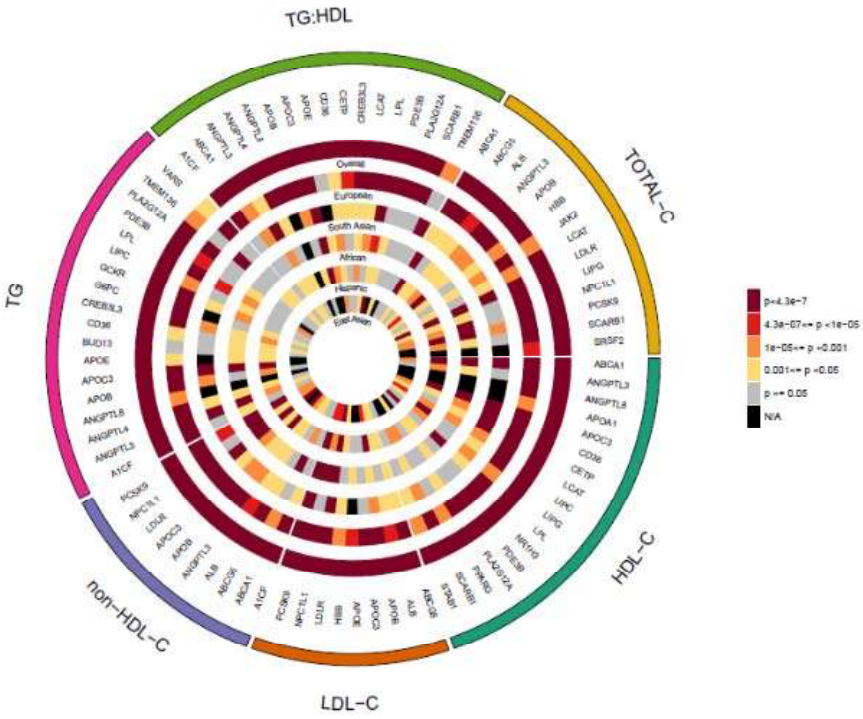


Figure 1: Flow chart of the different stages of the study. Exome sequence genotypes were derived from four major data sources: The Myocardial Infarction Genetics consortium (MIGen), the Trans-Omics from Precision Medicine (TOPMed), the UK Biobank and the Type 2 Diabetes Genetics (AMP-T2D-GENES) consortium. Single-variant association analyses were performed by ancestry and case-status in case-control studies and meta-analyzed. Single-variant summary estimates and covariance matrices were used in gene-based analyses using 6 different variant

groups and in multi-ancestry and each of the five main ancestries. AFR=African ancestry, EAS=East Asian ancestry, EUR=European ancestry, HIS=Hispanic ancestry, SAM=Samoan ancestry, SAS=South Asian ancestry.

Figure 2. Exome-wide significant associations with blood lipid phenotypes

A.



B.

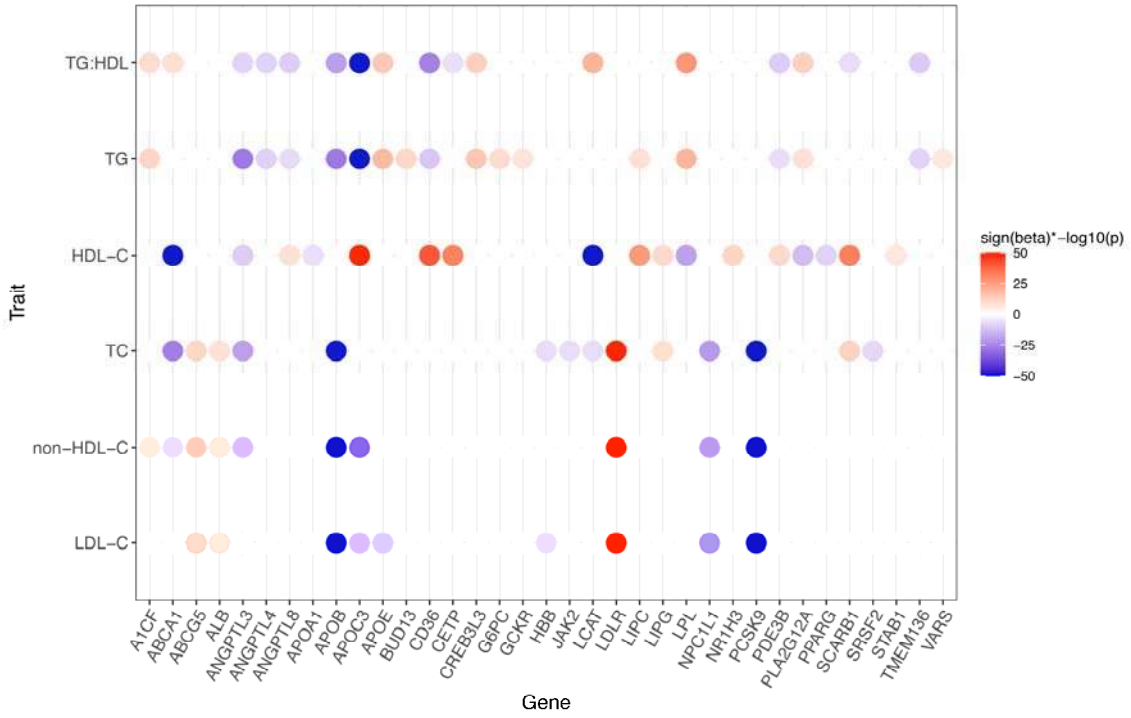


Figure 2: A) Circular plot highlighting the evidence of association between the exome-wide significant 35 genes with any of the six different lipid traits ($P < 4.3 \times 10^{-7}$). The most significant associations from any of the six different variant groups are

plotted. For almost all of the genes the most significant associations were obtained from the multi-ancestry meta-analysis. **B)** Strength of association of the 35 exome-wide significant genes based on the most significant variant grouping and ancestry across the six lipid phenotypes studied. Most of the genes indicated associations with more than one phenotype. Sign(beta)*-log10(p) displayed for associations that reached a $P < 4.3 \times 10^{-7}$. When the Sign(beta)*-log10(p) > 50, they were trimmed to 50.

Figure 3. Enrichment of Mendelian, GWAS, and drug target genes in the gene-based lipid associations

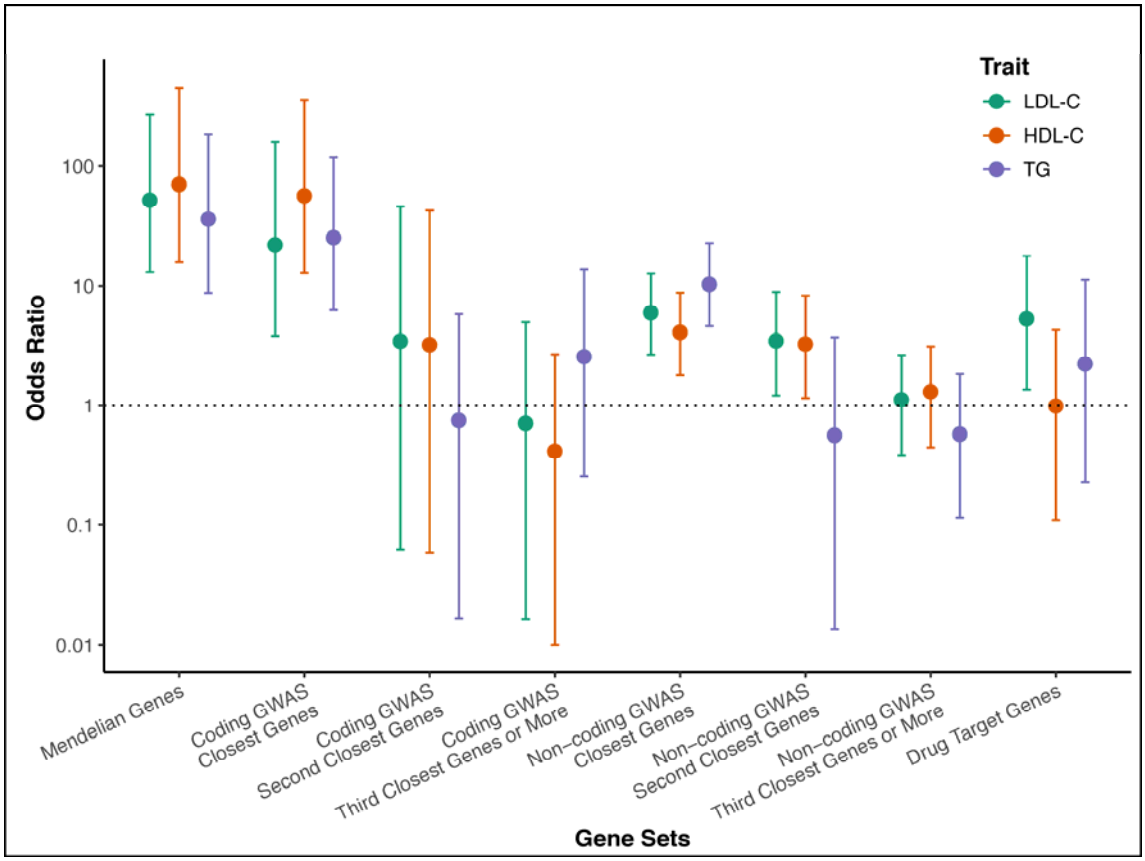


Figure 3: Enrichment of gene sets of Mendelian genes (n=21), GWAS loci for LDL-C (n=487), HDL-C (n=531), and triglycerides (TG) (n=471) genes and drug target genes (n=53).

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