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Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity

Turcot, V.; Lu, Y.C.; Highland, H.M.; Schurmann, C.; Justice, A.E.; Fine, R.S.; ... ; Rauramaa,

Citation

Turcot, V., Lu, Y. C., Highland, H. M., Schurmann, C., Justice, A. E., Fine, R. S., ... Rauramaa, . (2018). Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity. *Nature Genetics*, 50(1), 26-+. doi:10.1038/s41588-017-0011-x

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Note: To cite this publication please use the final published version (if applicable).



HHS Public Access

Author manuscript

Nat Genet. Author manuscript; available in PMC 2018 June 22.

Published in final edited form as:

Nat Genet. 2018 January ; 50(1): 26–41. doi:10.1038/s41588-017-0011-x.

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Correspondence to: Ruth J. F. Loos (ruth.loos@mssm.edu), Joel N. Hirschhorn (joelh@broadinstitute.org).

*These authors contributed equally to this work.

§These authors jointly supervised this work.

URLs

CDC 2000 Growth Charts: http://www.cdc.gov/growthcharts/cdc_charts.htm

CHOP cohort: <http://www.metabolic-programming.org/obesity/>

EC-DEPICT code: <https://github.com/RebeccaFine/obesity-ec-depict>

ENSEMBL: www.ensembl.org

EasyQC: www.genepi-regensburg.de/easyqc

EasyStrata: www.genepi-regensburg.de/easystrata

ExAC: <http://exac.broadinstitute.org/>

GCTA: <http://cns.genomics.com/software/gcta/>

GTEEx: <http://www.gtexportal.org/home/>

GTOOL: <http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html>

Impute2: https://mathgen.stats.ox.ac.uk/impute/impute_v2.html

INTERVAL Study: <http://www.intervalstudy.org.uk/>

PLINK v1.90: <https://www.cog-genomics.org/plink2>

QCTOOL: <http://www.well.ox.ac.uk/~gav/qctool/#overview>

RAREMETALWORKER: <http://genome.sph.umich.edu/wiki/RAREMETALWORKER>

RareMETALS: <http://genome.sph.umich.edu/wiki/RareMETALS>

RVTEST: <https://github.com/zhanxw/rvtests>

Shapeit2: https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html

UKHLS: <https://www.understandingsociety.ac.uk/>

UK10K Obesity Sample Sets - SCOOP: <http://www.uk10k.org/studies/obesity.html>

1000 Genomes Phase 1: <http://www.1000genomes.org/category/phase-1/>

AUTHOR CONTRIBUTIONS

Writing Group (wrote and edited manuscript)

P.D., M.F.F., T.M.F., M. Graff, H.M.H., J.N.H., A.E.J., G.L., C.M.L., A.E.L., R.J.F.L., Y. Lu, K.E.N., C.S., V.T., K.L.Y.

Data preparation group (program development and quality control of data from contributing cohorts for meta-analyses)

T.A., I.B.B., T.E., S. Feng, M. Graff, H.M.H., A.E.J., T. Karaderi, D.J.L., K.S.L., A.E.L., R.J.F.L., Y. Lu, E. Marouli, N.G.D.M.,

M.C.M.G., P. Mudgal, M.C.Y.N., M.A.R., S.S., C.S., K. Stirrups, V.T., S.V., S.M.W., T.W.W., K.L.Y., X.Z.

BMI meta-analyses (*discovery and follow-up, single-variant and gene-based*)

I.B.B., T.M.F., H.M.H., A.E.J., J.N.H., C.T.L., D.J.L., Y. Lu, R.J.F.L., C.S., V.T., K.L.Y.

Childhood data (analyses and interpretation)

I. Barroso, J.P.B., I.S.F., S.F.A.G., H.H., V.M., A.E.H., G.M.

Pleiotropy working group

G.A., M. Boehnke, J.P.C., P.D., F.D., J.C.F., H.M.H., S. Kathiresan, H. Kitajima, C.M.L., D.J.L., R.J.F.L., A.M., E. Marouli, G.M.,

M.I.M., P.B.M., G.M.P., J.R.B.P., K.S.R., X.S., T.F.V., S.W., J.W., S.M.W., C.J.W.

Phenome-wide association studies

L. Bastarache, J.C.D., T.L.E., A.G., A.M., M.I.M.

Gene-set enrichment analyses

R.S.F., J.N.H., T.H.P., D.L., S.B., Z.K.

Monogenic and syndromic gene enrichment analyses

H.M.H., A.K.M.

Fly Obesity Screen

A. Lempradl, J.A. Pospisilik

Overseeing of contributing studies

(1958 Birth Cohort) P.D.; (Add Health) P.G.L., K.M.H.; (ADDITION-DK) D.R.W., M.E.J.; (AGES) V. Gudnason, T.B.H.; (Airwave) P.E.; (AMC PAS) G.K.H.; (Amish) J.R.O.; (ARIC) E.B.; (ARIC, Add Health) K.E.N.; (ASCOT-UK, ASCOT-UK) H.R.W., P.B.M.; (ATBC) S.M.; (Athero-Express Biobank Study) H.M.d.R., G.P.; (ATVB, Ottawa) S. Kathiresan; (BioVU) T.L.E.; (BRAVE) E.D.A., R.C.; (BRIGHT) P.B.M.; (CARDIA) M.F., P.J.S.; (CHS) G.B.J.; (Cebu Longitudinal Health and Nutrition Survey) K.L.M.; (CGPS) B.G.N.; (CHD Exome + Consortium) A.S.B., J.M.M.H., D.F.R., J.D.; (CHES) R.V.; (CHOP) S.F.A.G., H.H.; (Clear/eMERGE (Seattle)) G.P.J.; (CROATIA_Korcula) V.V., O. Polasek, I.R.; (deCODE) K. Stefansson, U.T.; (DHS) D.W.B.; (DIABNORD) P.W.F.; (DIACORE) C.A.B.; (DPS) J.T., M.U.; (Duke) S. Kathiresan, Svati H. Shah; (EFSOCH) A.T. Hattersley, T.M.F.; (EGCUT) T.E.; (eMERGE (Seattle)) E.B.L.; (ENDO) A.P.M., K.T.Z.; (EPIC-Potsdam) M.B.S., H.B.; (EpiHealth) E.I., P.W.F.; (EUGENDA) A.I.d.H., S. Fauser; (EXTEND) A.T. Hattersley, T.M.F.; (Family Heart Study) I.B.B.; (Fenland, EPIC) R.A.S.; (Fenland, EPIC, InterAct) N.J.W., C.L.; (EPIC-CVD) J.D.; (FIA3) P.W.F.; (FINCAVAS) M. Kähönen, K.N.; (Finnish Twin Cohort) J. Kaprio; (FIN-D2D 2007) M.V.; (FINRISK) V. Salomaa; (FVG) P.G.; (GECCO) P.T.C., R.E.S.; (GeMEP) G. Tromp, D.J.C., H. Kuivaniemi; (GENDEP) G.B.; (Generation Scotland) C.H., S.P.; (Genetic Epidemiology Network of Arteriopathy (GENOA)) S.L.R.K., P.A.P.; (GLACIER) P.W.F., F.

Protein-altering variants associated with body mass index

Renström; (GoDARTS) C.N.A.P., A.D.M.; (GRAPHIC) N.J.S.; (GSK-STABILITY) D.M.W., L.W., H.D.W.; (Health) A. Linneberg; (Health ABC) T.B.H., Y. Liu; (HELIC MANOLIS) E.Z., G. Dedoussis; (HELIC Pomak) E.Z., G. Dedoussis; (HUNT-MI) C.J.W.; (ID1000/Spinoza (BBMRI-NL)) S.H. Scholte; (Inter99) T.H., T.J.; (INTERVAL Study) J.D.; (IRASFS) N.D.P., E.K.S., L.E.W.; (Jackson Heart Study (JHS)) J.G.W.; (KORA S4) K. Strauch, A. Peters, I.M.H.; (LASA (BBMRI-NL)) M.d.H.; (LBC1921) J.M.S.; (LBC1921/LBC1936) I.J.D.; (Leipzig-Adults) M. Blüher, P. Kovacs; (LOLIPOP-Exome) J.C.C., J.S.K.; (LOLIPOP-OmniEE) J.C.C., J.S.K.; (Marshfield Clinic Personalized Medicine Research Project) M.H.B., P.L.P.; (Massachusetts General Hospital Cardiology and Metabolic Patient Cohort (CAMP)) P.L.H.; (MESA) J.I.R., X.G.; (Montreal Heart Institute Biobank (MHIBB)) M.P.D., G.L., J.D.R., J.C.T.; (MORGAM Central Laboratory) M.P.; (MORGAM Data Centre) K.K.; (OBB) F. Karpe; (Ophthalmic Western Australia Biobank & Blue Mountains Eye Study) A.W.H.; (PCOS) A.P.M., C.M.L.; (PIVUS) C.M.L., L.L.; (PRIME - Belfast) F. Kee; (PRIME - Lille) P.A.; (PRIME - Strasbourg) M.M.; (PRIME - Toulouse) J.F.; (PROLAPSE (BBMRI-NL)) K.B.K.; (PROMIS) D.S.; (PROSPER) N.S.; (QC) M.A.R.; (QIMR) G.W.M., D.R.N., A.C.H., S.E.M.; (Raine) A.W.H.; (RISC) B.B., E.F., M.W.; (Rotterdam Study I) A.G.U., A.H.F., M.A.I.; (SCOOP) I. Barroso, I.S.F.; (SEARCH) A.M.D.; (SHIP/SHIP-Trend) H.J.G.; (SIBS) D.F.E.; (SOLID TIMI-52) D.M.W.; M.L.O. (SORBS) A.P.M., M.S., A. Tönjes; (Southampton) A.J.L.; (The Hoorn Diabetes Care System Cohort study (BBMRI-NL)) N.v.L.; (The Mother Child Cohort of Norway) P.R.N., S.J.; (The Mount Sinai BioMe Biobank) E.P.B., R.J.F.L.; (The NEO Study) D.O.M.K.; (The NBS and NBCS) K.K.A.; (The NHAPC study, The GBTDS study) X.L.; (The Western Australian Pregnancy Cohort (Raine) Study) C.E.P., T.A.M.; (TUDR) W.H.H.S.; (TwinsUK) T.D.S.; (UCLA-Utrecht) R.A.O.; (UCP (BBMRI-NL)) M.C.H.d.G.; (UKHLS) M. Kumari; (ULSAM) A.P.M.; (Utrecht Health Project) F.W.A.; (Vejle Biobank) I. Brandslund, C.C., O. Pedersen; (WGHS) D.I.C., P.M.R.; (Women's Health Initiative) P.L.A., A.P.R.; (WOSCOPS) I.F.; (WTCCC-UKT2D) M.I.M., K.R.O.; (YFS) T.L., O.T.R.

Genotyping of contributing studies

(1958 Birth Cohort) K.E.S., S. Kanoni; (Add Health) P.G.L., E.M.L., L.A. Lange, A.L.M.; (ADDITION-DK) A.P.G.; (Airwave) E.E., M.P.S.L.; (AMC PAS) S.S.; (Amish) L.M.Y.A., J.A. Perry; (ARIC) E.W.D., M.L.G.; (ASCOT-UK, ASCOT-SC) P.B.M.; (Athero-Express Biobank Study) S.W.v.d.L.; (BBMRI-NL) S.H.V.L.B., C.M.v.D., P.I.W.d.B.; (BRAVE) E.D.A.; (Cambridge Cancer Studies) J.G.D.; (CARDIA) M.F.; (CCHS) A. Tybjaerg-Hansen, A.V.; (CGPS) B.G.N., S.F.N.; (CHD Exome + Consortium) A.S.B., J.M.M.H., D.F.R., J.D., R.Y.; (CHOP) S.F.A.G., H.H.; (CIHDS) M. Benn, R.F.S.; (Clear/eMERGE (Seattle)) G.P.J.; (CROATIA_Korcula) V.V.; (DIABNORD) P.W.F.; (DIACORE) C.A.B., M. Gorski; (DPS) J.T.; (DR's EXTRA) T.A.L., R.R.; (Duke) S. Kathiresan; (EGCUT) T.E., L. Milani; (ENDO) A.P.M.; (EPIC-Potsdam) M.B.S., K.M.; (EpiHealth) E.I., P.W.F.; (EUGENDA) A.I.d.H.; (Family Heart Study) K.D.T.; (Fenland, EPIC) R.A.S.; (Fenland, EPIC, InterAct) N.J.W., C.L.; (FINCAVAS) M. Kähönen; (Finnish Twin Cohort) A. Loukola; (FUSION) M. Boehnke, F.S.C.; (FVG) I.G.; (GECCO) P.T.C., R.E.S.; (GeMEP) G. Tromp, D.J.C., H. Kuivaniemi; (Generation Scotland) C.H.; (Genetic Epidemiology Network of Arteriopathy (GENOA)) S.L.R.K.; (GLACIER) P.W.F.; (GoDARTS) C.N.A.P.; (GRAPHIC) N.J.S.; (GSK-STABILITY) D.M.W., M.L.O.; (Health) J.B.J.; (Health ABC) T.B.H., Y. Liu; (Health and Retirement Study (HRS)) J.D.F., Wei Zhao; (HELIC MANOLIS) L. Southam; (HELIC Pomak) L. Southam; (ID1000/Spinoza (BBMRI-NL)) A.R.H.; (Inter99) T.H., N.G.; (INTERVAL Study) C.M., J.S., W.O., D.J.R., A.B.; (IRASFS) N.D.P.; (KORA) M.M.N.; (KORA S4) K. Strauch; (LBC1921/LBC1936) G. Davies; (Leipzig-Adults) A.M.; (LOLIPOP-Exome) J.C.C., J.S.K.; (LOLIPOP-OmniEE) J.C.C., J.S.K.; (Marshfield Clinic Personalized Medicine Research Project) M.H.B.; (MESA) J.I.R., Y.D.I.C., K.D.T.; (METSIM) J. Kuusisto, M.L.; (Montreal Heart Institute Biobank (MHIBB)) M.P.D.; (The NHAPC study, The GBTDS study) Y.W.; (OBB) F. Karpe; (Ophthalmic Western Australia Biobank & Blue Mountains Eye Study) A.W.H.; (PCOS) A.P.M.; (PIVUS) C.M.L.; (PROLAPSE (BBMRI-NL)) K.B.K.; (QIMR) G.W.M., D.R.N.; (Raine) A.W.H.; (Rotterdam Study I) A.G.U., M.C.M.G., F. Rivadeneira; (SDC) H.V.; (SEARCH) A.M.D.; (SHIP/SHIP-Trend) U.V.; (SOLID TIMI-52) D.M.W., M.L.O.; (SORBS) A.P.M.; (Southampton) A.J.L., H.L.G.; (SR) S.C., A.D.E.; (The Hoorn Diabetes Care System Cohort study (BBMRI-NL)) N.v.L.; (The Mother Child Cohort of Norway) O.H.; (The Mount Sinai BioMe Biobank) E.P.B., R.J.F.L., Y. Lu, C.S.; (The NEO Study) R.L.G.; (The NBS and NBCS) L.A.K.; (The NHAPC study, The GBTDS study) X.L., H. Li, Y.H.; (The Western Australian Pregnancy Cohort (Raine) Study) C.E.P.; (TUDR) E.K.; (TwinsUK) A.P.M.; (UCLA-Utrecht) R.A.O.; (UCP (BBMRI-NL)) M.C.H.d.G.; (UKHLS) M. Kumari; (ULSAM) A.P.M.; (Utrecht Health Project) M.L.B.; (WGHS) D.I.C., A.Y.C.; (Women's Health Initiative) C.K., A.P.R.; (WOSCOPS) C.J.P.; (WTCCC-UKT2D) M.I.M.; (YFS) T.L., L.P.L.

Phenotyping of contributing studies

(Add Health) P.G.L., K.M.H.; (ADDITION-DK) D.R.W.; (Airwave) E.E.; (AMC PAS) S.S.; (Amish) L.M.Y.A.; (ARIC) E.W.D.; (ARIC, Add Health) K.E.N.; (ASCOT-SC) M.J.C.; (ATBC) S.M.; (BBMRI-NL) S.H.V.; (BioVU) T.L.E., D.R.V.E.; (Blue Mountains Eye Study) P. Mitchell; (BRAVE) E.D.A.; (BRIGHT) M.J.C.; (CARDIA) C.E.L., (CARL) A. Robino; (CCHS) G.B.J., A. Tybjaerg-Hansen, A.V.; (Cebu Longitudinal Health and Nutrition Survey) N.R.L.; (CGPS) B.G.N., P.R.K.; (CHD Exome + Consortium) A.S.B., J.M.M.H., D.F.R., J.D., R.Y.; (CHES) R.V., R.M.C.; (CHOP) S.F.A.G., H.H., J.P.B.; (CIHDS) R.F.S., L.E.B.; (Clear/eMERGE (Seattle)) G.P.J., A.A.B.; (CROATIA_Korcula) O. Polasek, I.R.; (DIABNORD) O.R.; (DIACORE) C.A.B., M.R.; (DPS) A.U.J., J. Lindström; (DR's EXTRA) P. Komulainen, T.A.L., R.R.; (Duke) S. H. Shah; (EFSOCH) A.T. Hattersley; (EGCUT) D.E., A.F.; (ENDO) K.T.Z.; (EPIC-Potsdam) H.B.; (EPIC-CVD) A.B.; (EpiHealth) E.I.; (EUGENDA) S. Fauser; (EXTEND) A.T. Hattersley; (Family Heart Study) M.F.F.; (Fenland, EPIC, InterAct) N.J.W.; (FIA3) J.H.J.; (FINCAVAS) M. Kähönen, K.N.; (Finnish Twin Cohort) J. Kaprio; (FINRISK) V. Salomaa; (FUSION) A.J.S., N.N.; (FVG) E.C.; (GECCO) P.T.C., R.E.S.; (GeMEP) G. Tromp; (Generation Scotland) C.H.; (Genetic Epidemiology Network of Arteriopathy (GENOA)) S.L.R.K.; (GLACIER) F. Renström; (GoDARTS) C.N.A.P., A.D.M.; (GRAPHIC) N.J.S.; (GSK-STABILITY) L.W., H.D.W.; (Health) A. Linneberg, B.H.T.; (Health ABC) T.B.H., Y. Liu; (Health and Retirement Study (HRS)) J.D.F.; (HELIC MANOLIS) L. Southam, A.E.F., E.T.; (HELIC Pomak) L.

implicate pathways that control energy intake and expenditure underpinning obesity

A full list of authors and affiliations appears at the end of the article.

Abstract

Genome-wide association studies (GWAS) have identified >250 loci for body mass index (BMI), implicating pathways related to neuronal biology. Most GWAS loci represent clusters of common, non-coding variants from which pinpointing causal genes remains challenging. Here, we

Southam, A.E.F., M. Karaleftheri; (HUNT-MI) O.L.H.; (BBMRI-NL)) S.H. Scholte, A.R.H.; (Inter99) T.J., N.G.; (INTERVAL Study) C.M., J.S., W.O., D.J.R.; (IRASFS) N.D.P., B.K., L.E.W.; (KORA) M.M.N.; (KORA S4) A. Peters; (LBC1921) J.M.S., A. Pattie; (LBC1921/LBC1936) I.J.D.; (LBC1936) J.C.; (Leipzig-Adults) M. Blüher, P. Kovacs; (LOLIPOP-Exome) J.C.C., J.S.K.; (LOLIPOP-OmnIEE) J.C.C., J.S.K.; (Marshfield Clinic Personalized Medicine Research Project) P.L.P., T.N.P.; (Massachusetts General Hospital Cardiology and Metabolic Patient Cohort (CAMP)) S.A.L., P.T.E.; (MESA) M.A.; (METSIM) X.S.; (Montreal Heart Institute Biobank (MHIBB)) G.L., K.S.L., V.T.; (MORGAM Data Centre) K.K.; (The NHAPC study, The GBTDS study) X.L.; (NESCOG (BBMRI-NL)) T.J.P.; (OBB) F. Karpe, M.N.; (Ophthalmic Western Australia Biobank & Blue Mountains Eye Study) A.W.H.; (PCOS) C.M.L.; (PIVUS) L.L.; (PRIME - Belfast) F. Kee; (PRIME - Lille) P.A.; (PRIME - Strasbourg) M.M.; (PRIME - Toulouse) J.F.; (PROLAPSE (BBMRI-NL)) K.B.K.; (PROSPER) S.T.; (QIMR) G.W.M., D.R.N., A.C.H., S.E.M.; (Raine) A.W.H.; (RISC) B.B., E.F.; (Rotterdam Study I) O.H.F., M.A.I., M.C.M.G., F. Rivadeneira; (SDC) E.R.B.P.; (SHIP, SHIP-TREND) N.L., (SORBS) M.S., A. Tönjes; (Southampton) A.J.L., H.L.G.; (SR) M. Brumat; (The Mother Child Cohort of Norway) P.R.N., O.H.; (The Mount Sinai BioMe Biobank) E.P.B., Y. Lu, C.S.; (The NEO Study) R.d.M.; (The NBS and NBS) K.K.A., L.A.K., T.E.G.; (The NHAPC study, The GBTDS study) X.L., H. Li, L. Sun, F.W.; (The Western Australian Pregnancy Cohort (Raine) Study) C.E.P., T.A.M.; (TUDR) W.H.H.S., K.H.L.; (TwinsUK) T.D.S., K.S.S.; (UCLA-Utrecht) R.S.K.; (UCP (BBMRI-NL)) M.C.H.d.G.; (UKGPCS) A. Lophatananon; (UKHLS) M. Kumari; (ULSAM) V. Giedraitis; (Utrecht Health Project) M.L.B.; (WGHS) D.I.C., P.M.R.; (Women's Health Initiative) C.K.; (WOSCOPS) C.J.P.; (WTCCC-UKT2D) M.I.M., K.R.O.; (YFS) T.L., O.T.R.

Data analysis of contributing studies

(1958 Birth Cohort) K.E.S., S.E.A.; (AGES) A.V.S.; (Airwave) E.E., M.P.S.L.; (AMC PAS) S.S.; (Amish) J.R.O., L.M.Y.A., J.A. Perry; (ARIC, Add Health) K.E.N., K.L.Y., M. Graff; (ASCOT-UK, ASCOT-SC) H.R.W.; H.Y.; (Athero-Express Biobank Study) S.W.v.d.L.; (ATVB, Ottawa) H.T.; (BBMRI-NL) L. Broer; (BioVU) T.L.E., A.G.; (BRAVE) R.C., D.S.A.; (BRIGHT) H.R.W.; (Cambridge Cancer Studies) J.G.D., A. Pirie, D.J.T.; (CARDIA) M.F., L.-A. Lin; (CARL) A. Robino, M.C., D.V.; (Cebu Longitudinal Health and Nutrition Survey) Y. Wu; (CHD Exome + Consortium) A.S.B., J.M.M.H., D.F.R., R.Y., P.S.; (CHES) Y.J.; (CHOP) J.P.B.; (CROATIA_Korcula) V.V.; (deCODE) V. Steinthorsdottir, G. Thorleifsson; (DHS) A.J.C., P. Mudgal, M.C.Y.N.; (DIABNORD) T.V.V.; (DIACORE) C.A.B., M. Gorski, M.R.; (Duke) A.P.P.; (EFSOCH) H.Y.; (EGCUT) T.E.; (eMERGE (Seattle)) T.S.C.; (ENDO) A.M., Tugce Karaderi, N.R.R.; (EPIC) J.H.Z.; (EPIC-Potsdam) K.M.; (EPIC-CVD) A.S.B., J.M.M.H., D.F.R., R.Y., P.S.; (EpiHealth) S.G.; (EUGENDA) J.C.G.; (EXTEND) H.Y.; (Family Heart Study) M.F.F.; (Fenland) J. Luan; (Fenland, EPIC) R.A.S.; (Fenland, InterAct) S.M.W.; (FIA3) T.V.V.; (FINCAVAS) J. Hernesniemi; (FIN-D2D 2007) H.P., M.U., M.V.; (Finnish Twin Cohort) L.H.; (FINRISK 2007 (T2D)) T. Korhonen, S.M., L. Moilanen, H.M.S.; (Finrisk Extremes and QC) S.V.; (Framingham Heart Study) C.T.L., N.L.H.C.; (FVG) I.G.; (GECCO) M.D.; (GeMEP) G. Tromp; (GENDEP) K.E.T., R.U.; (Generation Scotland) C.H., J.E.H.; (Genetic Epidemiology Network of Arteriopathy (GENOA)) L.F.B.; (GIANT-Analyst) A.E.J.; (GLACIER) T.V.V.; (GoDARTS) A.M.; (GRAPHIC) N.J.S., N.G.D.M., C.P.N.; (GSK-STABILITY) D.M.W., A.J.S.; (Health) J.B.J.; (Health ABC) M.A.N.; (Health and Retirement Study (HRS)) Wei Zhao, E.B.W.; (HELIC MANOLIS) L. Southam; (HELIC Pomak) L. Southam; (HUNT-MI) W. Zhou; (Inter99) N.G.; (INTERVAL Study) A.S.B.; (IRASFS) N.D.P., B.K.; (Jackson Heart Study (JHS)) L.A. Lange, J. Li; (KORA S4) T.W.W.; (LBC1921/LBC1936) G. Davies; (Leipzig-Adults) A.M.; (LOLIPOP-Exome) J.C.C., J.S.K., W. Zhang; (LOLIPOP-OmnIEE) J.C.C., J.S.K., W. Zhang; (Massachusetts General Hospital Cardiology and Metabolic Patient Cohort (CAMP)) S.A.L., H. Lin; (MESA) J.I.R., X.G., J.Y.; (METSIM) J. Kuusisto, M.L., X.S.; (MONICA-Brianza) G.V.; (Montreal Heart Institute Biobank (MHIBB)) G.L., K.S.L., V.T.; (The NHAPC study, The GBTDS study) P.Y.; (OBB) A.M.; (Ophthalmic Western Australia Biobank & Blue Mountains Eye Study) G.C.P.; (PCOS) A.M., T. Karaderi, N.R.R.; (PIVUS) A.M., Tugce Karaderi, N.R.R.; (PROLAPSE (BBMRI-NL)) K.B.K.; (PROMIS) A. Rasheed, Wei Zhao; (PROSPER) J.W.J.; (QC GoT2D/T2D-GENES (FUSION, METSIM, etc)) A.E.L.; (QIMR) Y.S.; (RISC) H.Y.; (Rotterdam Study I) M.C.M.G., F. Rivadeneira; (SCOOP) V.M., A.E.H., G.M.; (SDC) C.T.H.; (SHIP/SHIP-Trend) A. Teumer; (SOLID TIMI-52) D.M.W., A.J.S.; (SORBS) A.P.M.; (Southampton) J.G.; (The Hoom Diabetes Care System Cohort study (BBMRI-NL)) N.v.L.; (The Mother Child Cohort of Norway) S.J., O.H.; (The Mount Sinai BioMe Biobank) Y. Lu, C.S.; (The NEO Study) R.L.G.; (The NHAPC study, The GBTDS study) X.L., H. Li, Y.H.; (The Western Australian Pregnancy Cohort (Raine) Study) C.A.W.; (UCLA-Utrecht) L.M.O.L.; (UCP (BBMRI-NL)) M.C.H.d.G.; (UK Biobank) A.R.W., T.M.F.; (UKGPCS) A. Lophatananon; (UKHLS) M. Kumari; (UKOPS) J.P.T.; (ULSAM) A.M., T. Karaderi, N.R.R.; (Utrecht Health Project) J.v.S.; (WGHS) D.I.C., A.Y.C.; (Women's Health Initiative) P.L.A., J. Haessler; (WOSCOPS) I.F., S.P.; (WTCCC-UKT2D) W.G.; (YFS) L.P.L.

combined data from 718,734 individuals to discover rare and low-frequency (MAF<5%) coding variants associated with BMI. We identified 14 coding variants in 13 genes, of which eight in genes (*ZBTB7B*, *ACHE*, *RAPGEF3*, *RAB21*, *ZFH3*, *ENTPD6*, *ZFR2*, *ZNF169*) newly implicated in human obesity, two (*MC4R*, *KSR2*) previously observed in extreme obesity, and two variants in *GIPR*. Effect sizes of rare variants are ~10 times larger than of common variants, with the largest effect observed in carriers of an *MC4R* stop-codon (p.Tyr35Ter, MAF=0.01%), weighing ~7kg more than non-carriers. Pathway analyses confirmed enrichment of neuronal genes and provide new evidence for adipocyte and energy expenditure biology, widening the potential of genetically-supported therapeutic targets to treat obesity.

Obesity is a heritable disease and represents a major unmet public health problem with only a few safe and long-term effective therapies¹ and intervention strategies². To understand the genetic basis of obesity and identify potential targets for new therapies, genome-wide association studies (GWAS) for body mass index (BMI) and obesity risk have identified >250 common variants over the past decade³⁻⁷. Consistent with single-gene disorders of obesity⁸, tissue expression and gene-set enrichment analyses for genes in BMI-associated loci have shown that the central nervous system (CNS) plays a critical role in body weight regulation⁵. While the numerous GWAS loci have provided insight into broad biological mechanisms underlying body weight regulation, pinpointing the causal gene(s)/variant(s) remains a major challenge⁹, as GWAS-identified variants are typically non-coding and may affect genes at long distance. The association of intronic *FTO* variants with BMI illustrates the challenges of identifying causal regulatory effects. The proposed causal variant was found to regulate the expression of nearby *RPGRIP1L* in some studies¹⁰⁻¹², whereas others found that it regulates distant *IRX3/IRX5* genes in specific cell types^{13,14}.

To expedite mapping of obesity-related genes, we performed an exome-wide search for low-frequency (LF, minor allele frequency [MAF]=1-5%) and rare (R, MAF<1%) single nucleotide variants (SNVs) associated with BMI using exome-targeted genotyping arrays. A total of 125 studies ($N_{\text{individuals}}=718,734$) performed single-variant association between up to 246,328 SNVs and BMI. In addition, we performed gene-based meta-analyses to aggregate rare and LF (R/LF) coding SNVs across 14,541 genes. Using genetic, functional and computational follow-up analyses, we gained insights into the function of BMI-implicated genes, and the biological pathways through which they may influence body weight.

RESULTS

Fourteen rare and low-frequency coding variants in 13 genes

Our study comprises a discovery and a follow-up stage (Supplementary Figure 1, Supplementary Tables 1-3, Online Methods). In our primary analysis, the discovery stage includes data from 123 studies ($N_{\text{max}}=526,508$) across five ancestry groups, predominantly European (~85%). Each study performed single-variant association analyses of coding variants present on the exome array, including up to 13,786 common (MAF>5%) and 215,917 R/LF coding SNVs (exons and splicing sites). Summary statistics were combined using fixed-effect meta-analyses. SNV-associations of R/LF variants that reached suggestive

significance ($P < 2.0 \times 10^{-6}$) were taken forward for follow-up in two European cohorts, deCODE ($N_{max}=72,613$) and UK Biobank ($N_{max}=119,613$ [interim release]). Overall significance was assessed after combining results of discovery and follow-up studies into a final meta-analysis (all-ancestries, sex-combined, additive model, $N_{max}=718,734$); SNV-associations that reached $P < 2 \times 10^{-7}$ were considered array-wide significant^{15,16} (Table 1, Supplementary Table 4, Supplementary Figures 2–4). In secondary analyses, we performed sex-specific analyses, analyses limited to individuals of European ancestry, and analyses using a recessive model.

In our primary analysis of R/LF variants, we identified five rare SNVs in three genes (*KSR2*, 2 in *MC4R*, 2 in *GIPR*) and nine LF SNVs in eight genes (*ZBTB7B*, 2 in *ACHE*, *RAPGEF3*, *PRKAG1*, *RAB21*, *HIP1R*, *ZFH3*, *ENTPD6*) (Table 1, Box 1, Supplementary Table 5, Supplementary Figure 3a). In secondary analyses, we identified two additional LF SNVs; one in all-ancestry women-only (*ZFR2*) and one in European ancestry only analyses (*ZNF169*) (Table 1, Supplementary Tables 6–8, Supplementary Figures 3b, 3c). Of these 16 SNVs, located in 13 genes, the two SNVs in *MC4R* ($r^2=1$; $D'=1$) and two in *ACHE* ($r^2=0.98$; $D'=0.99$) were in high LD, whereas the two SNVs in *GIPR* ($r^2=0$; $D'=0.16$) were independent of each other. Hence, the 16 SNVs represent 14 independent SNVs (4 rare, 10 LF), of which eight locate in genes not previously implicated in BMI (*ZBTB7B*, *ACHE*, *RAPGEF3*, *RAB21*, *ZFH3*, *ENTPD6*, *ZFR2*, *ZNF169*), and six are located in five loci that were previously identified by GWAS (*PRKAG1/BCDIN3D*, *HIP1R/CLIP1*, *MC4R*, *GIPR/QPCTL*)⁵ and/or through sequencing of severe early-onset obesity cases (*MC4R*, *KSR2*)^{17–19} (Supplementary Figure 5). Conditional analyses established that coding SNVs in *PRKAG1*, *MC4R* and *GIPR* are independent of the common lead variants in GWAS loci (rs7138803, rs17782313, rs2287019, respectively), whereas the SNV in *HIP1R* and GWAS locus near *CLIP1* (rs11057405) represent the same signal (Online Methods, Supplementary Tables 9, 10, Supplementary Figure 5).

Next, we performed gene-based association tests (SKAT, VT; broad, strict) in up to 14,541 genes²⁰ to examine whether these aggregated analyses would yield new evidence for multiple R/LF coding SNVs in the same gene affecting BMI (Online Methods). Using broad SNV inclusion criteria, associations for 13 genes reached array-wide significance ($P < 2.5 \times 10^{-6}$)^{15,16}, four of which had not been highlighted in single-variant analyses (Table 2, Supplementary Table 11). Conditional analyses showed that only for *GIPR* was the gene-based association driven by multiple SNVs (Table 2, Supplementary Table 12). For all other genes, associations were driven by a single SNV only, but these SNVs had not reached array-wide significance in single-variant analyses.

Taken together, we identified 14 R/LF coding SNVs in 13 genes that are independently associated with BMI; four rare SNVs in three genes, and 10 LF SNVs in 10 genes. One SNV (*ZFR2*) showed a sex-specific effect, whereas no ancestry-specific effects were observed (Supplementary Note, Supplementary Tables 6–8, Supplementary Figure 6). Eight (*ACHE*, *ENTPD6*, *RAB21*, *RAPGEF3*, *ZBTB7B*, *ZFH3*, *ZFR2*, *ZNF169*) of these 13 genes have not been previously implicated in body weight regulation (Table 1).

Novel common coding variants associated with BMI

Although the main focus of our study was on R/LF coding SNVs, we also identified 92 common coding variants ($P < 2.0 \times 10^{-7}$; Supplementary Tables 4; Supplementary Figures 4, 7), of which 41 were novel (Supplementary Table 9, Supplementary Note). These novel common loci had not been identified in previous GWAS efforts, because our current sample size is more than twice as large as the most recent GWAS meta-analysis⁵, and also because some SNVs were not tested before, as they were not present on the HapMap reference panel and/or were on the X-chromosome, which was not analyzed. Because of the increased samples size, effect sizes of the 41 novel common loci are smaller (on average 0.014 SD/allele, [range: 0.010–0.024]) than of previously established common loci (0.021 SD/allele, [0.010–0.050]) (Supplementary Figure 7).

Impact of R/LF SNVs on BMI and obesity risk

The minor allele for half of the 14 R/LF SNVs is associated with lower BMI (Table 1, Figure 1). The effects of LF SNVs range between 0.024 and 0.066 SD/allele, equivalent to ~ 0.11 to 0.30 kg/m^2 in BMI or ~ 0.315 to 0.864 kg in body weight for a 1.7m tall person. Effects of rare SNVs range between 0.06 and 0.54 SD per allele, equivalent to 0.26 to 2.44 kg/m^2 or 0.74 kg to 7.05 kg per allele (Table 1, Figure 1). By comparison, these rare SNV effect sizes are on average ten times larger than those for previously identified GWAS loci (effect_{mean}=0.019 SD/allele, $\sim 0.086 \text{ kg/m}^2$ or $\sim 0.247 \text{ kg/allele}$) of which the largest effect is seen for the *FTO* locus (0.08 SD/allele, $\sim 0.35 \text{ kg/m}^2$ or 1 kg/allele) and those for other GWAS loci range between 0.010 and 0.056 SD/allele (~ 0.045 to 0.25 kg/m^2 , or 0.130 to 0.728 kg)⁵.

Effect sizes increase as MAF decreases, in particular for SNVs with a MAF < 0.5% (~ 1 heterozygote carrier in 100 people), consistent with the statistical power of our sample (Figure 1). For example, the nonsense p.Tyr35Ter *MC4R* SNV (rs13447324, MAF=0.01%) is present in ~ 1 in 5,000 individuals and results in a $\sim 7 \text{ kg}$ higher body weight for a 1.7m tall person. The two *GIPR* SNVs contribute independently to a lower body weight; carriers (1 in ~ 455 individuals) of p.Arg190Gln (rs139215588) weigh $\sim 1.92 \text{ kg}$ (0.148 SD BMI) less than non-carriers and carriers (1 in ~ 385 individuals) of p.Glu288Gly (rs143430880) weigh $\sim 1.99 \text{ kg}$ (0.153 SD BMI) less. Among 115,611 individuals of the UK Biobank, one apparently healthy 61-year-old woman, with no reported illnesses, carried both rare *GIPR* alleles and weighed $\sim 11.2 \text{ kg}$ less (equivalent to -0.86 SD BMI or 3.87 kg/m^2) than the average non-carrier of the same height (Supplementary Figure 8). The possible synergistic effect of the two *GIPR* alleles needs confirmation by additional individuals that carry both variants.

Even though effect sizes of LF and, in particular, rare SNVs tend to be larger than those of common GWAS-identified loci⁵, the 14 SNVs combined explain $< 0.1\%$ of BMI variation, because of their low population frequency (Table 1, Online Methods). Also, although the effects of the four rare SNVs (*KSR2*, *MC4R*, 2 in *GIPR*) are large by GWAS standards, penetrance for obesity is still expected to be low. Indeed, using data from the UK Biobank ($N_{\text{max}}=119,781$), we compared the prevalence of normal-weight (18.5 kg/m^2 BMI $< 25 \text{ kg/m}^2$) and obesity (BMI $\geq 30 \text{ kg/m}^2$) between carriers and non-carriers (Supplementary Table 13, Online Methods). For *GIPR* (p.Arg190Gln, p.Glu288Gly), both BMI-decreasing

SNVs, carriers tended ($P < 0.05$) to have a lower obesity prevalence (21.2%, 20.1%, respectively), compared to non-carriers (25.1%, 25%). For *MC4R* p.Tyr35Ter and *KSR2* p.Arg525Gln, the prevalence of obesity between carriers (30%, 25.7%, resp.) and non-carriers (25.1%, 25.3%) was not significantly different.

We examined whether R/LF SNVs affect obesity risk early on in life by combining data from three case-controls studies of childhood obesity ($N_{cases}=4,395$; $N_{controls}=13,072$) (Online Methods, Supplementary Table 14). Associations for 10 of 13 SNVs were directionally consistent with those observed for BMI in adults (77%, $P_{binomial}=0.046$), three of which (*ZBTB7B*, *PRKAG1*, *RAB21*) reached nominal significance ($P < 0.05$). While no carriers of the *MC4R* mutations were available for analyses, the role of *MC4R* in body weight regulation in childhood was established almost two decades ago^{17,19,21}.

Impact of R/LF SNVs on cardiometabolic and other traits

To examine whether identified SNVs affect other traits, we obtained results from multiple large-scale genetic consortia (GIANT¹⁵, MAGIC, GoT2D/T2D-GENES¹⁶, GLGC, ICBP²², REPROGEN²³) (Supplementary Table 15, Supplementary Figure 9), and performed phenome-wide association (PheWAS) analyses using electronic medical record (EMR) data from BioVu and UK Biobank (Online Methods, Supplementary Table 16). The BMI-increasing allele of *ZBTB7B* p.Pro190Ser is associated with greater height, and those of *PRKAG1*, *ACHE*, and *RAPGEF3* SNVs are associated with shorter height, but association with other traits differ. Specifically, *PRKAG1* p.Thr38Ser Ser-allele carriers appear heavier and shorter, have lower HDL-cholesterol levels, earlier age at menarche (reported before²³) and higher systolic blood pressure, which is in agreement with PheWAS analyses showing an increased risk of “malignant essential hypertension” and “hypertension” (Supplementary Table 16). While carriers of the *RAPGEF3* p.Leu300Pro Pro-allele are also heavier and shorter, they have a lower WHR_{adjBMI} ²⁴ and lower fasting insulin levels (Supplementary Table 15), consistent with PheWAS results that show lower odds of “secondary diabetes mellitus” (Supplementary Table 16). Thus, while all SNVs are associated with BMI, their patterns of association with other traits suggest they may affect different physiological pathways.

Gene set enrichment analyses

To test whether the R/LF variants implicate biological pathways, we performed gene set enrichment analyses. Similar to our previous analysis of GWAS for BMI⁵, we analyzed coding variants that reached $P < 5 \times 10^{-4}$, using a DEPICT version adapted for exome-array analysis¹⁵ (Online Methods, Supplementary Note). We used 50 R/LF coding variants as input (all $P < 5 \times 10^{-4}$; Online Methods) and observed significant enrichment (Figure 2, Supplementary Table 17, Supplementary Figure 10a). Many of these relate to neuronal processes, such as neurotransmitter release and synaptic function (e.g. glutamate receptor activity, regulation of neurotransmitter levels, synapse part), consistent with previous findings from GWAS⁵. When we excluded variants near (± 1 Mb) previously identified GWAS loci, we still observed 29 significantly enriched gene sets (in 12 meta-gene sets) (Supplementary Table 18, Supplementary Figure 10b), thereby providing an independent confirmation of the GWAS gene set enrichment results. In addition to neuronal-related gene

sets, the analyses with R/LF coding variants newly identified a cluster of metabolic pathways related to insulin action and adipocyte/lipid metabolism (e.g. enhanced lipolysis, abnormal lipid homeostasis, increased circulating insulin level; Figure 2). Finally, we observed that R/LF BMI-associated coding variants are more effective at identifying enriched gene sets compared to common coding variants. Specifically, adding 192 common coding SNVs (all $P < 5 \times 10^{-4}$) to the analysis decreased the number of enriched gene sets from 471 (106 meta-gene sets) seen with R/LF coding SNVs to 62 (24 meta-gene sets) (Supplementary Table 19, Supplementary Figure 10c). We observed fewer significant genes sets with the combined common and R/LF analysis, despite including more total coding variants and a higher fraction of array-wide significant coding variants. One possible explanation is that R/LF coding variants may fall in the causal gene more often than do common coding variants, which suggests that the R/LF variants are more likely to be causal, rather than simply in LD with causal variants.

We also used gene set enrichment analysis to prioritize candidate genes. Among the genes with R/LF coding variants associated with BMI at $P < 5 \times 10^{-4}$, a subset is prominently represented in the CNS-related enriched gene sets (Figure 2) and is proposed to influence neurotransmission and/or synaptic organization, function and plasticity. These include genes in regions with suggestive evidence of association from GWAS (e.g. *CARTPT*, *MAP1A*, *ERC2*) and genes in regions not previously implicated by GWAS (e.g. *CALY*, *ACHE*, *PTPRD*, *GRIN2A*). The non-neuronal metabolic gene sets implicate two genes (*CIDEA*, *ADH1B*) that are markers of brown or “beige” adipose tissue^{25,26}, providing new supporting evidence for a causal role of this aspect of adipocyte biology.

***Drosophila* fly results**

To test for potential adiposity-driving effects of gene regulation, we performed tissue-specific RNAi-knockdown experiments in *Drosophila*. We generated adipose-tissue (cg-Gal4) and neuronal (elav-Gal4) specific RNAi-knockdown crosses for nine of the 13 candidate genes for which fly orthologues exist (Supplementary Table 20) and performed whole body triglyceride analysis in young adult male flies. Triglycerides, the major lipid storage form in animals, were chosen as a direct measure of fly adiposity. Both neuronal and fat-body knockdown of *zfh2*, the orthologue of *ZFHX3*, resulted in significantly increased triglyceride levels. Adipose-tissue specific, but not neuronal, knockdown of *epac* (*RAPGEF3*) was lethal. Tissue-specific loss-of-function of the other seven genes tested did not affect triglyceride levels.

R/LF coding SNVs in monogenic and syndromic genes

We identified 39 genes in the literature that have been convincingly implicated in monogenic obesity or syndromes of which obesity is one of the main features (Supplementary Table 21, 22, Supplementary Figure 11). Of the 652 R/LF SNVs in these 39 monogenic and/or syndromic genes, five R/LF SNVs were significantly associated with BMI (Bonferroni-corrected P -value = 7.7×10^{-5} (=0.05/652)). Beside SNVs in *MC4R* (p.Tyr35Ter, Asp37Val) and *KSR2* (Arg525Gln), already highlighted in the single-variant analyses, we identified an additional SNV in *MC4R* (p.Ile251Leu) and one in *BDNF* (p.Glu6Lys). *MC4R* p.Ile251Leu has been previously shown to protect against obesity²⁷, whereas *BDNF* p.Glu6Lys,

independent of previously GWAS-identified SNVs ($r^2=0.01$, $D'=1.0$)⁵, has not been implicated in body weight regulation before. We examined whether the 652 R/LF SNVs showed enrichment for association with BMI compared to R/LF coding SNVs in all other genes, but found no evidence to support this.

DISCUSSION

In this meta-analysis of exome-targeted genotyping data, we identified 14 R/LF coding variants in 13 genes associated with BMI. Eight of these genes (*ACHE*, *ENTPD6*, *RAB21*, *RAPGEF3*, *ZBTB7B*, *ZFH3*, *ZFR2*, *ZNF169*) have not been previously implicated in human obesity, but evidence from animal studies provides support for a role in energy metabolism for some of these, such as *ACHE*^{28,29}, *RAPGEF3*^{30–33}, and *PRKAG1*^{34–39}. Others fall into established BMI GWAS loci (*PRKAG1/BCDIN3D*, *HIP1R/CLIP1*, *MC4R*, *GIPR/QPCTL*)⁵ and/or were previously implicated in severe early-onset obesity (*MC4R*, *KSR2*)^{17–19} and using this exome-targeted approach, we pinpoint R/LF variants in these loci that play a role in obesity in the general population. Pathway analyses confirm a key role for neuronal processes, and newly implicate adipocyte and energy expenditure biology.

Consistent with other polygenic traits^{15,23,40–43}, we show that large sample sizes are needed to identify R/LF variants. Observed effect sizes reflect the statistical power of our sample size, and are particularly large for SNVs with a MAF < 0.05%. The existence of rare alleles with larger effects on BMI than have been observed for common alleles might reflect negative or stabilizing selection on the extremes of BMI. However, rare variants with smaller effects almost certainly exist; larger samples will be needed to uncover these. Our study was limited to coding variants on the exome-array; large-scale sequencing studies will be needed to test for variants not covered by exome-arrays.

The strongest association was observed for a stop-codon (p.Tyr35Ter, rs13447324, MAF=0.01%) in *MC4R*, with carriers weighing on average 7kg more than non-carriers. *MC4R* is widely expressed in the CNS and is an established key player in energy balance regulation^{44,45}. Mouse and human studies showed already two decades ago that *MC4R*-deficiency results in extreme obesity, mainly through increased food intake^{46–49}. p.Tyr35Ter, which results in *MC4R*-deficiency⁵¹, was one of the first *MC4R* mutations discovered in monogenic cases of obesity^{17,19}, in whom the mutation is >20× more prevalent than in the general population^{17,50,52,53}. Here, we show that p.Tyr35Ter plays a role outside the setting of early-onset and extreme obesity. Despite its large effect, penetrance is low, and does not fit the model of a fully penetrant Mendelian variant.

While significant R/LF coding variants are strong candidates for being causal, the strongest implication of causal genes is provided by association with multiple independent coding variants, as we demonstrate for *GIPR*. We identified two rare variants in *GIPR* (p.Arg190Gln, rs139215588, MAF=0.11%; p.Glu288Gly, rs143430880, MAF=0.13%) independently associated with lower BMI; carriers of either variant weigh ~2 kg less than non-carriers. Common variants in/near *GIPR* have been found to associate with lower BMI⁵⁵ and delayed glucose and insulin response to an oral glucose challenge⁵⁴. However, the two rare variants influence BMI independently of these common ones and are not associated

with type 2 diabetes or glycemic traits tested. Rodent models have provided strong evidence for a role of GIPR in body weight regulation. *Gipr*-deficient mice are protected from diet-induced obesity⁵⁶ and have an increased resting metabolic rate⁵⁷. Blocking GIP-signaling using a vaccination approach in mice on a high-fat diet reduces weight gain, mainly through reduced fat accumulation, mediated through increased energy expenditure⁵⁸. Manipulation of incretins (GIP, GLP1) and their receptors has complex effects on obesity and insulin secretion/action that may differ between human and mice⁵⁹. The human genetic data suggest that inhibition of GIPR-signaling might present a therapeutic target for the treatment of obesity⁶⁰.

A fourth rare variant, in *KSR2*, (p.Arg525Gln, rs56214831, MAF=0.82%) increases body weight by ~740g/allele. *KSR2* is another gene previously implicated in energy metabolism and obesity^{18,61,62}. In a recent study, mutation carriers were hyperphagic, had a reduced basal metabolic rate and severe insulin resistance¹⁸. Consistent with human data, *Ksr2*^{-/-} mice were obese, hyperphagic, and had a reduced energy expenditure^{18,61-63}. *KSR2* is almost exclusively expressed in the brain and interacts with multiple proteins⁶⁴, including AMP-activated protein kinase (AMPK), a key regulator of energy homeostasis^{61,62}. Interestingly, *KSR2* is one of the first genes implicated in severe, early-onset obesity in which mutations not only affect food intake but also basal metabolic rate, and is thought to act via neuronal effects¹⁸ (Figure 2).

Despite convincing associations of these four rare variants in *MC4R*, *GIPR* and *KSR2*, their penetrance for obesity is low (Supplementary Table 13). This is consistent with the polygenic and multifactorial nature of obesity, where variants across a range of frequencies and effect sizes contribute to the phenotype in any one person. Despite low predictive power, it remains possible that the identities of particular variants in any one person may contribute to different balances of underlying physiologies and hence, different responses to treatments. This was illustrated in two patients with monogenic obesity due to *POMC* mutations; these patients lack the main activator of MC4R and were effectively treated with an MC4R-agonist⁶⁵.

Of the coding variants in newly identified genes, some have well-known connections to obesity. For example, *PRKAG1* encodes the γ 1-subunit of AMPK, a critical cellular energy sensor³⁴. In the hypothalamus, AMPK integrates hormonal and nutritional signals with neuronal networks to regulate food intake and whole-body energy metabolism³⁵⁻³⁷. Furthermore, hypothalamic AMPK is a key regulator of brown adipose tissue in mice^{36,38,39}. The BMI-decreasing allele at the associated *PRKAG1* variant (p.Thr38Ser, rs1126930, MAF=3.22%) has additional beneficial effects on blood pressure, providing additional genetic support for modulation of AMPK as an ongoing therapeutic avenue for treatment.

ACHE, in which p.His353Asn (rs1799805, MAF=3.9%) is associated with increased BMI, is another candidate gene related to neuronal biology, involved in the signaling of acetylcholine at neuromuscular junction and brain cholinergic synapses^{67,68}. Inhibitors of ACHE, used to treat moderate-to-severe Alzheimer's Disease⁶⁹, results in weight loss in humans and Ache-deficient mice have delayed weight gain^{28,29}. However, these may be

indirect consequences of adverse gastrointestinal and neuromuscular effects, respectively^{28,29,70,71}.

Another LF coding variant (p.Leu300Pro, rs145878042, MAF=1.1%) is located in *RAPGEF3*, and has strong effects on multiple other phenotypes. The BMI-increasing 300Pro-allele is associated with shorter height, lower WHR_{adjBMI} and lower insulin levels, suggesting that this variant has multiple physiologic consequences. Data from animal models also suggest complex effects of *RAPGEF3* on adipocyte biology, energy balance and glucose metabolism^{30–33}. For example, in one study, global deletion of *Rapgef3* in mice on a high-fat diet are resistant to obesity due to reduced food intake and have an increased glucose tolerance³¹. However, in a similar study, *Rapgef3*^{-/-} mice develop severe obesity, increased respiratory exchange ratio and impaired glucose tolerance³³. Adipose tissue-specific *Rapgef3* knockout mice on a high-fat diet are also more prone to obesity, show increased food intake, reduced energy expenditure, impaired glucose tolerance, and reduced circulating leptin levels⁷². More research is needed to understand the consequences of *RAPGEF3* manipulation.

The remaining genes with significant associations, *ENTPD6*, *HIP1R*, *RAB21*, *ZFR2*, *ZBTB7*, and *ZFHX3*, have no clear prior evidence for a role in energy homeostasis, and in-depth functional follow up is needed to gain insight in how they affect body weight. Here, we performed gene set enrichment analyses to better understand the biology implicated by our genetic data, and confirm the importance of neuronal processes, in particular synaptic function and neurotransmitter release, providing an independent validation of previous GWAS findings⁵. The combination of gene set enrichment and association analyses of coding variants also enables us to highlight candidate genes that are both within these gene sets and show association with BMI at R/LF coding variants. These include genes reaching array-wide significance (e.g. *ACHE*, *ZFR2*), and others with clear prior evidence for a role in body weight regulation (e.g. *CARTPT*⁷³), but that had not been highlighted in our single-variant or gene-based association analyses. Of note, the enrichment signals were stronger with R/LF coding variants only than with *all* coding variants, suggesting that R/LF variants are more likely to be causal and may more often point directly to relevant genes, whereas common coding variants may more often be proxies for common noncoding variants that affect nearby genes.

In addition, our gene set enrichment analyses now provide supporting evidence for a role of non-neuronal mechanisms as well. Specifically, *CIDEA* and *ADH1B* are both strongly predicted to be members of enriched gene sets related to insulin action and adipocyte biology, and both are markers that distinguish brown from white fat depots in mice²⁵ and humans²⁶. *CIDEA* is predominantly expressed in adipose tissue and known as a key regulator of energy metabolism²⁵. *Cidea*-deficient mice are resistance to diet-induced obesity with increased lipolysis and mitochondrial uncoupling²⁵. The connection of *ADH1B* to obesity is less clear, but the gene is highly expressed in human adipocytes, has been implicated by gene expression analyses in obesity and insulin resistance, and functions early in a potentially relevant metabolic pathway (retinoid biosynthesis)^{25,26,74,75}. Similar pathways were implicated by recent work dissecting the signal near *FTO*¹³. However,

because SNV-association signals at *ADH1B* and *CIDEA* did not reach array-wide significance, additional genetic analysis of their role in obesity would be warranted.

In summary, we performed association analyses between R/LF variants and BMI in >700,000 individuals, and identified 14 variants in 13 genes, in 5 known and 8 novel genes. While each variant contributes little to BMI variation in the general population, they may have substantial impact on body weight at an individual level. Furthermore, prior literature for these genes and unbiased gene set enrichment analysis indicate a strong role for neuronal biology and also provide new support for a causal role of aspects of adipocyte biology. The identified genes provide potential targets that may lead to new and more precise approaches for the treatment of obesity, which has seen minimal innovation in the past 30 years¹.

ONLINE METHODS

Study design & participants

The discovery cohort consisted of 123 studies (163 datasets) comprising 526,508 adult (>18 yrs) individuals of the following ancestries (Supplementary Figure 1): 1) European (N = 449,889), 2) South Asian (N = 29,398), 3) African (N = 27,610), 4) East Asian (N = 8,839), and 5) Hispanic (N = 10,772). All participating institutions and coordinating centers approved this project and informed consent was obtained from all study participants. Discovery meta-analyses were carried out in each ancestry separately and in the All-ancestries combined group, for both sex-specific and sex-combined analyses. SNVs for which associations reach suggestive significance ($P < 2.0 \times 10^{-6}$) in the discovery analyses, were taken forward for follow-up in 192,226 individuals of European ancestry from the UK BioBank and deCODE. Conditional analyses were conducted in the All-ancestries and European descent groups. Study-specific design, sample quality control and descriptive statistics are provided in Supplementary Tables 1–3.

Phenotype

Body mass index (BMI: weight [in kilograms] / height [in meters]²) was corrected for age, age² and genomic principal components (PC, derived from GWAS data, the variants with MAF > 1% on ExomeChip, or ancestry informative markers available on the ExomeChip), as well as any additional study-specific covariates (e.g. recruiting center), in a linear regression model. For studies with non-related individuals, residuals were calculated separately by sex, whereas for family-based studies sex was included as a covariate in the model. Additionally, residuals for case/control studies were calculated separately. Finally, residuals were subject to inverse normal transformation⁹⁶.

Genotype calling

The majority of studies followed a standardized protocol and performed genotype calling using the designated manufacturer software, which was then followed by zCall⁹⁷. For 10 studies, participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, the raw intensity data for the samples from seven genotyping centers were assembled into a single project for joint calling⁹⁸. Study-specific

quality control (QC) measures of the genotyped variants were implemented before association analysis (Supplementary Table 2).

Statistical analyses

Study-level association analyses—Individual cohorts were analyzed separately for each ancestry, in sex-combined and sex-specific groups, with either RAREMETALWORKER (see URL links at the end of the Online Methods) or RVTEST⁹⁹ (Supplementary Table 2), to associate inverse normal transformed BMI with genotype accounting for potential cryptic relatedness (kinship matrix) in a linear mixed model. These software tools are designed to perform score-statistics based rare-variant association analyses, can accommodate both unrelated and related individuals, and provide single-variant results and variance-covariance matrices. The covariance matrix captures linkage disequilibrium (LD) relationships between markers within 1 Mb, which is used for gene-level meta-analyses and conditional analyses¹⁰⁰. Single-variant analyses were performed for both additive and recessive models.

Centralized quality-control—A centralized quality-control procedure, implemented in EasyQC¹⁰¹, was applied to individual cohort association summary statistics to identify cohort-specific problems: (1) assessment of possible problems in BMI transformation, (2) comparison of allele frequency alignment against 1000 Genomes Project phase 1 reference data to pinpoint any potential strand issues, and (3) examination of quantile-quantile (QQ) plots per study to identify any problems arising from population stratification, cryptic relatedness and genotype biases.

Meta-analyses—Meta-analyses were carried out by two different analysts at two sites in parallel. We excluded variants with a call rate < 95%, Hardy-Weinberg equilibrium P -value < 1×10^{-7} , or large allele frequency deviations from reference populations (> 0.6 for all-ancestry analyses and > 0.3 for ancestry-specific population analyses). Significance for single-variant analyses was defined at the array-wide level (a Bonferroni-corrected threshold of $P < 2 \times 10^{-7}$ for ~250,000 SNVs). To test for sex-differences of the significant variants ($P < 2 \times 10^{-7}$), we calculated the P -diff for each SNP, which tests for differences between women-specific and men-specific beta estimates using EasyStrata¹⁰². For gene-based analyses, we applied the sequence kernel association test (SKAT)¹⁰³ and the Variable Threshold (VT)¹⁰⁴ gene-based methods using two different sets of criteria (broad and strict) to select predicted damaging R/LF variants with MAF < 5%, based on coding variant annotation from five prediction algorithms (PolyPhen2 HumDiv and HumVar, LRT, MutationTaster and SIFT)²⁰. Our *broad* gene-based tests included nonsense, stop-loss, splice site, and missense variants that are annotated as damaging by at least one algorithm mentioned above. Our *strict* gene-based tests included only nonsense, stop-loss, splice site, and missense variants annotated as damaging by all five algorithms. Statistical significance for gene-based tests was set at a Bonferroni-corrected threshold of $P < 2.5 \times 10^{-6}$ for about 20,000 genes^{16,105}. Single-variant and gene-based meta-analyses were both performed using RareMETALS R-package¹⁰⁶. As our secondary analyses are nested and/or highly correlated with our primary analysis, we chose the same, already stringent, Bonferroni-corrected significance threshold for both analyses.

Genomic inflation—Although the overall λ_{GC} value is in the normal range for all coding variants ($\lambda_{GC} = 1.1$, Supplementary Table 23), we observed a marked genomic inflation of the test statistics even after adequate control for population stratification (linear mixed model) arising from common markers ($\lambda_{GC} = 1.99$, Supplementary Figure 2a and Supplementary Table 23). Such inflation is expected for a highly polygenic trait like BMI, as was previously confirmed for height¹⁵, and is consistent with our very large sample size^{5,107}. Furthermore, some of the inflation may be due to the design of the ExomeChip, which besides R/LF coding SNVs also contains (common and non-coding) SNVs that include previously identified GWAS loci for all traits, including for BMI and BMI-related traits, reported in the GWAS catalogue at the time of its design.

After removing established loci (± 1 Mb), the excess of significant associations is markedly reduced and inflation reduced (Supplementary Figures 2c and 2d).

Furthermore, to exclude the possibility that some of the observed associations between BMI and R/LF SNVs could be due to allele calling problems in the smaller studies, we performed a sensitivity meta-analysis with primarily European ancestry studies totaling $>5,000$ participants. We found very concordant effect sizes, suggesting that smaller studies do not bias our results (Supplementary Figure 12).

Follow-up Analysis—We sought additional evidence for association of the top signals ($P < 2.0 \times 10^{-6}$) identified in the discovery meta-analysis using two independent studies from the UK (UK Biobank, interim release, $N = 119,613$) and Iceland (deCODE, $N = 72,613$), respectively (Supplementary Tables 1–3). We used the same QC and analytical methodology as described above. We used the inverse-variance weighted fixed effects meta-analysis in METAL¹⁰⁸, to combine the discovery and follow-up association results. Significant associations were defined at $P < 2 \times 10^{-7}$ in the combined meta-analysis of discovery, UK Biobank and deCODE results.

Effect of study design—To investigate the potential effect of study design of the participating studies, we tested for heterogeneity between population-based, all case-control studies; T2D case-control studies (Supplementary Table 26). None of these comparisons showed significant evidence of heterogeneity ($P < 7.4 \times 10^{-5}$, correcting for multiple testing).

Conditional analyses—The RareMETALS R-package¹⁰⁶ was used to identify independent BMI associated signals across the all-ancestry meta-analysis results in the discovery phase. RareMETALS performs conditional analyses by using covariance matrices from each individual cohort to distinguish true signals from the shadows of adjacent significant variants in LD. The conditional associations of all the variants within 1 Mb of each R/LF coding variant were analyzed to identify [1] nearby secondary signals and [2] to determine independence from nearby non-coding variants or previously identified GWAS loci (previously defined as a window of 1 Mb surrounding the lead SNP). Gene-based conditional analyses were also performed in RareMETALS.

Due to the selective coverage of variants on the ExomeChip, we also conducted the respective conditional analyses in the UK Biobank dataset that included 847,441 genome-

wide genotyped markers, and 72,355,667 variants imputed against UK10k haplotype reference panel, merged with the 1000 Genomes Phase 3 reference panel. Where available, directly genotyped variants were used for conditional analyses. Otherwise, imputed variants with good imputation quality (IMPUTE2 info score > 0.6) were used. We used QCTOOL to extract variants of interest from the original imputed data set. Subsequently, GTOOL was used to convert to PLINK format (genotype calling threshold 0.99) and merged with the directly genotyped variants for conditional analyses in PLINK v1.90b3.35 64-bit (25 Mar 2016).

Conversions of effect size and explained variants—We assumed that 1 SD = 4.5 kg/m² BMI-units, based on population based data, and 1.7m as the average height of a person to convert effects sizes in SD-units into body weight. The variance explained by each variant was calculated using the effect allele frequency (f) and beta (β) from the meta analyses using the formula¹⁰⁹ of explained variance = $2f(1-f)\beta^2$.

Penetrance analysis—We examined the penetrance for the four rare SNVs, p.Arg525Gln (rs56214831) in *KSR2*, p.Tyr35Ter (rs13447324) in *MC4R*, and p.Arg190Gln (rs139215588) and p.Glu288Gly (rs143430880) in *GIPR* in European ancestry data from the UKBiobank (N up to 120,000). For each variant, we compared the prevalence of underweight (BMI < 18.5 kg/m²), normal weight (18.5 kg/m² ≤ BMI < 25 kg/m²), overweight (25 kg/m² ≤ BMI < 30 kg/m²) and obesity (BMI ≥ 30 kg/m²) of non-carriers with non-carriers. We used a Pearson χ^2 test to test for difference between distributions, and a χ^2 for linear trend to test whether distributions of carriers were shifted compared to non-carriers. For p.Arg525Gln in *KSR2* and p.Tyr35Ter in *MC4R*, we hypothesized that obesity prevalence was higher in carriers than in non-carriers, whereas for the two *GIPR* variants, we hypothesized that the prevalence of normal weight was higher in carriers than non-carriers.

Associations with obesity for the coding rare and low-frequency loci in children—For each of the 14 R/LF SNVs, we tested for association with childhood obesity in the CHOP cohort (Childhood Obesity: Early Programming by Infant Nutrition), the Severe Childhood Onset Obesity Project (SCOOP), the UK Household Longitudinal Study (UKHLS) and INTERVAL Study (INTERVAL). Summary statistics across the studies were combined using a fixed effects inverse-variance meta-analysis with METAL¹⁰⁸.

In the CHOP study, cases (1,358 boys, 1,060 girls) were defined as having a BMI > 95th percentile at any point in their childhood. Controls (1,412 boys, 1,143 girls) were defined as having < 50th percentile consistently throughout childhood. The BMI percentiles are based on the CDC 2000 Growth Charts. All children were classified based on their BMI measurements between the ages of 2 and 18. All individuals are of European ancestry and were collected at the Children's Hospital of Philadelphia. Informed consent was obtained from all study participants and study protocols were approved by the local ethics committees. Genotypes were obtained using the HumanHap550v1, HumanHap550v3, and Human610-Quad high-density SNP arrays from Illumina. The intersection of all SNPs on the arrays was used in all subsequent pre-imputation analyses. Before imputation, we excluded SNPs with a Hardy-Weinberg equilibrium P -value < 1.0×10^{-6} , call rate of < 95%

or MAF of < 1%. The genotypes were then pre-phased using Shapeit2 and imputed using the 1000 Genomes Phase 1 integrated variant set with Impute2. After imputation, SNPs were excluded if the INFO score was < 0.4. Boys and girls were analyzed separately using a logistic regression of case and control status, adjusting for three eigenvectors, and summary statistics were combined using a fixed effects inverse-variance meta-analysis with METAL¹⁰⁸.

SCOOP is a sub-cohort of the Genetics Of Obesity Study (GOOS) cohort. It includes >1,500 UK European ancestry individuals with severe, early onset obesity (BMI Standard Deviation Score > 3 and obesity onset before the age of 10 years), in whom known monogenic causes of obesity have been excluded (cases with *MC4R* mutations were excluded). Two case-control analyses with SCOOP cases were performed: 1) SCOOP vs. UKHLS for which array (Illumina HumanCoreExome) data was available, and 2) SCOOP vs. INTERVAL, for whom whole-exome sequencing data was available.

For the array based analyses, UKHLS controls were genotyped on the Illumina HumanCoreExome-12v1-0 Beadchip. SCOOP cases and 48 UKHLS controls were genotyped on the Illumina HumanCoreExome-12v1-1 Beadchip. The 48 overlapping UKHLS samples were used for quality control to ensure there were no systematic differences and bias between the two versions of the chip. SCOOP and UKHLS samples were phased with SHAPEITv2, and imputed with IMPUTE2 using the combined UK10K-1000G Phase III reference panel. For the WES analyses, SCOOP vs. INTERVAL controls were WES within the UK10K-EXOME project (Agilent v3) and the INTERVAL project (Agilent v5) respectively and were then jointly called and QC-ed on the union of the sequencing baits. Individuals overlapping or related between the array based and WES studies were removed.

After QC, 1,456 SCOOP and 6,460 UKHLS (BMI range 19–30), and 521 SCOOP and 4,057 INTERVAL individuals were available for the two analyses; all were unrelated, of high quality, and of European ancestry. For both analyses (i.e. SCOOP vs. UKHLS and SCOOP vs. INTERVAL), a maximum likelihood frequentist association test with the additive genetic model was implemented in SNPTEST v2.5. In the SCOOP vs. UKHLS analysis, sex and the first six PCs were included as covariates and variants with a SNPTEST INFO score < 0.4 and HWE $p < 10^{-6}$ were removed. For the SCOOP vs INTERVAL analysis, we performed an unadjusted analysis (adjustment for PCs did not change sufficiently the results) and variants were limited to those covered at $\geq 7\times$ in at least 80% of each sequencing cohort, meeting the VQSR threshold of -2.52 , missingness < 80%, HWE P -value $< 10^{-8}$, and GQ ≥ 30 .

Cross-trait analyses—We evaluated each of the 14 R/LF SNVs for their association with other relevant obesity-related traits and conditions. We performed lookups in ExomeChip meta-analysis results from other consortia, including; our own GIANT consortium (height¹⁵, WHR adjusted for BMI²⁴), MAGIC (HbA1c, Fasting Insulin, Fasting Glucose, 2-hour glucose), GLGC (HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglycerides and total cholesterol)), IBPC⁴⁰ (systolic and diastolic blood pressure), REPROGEN²³ (age at menarche and menopause) and GoT2D/T2D-GENES¹⁶ (type 2 Diabetes). Associations were considered significant at $P < 2.0 \times 10^{-5}$, accounting for multiple testing.

Phenome-wide association analysis (PheWAS)—To evaluate the potential for pleiotropic effects for SNPs discovered from primary analyses, we performed phenome-wide association studies (PheWASs) using genotype and phenotype data from two independent sources of electronic health records (EHR): Vanderbilt University Medical Center Biorepository (BioVU) and the United Kingdom BioBank (UKBB). Phenotype selection and analysis strategy were synchronized across sites. A total of 1502 hierarchical phenotype codes from EHRs were curated by grouping International Classification of Disease, Ninth Revision (ICD-9) clinical/billing codes as previously described¹¹⁰. Phenotype codes with 20 or more cases and with minor allele count of 5 or greater in cases and controls were eligible for analysis. Series of logistic regression analyses were then performed in individuals of European ancestry for each eligible phenotype-genotype combination while adjusting for 5 genetic ancestry PCs. Odds ratios from genotype-phenotype combinations present in both BioVU and UKBB were then aggregated using inverse-variance weighted fixed-effects meta-analysis. Associations with p-values corresponding to false discovery rate (FDR) cut off of less than 10% were considered statistically significant.

Gene set enrichment analysis—We adapted DEPICT, a gene set enrichment analysis method for GWAS data, for use with the ExomeChip ('EC-DEPICT'). DEPICT's primary innovation is the use of "reconstituted" gene sets, where many different types of gene sets (e.g. canonical pathways, protein-protein interaction networks, and mouse phenotypes) were extended through the use of large-scale microarray data (see¹¹¹ for details). EC-DEPICT computes *P*-values based on Swedish ExomeChip data (Malmö Diet and Cancer [MDC], All New Diabetics in Scania [ANDIS], and Scania Diabetes Registry [SDR] cohorts, N=11,899) and, unlike DEPICT, takes as input only coding variants and only the genes directly containing those variants, rather than all genes within a specified amount of linkage disequilibrium (Supplementary Note).

Four analyses were performed for the BMI EC variants: [1] all coding variants with $P < 5 \times 10^{-4}$, [2] all coding variants with $P < 5 \times 10^{-4}$ independent of known GWAS variants⁵, [3] all coding R/LF variants with $P < 5 \times 10^{-4}$, and [4] all coding R/LF variants with $P < 5 \times 10^{-4}$ independent of known GWAS variants. Affinity propagation clustering³ was used to group highly correlated gene sets into "meta-gene sets". For each meta-gene set, the member gene set with the best *P*-value was used as representative for purposes of visualization (Supplementary Note). DEPICT for ExomeChip was written using the Python programming language (See URLs).

Drosophila RNAi knockdown experiments—For each of the 13 genes in which R/LF coding variants were associated with BMI, we searched for its corresponding orthologues in *Drosophila* in the ENSEMBL orthologue database. Orthologues were available for nine genes, but missing for *ZBTB7B*, *MC4R*, *GIPR*, and *ZNF169*. For each of the nine genes, we generated adipose-tissue (cg-Gal4) and neuronal (elav-Gal4) specific RNAi-knockdown crosses, leveraging upstream activation sequence (UAS)-inducible short-hairpin knockdown lines, available through the Vienna Drosophila Resource Center (VDRC). We crossed male UAS-RNAi flies and elav-GAL4 or CG-GAL4 virgin female flies. All fly experiments were carried out at 25 °C. Five-to-seven-day-old males were sorted into groups of 20, weighed

and homogenated in PBS with 0,05% Tween with Lysing Matrix D in a beadshaker. The homogenate was heat-inactivated for 10 min in a thermocycler at 70 °C. 10µl of the homogenate was subsequently used in triglyceride assay (Sigma, Serum Triglyceride Determination Kit) which was carried out in duplicates according to protocol, with one alteration: the samples were cleared of residual particulate debris by centrifugation before absorbance reading. Resulting triglyceride values were normalized to fly weight and larval/population density. We used the non-parametric Kruskal-Wallis test to compare wild type with knockdown lines.

Enrichment analysis in monogenic genes of obesity—We identified 39 genes with strong evidence that disruption causes monogenic or syndromic forms of obesity (Supplementary Table 21). To test whether these genes are enriched for R/LF coding variant associations with BMI, we conducted simulations by matching each of the 39 genes with other genes based on gene length and number of variants tested, to create a matched set of genes. We generated 1,000 matched gene sets from our data and assessed how often the number of R/LF coding variants that exceeded given significance thresholds was greater in our monogenic/syndromic obesity gene set compared to the matched gene sets.

DATA AVAILABILITY

Summary statistics can be downloaded from http://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Valérie Turcot^{1,*}, Yingchang Lu^{2,3,4,*}, Heather M Highland^{5,6,*}, Claudia Schurmann^{3,4,*}, Anne E Justice^{5,*}, Rebecca S Fine^{7,8,9,*}, Jonathan P Bradfield^{10,11}, Tõnu Esko^{7,9,12}, Ayush Giri¹³, Mariaelisa Graff⁵, Xiuqing Guo¹⁴, Audrey E Hendricks^{15,16}, Tugce Karaderi^{17,18}, Adelheid Lempradl¹⁹, Adam E Locke^{20,21}, Anubha Mahajan¹⁷, Eirini Marouli²², Suthesh Sivapalaratnam^{23,24,25}, Kristin L Young⁵, Tamuno Alfred³, Mary F Feitosa²⁶, Nicholas GD Masca^{27,28}, Alisa K Manning^{7,24,29,30}, Carolina Medina-Gomez^{31,32}, Poorva Mudgal³³, Maggie CY Ng^{33,34}, Alex P Reiner^{35,36}, Sailaja Vedantam^{7,8,9}, Sara M Willems³⁷, Thomas W Winkler³⁸, Goncalo Abecasis²⁰, Katja K Aben^{39,40}, Dewan S Alam⁴¹, Sameer E Alharthi^{22,42}, Matthew Allison⁴³, Philippe Amouyel^{44,45,46}, Folkert W Asselbergs^{47,48,49}, Paul L Auer⁵⁰, Beverley Balkau⁵¹, Lia E Bang⁵², Inês Barroso^{15,53,54}, Lisa Bastarache⁵⁵, Marianne Benn^{56,57}, Sven Bergmann^{58,59}, Lawrence F Bielak⁶⁰, Matthias Blüher^{61,62}, Michael Boehnke²⁰, Heiner Boeing⁶³, Eric Boerwinkle^{64,65}, Carsten A Böger⁶⁶, Jette Bork-Jensen⁶⁷, Michiel L Bots⁶⁸, Erwin P Bottinger³, Donald W Bowden^{33,34,69}, Ivan Brandslund^{70,71}, Gerome Breen^{72,73}, Murray H Brilliant⁷⁴, Linda Broer³², Marco Brumat⁷⁵, Amber A Burt⁷⁶, Adam S Butterworth^{77,78}, Peter T Campbell⁷⁹, Stefania Cappellani⁸⁰, David J

Carey⁸¹, Eulalia Catamo⁸⁰, Mark J Caulfield^{22,82}, John C Chambers^{83,84,85}, Daniel I Chasman^{7,86,87,88}, Yii-Der Ida Chen¹⁴, Rajiv Chowdhury⁷⁷, Cramer Christensen⁸⁹, Audrey Y Chu^{87,90}, Massimiliano Cocca⁹¹, Francis S Collins⁹², James P Cook⁹³, Janie Corley^{94,95}, Jordi Corominas Galbany^{96,97}, Amanda J Cox^{33,34,98}, David S Crosslin⁹⁹, Gabriel Cuellar-Partida^{100,101}, Angela D'Eustacchio⁸⁰, John Danesh^{15,77,78,102}, Gail Davies^{94,95}, Paul IW de Bakker^{68,103}, Mark CH de Groot^{104,105}, Renée de Mutsert¹⁰⁶, Ian J Deary^{94,95}, George Dedoussis¹⁰⁷, Ellen W Demerath¹⁰⁸, Martin den Heijer¹⁰⁹, Anneke I den Hollander⁹⁷, Hester M den Ruijter¹¹⁰, Joe G Dennis¹¹¹, Josh C Denny⁵⁵, Emanuele Di Angelantonio^{77,78}, Fotios Drenos^{112,113}, Mengmeng Du^{114,115}, Marie-Pierre Dubé^{1,116}, Alison M Dunning¹¹⁷, Douglas F Easton^{111,117}, Todd L Edwards¹³, David Ellinghaus¹¹⁸, Patrick T Ellinor^{7,24,30}, Paul Elliott¹¹⁹, Evangelos Evangelou^{84,120}, Aliki-Eleni Farmaki^{107,121}, I. Sadaf Farooqi^{53,54}, Jessica D Faul¹²², Sascha Fauser¹²³, Shuang Feng²⁰, Ele Ferrannini^{124,125}, Jean Ferrieres¹²⁶, Jose C Florez^{7,24,29,30}, Ian Ford¹²⁷, Myriam Fornage¹²⁸, Oscar H Franco³¹, Andre Franke¹¹⁸, Paul W Franks^{129,130,131}, Nele Friedrich¹³², Ruth Frikke-Schmidt^{57,133}, Tessel E. Galesloot⁴⁰, Wei Gan¹⁷, Ilaria Gandin¹³⁴, Paolo Gasparini^{75,135}, Jane Gibson¹³⁶, Vilmantas Giedraitis¹³⁷, Anette P Gjesing⁶⁷, Penny Gordon-Larsen^{138,139}, Mathias Gorski^{38,66}, Hans-Jörgen Grabe^{140,141}, Struan FA Grant^{10,142,143}, Niels Grarup⁶⁷, Helen L Griffiths¹⁴⁴, Megan L Grove⁶⁴, Vilmundur Gudnason^{145,146}, Stefan Gustafsson¹⁴⁷, Jeff Haessler³⁶, Hakon Hakonarson^{10,142}, Anke R Hammerschlag¹⁴⁸, Torben Hansen⁶⁷, Kathleen Mullan Harris^{138,149}, Tamara B Harris¹⁵⁰, Andrew T Hattersley¹⁵¹, Christian T Have⁶⁷, Caroline Hayward¹⁵², Liang He^{153,154}, Nancy L Heard-Costa^{90,155}, Andrew C Heath¹⁵⁶, Iris M Heid^{38,157}, Øyvind Helgeland^{158,159}, Jussi Hernesniemi^{160,161,162}, Alex W Hewitt^{163,164,165}, Oddgeir L Holmen¹⁶⁶, G Kees Hovingh¹⁶⁷, Joanna MM Howson⁷⁷, Yao Hu¹⁶⁸, Paul L Huang²⁴, Jennifer E Huffman¹⁵², M Arfan Ikram^{31,169,170}, Erik Ingelsson^{147,171}, Anne U Jackson²⁰, Jan-Håkan Jansson^{172,173}, Gail P Jarvik^{76,174}, Gorm B Jensen¹⁷⁵, Yucheng Jia¹⁴, Stefan Johansson^{159,176}, Marit E Jørgensen^{177,178}, Torben Jørgensen^{57,179,180}, J Wouter Jukema^{181,182}, Bratati Kahali^{183,184,185,186}, René S Kahn¹⁸⁷, Mika Kähönen^{188,189}, Pia R Kamstrup⁵⁶, Stavroula Kanoni²², Jaakko Kaprio^{154,190,191}, Maria Karaleftheri¹⁹², Sharon LR Kardia⁶⁰, Fredrik Karpe^{193,194}, Sekar Kathiresan^{7,24,88}, Frank Kee¹⁹⁵, Lambertus A Kiemeny⁴⁰, Eric Kim¹⁴, Hidetoshi Kitajima¹⁷, Pirjo Komulainen^{196,197,198}, Jaspal S Kooner^{83,85,199,200}, Charles Kooperberg³⁶, Tellervo Korhonen^{191,201,202}, Peter Kovacs⁶¹, Helena Kuivaniemi^{81,203}, Zoltán Kutalik^{59,204}, Kari Kuulasmaa¹⁹¹, Johanna Kuusisto²⁰⁵, Markku Laakso²⁰⁵, Timo A Lakka^{196,197,198}, David Lamparter^{58,59}, Ethan M Lange²⁰⁶, Leslie A Lange²⁰⁶, Claudia Langenberg³⁷, Eric B Larson^{76,207,208}, Nanette R Lee^{209,210}, Terho Lehtimäki^{161,162}, Cora E Lewis²¹¹, Huaixing Li¹⁶⁸, Jin Li²¹², Ruifang Li-Gao¹⁰⁶, Honghuang Lin²¹³, Keng-Hung Lin²¹⁴, Li-An Lin¹²⁸, Xu Lin¹⁶⁸, Lars Lind²¹⁵, Jaana Lindström¹⁹¹, Allan Linneberg^{180,216,217}, Ching-Ti Liu²¹⁸, Dajiang J Liu²¹⁹, Yongmei Liu²²⁰, Ken Sin Lo¹, Artitaya Lophatananon²²¹, Andrew J Lotery¹⁴⁴, Anu Loukola^{154,190}, Jian'an Luan³⁷, Steven A Lubitz^{7,24,30}, Leo-Pekka Lyytikäinen^{161,162}, Satu Männistö¹⁹¹, Gaëlle Marenne¹⁵, Angela L Mazul⁵, Mark I McCarthy^{17,193,194}, Roberta McKean-

Cowdin²²², Sarah E Medland¹⁰¹, Karina Meidtner^{223,224}, Lili Milani¹², Vanisha Mistry^{53,54}, Paul Mitchell²²⁵, Karen L Mohlke²⁰⁶, Leena Moilanen²²⁶, Marie Moitry^{227,228}, Grant W Montgomery^{101,229}, Dennis O Mook-Kanamori^{106,230}, Carmel Moore^{78,231}, Trevor A Mori²³², Andrew D Morris²³³, Andrew P Morris^{17,93}, Martina Müller-Nurasyid^{157,234,235}, Patricia B Munroe^{22,82}, Mike A Nalls^{236,237}, Narisu Narisu⁹², Christopher P Nelson^{27,28}, Matt Neville^{193,194}, Sune F Nielsen^{56,57}, Kjell Nikus¹⁶⁰, Pål R Njølstad^{158,159}, Børge G Nordestgaard^{56,57}, Dale R Nyholt^{101,238}, Jeffrey R O'Connel²³⁹, Michelle L. O'Donoghue²⁴⁰, Loes M Olde Loohuis²⁴¹, Roel A Ophoff^{187,241}, Katharine R Owen^{193,194}, Chris J Packard¹²⁷, Sandosh Padmanabhan¹²⁷, Colin NA Palmer²⁴², Nicholette D Palmer⁶⁹, Gerard Pasterkamp^{110,243}, Aniruddh P Patel^{7,24,88}, Alison Pattie⁹⁵, Oluf Pedersen⁶⁷, Peggy L Peissig⁷⁴, Gina M Peloso²¹⁸, Craig E Pennell²⁴⁴, Markus Perola^{191,245,246}, James A Perry²³⁹, John RB Perry³⁷, Tune H Pers^{67,247}, Thomas N Person⁷⁴, Annette Peters^{224,235,248}, Eva RB Petersen²⁴⁹, Patricia A Peyser⁶⁰, Ailith Pirie¹¹⁷, Ozren Polasek^{233,250}, Tinca J Polderman¹⁴⁸, Hannu Puolijoki²⁵¹, Olli T Raitakari^{252,253}, Asif Rasheed²⁵⁴, Rainer Rauramaa^{196,197,198}, Dermot F Reilly²⁵⁵, Frida Renström^{129,256}, Myriam Rheinberger⁶⁶, Paul M Ridker^{87,88,240}, John D Rioux^{1,116}, Manuel A Rivas^{7,257}, David J Roberts^{78,258,259}, Neil R Robertson^{17,193}, Antonietta Robino⁸⁰, Olov Rolandsson^{172,260}, Igor Rudan²³³, Katherine S Ruth²⁶¹, Danish Saleheen^{254,262}, Veikko Salomaa¹⁹¹, Nilesh J Samani^{27,28}, Yadav Sapkota¹⁰¹, Naveed Sattar¹²⁷, Robert E Schoen²⁶³, Pamela J Schreiner²⁶⁴, Matthias B Schulze^{223,224}, Robert A Scott³⁷, Marcelo P Segura-Lepe⁸⁴, Svati H Shah²⁶⁵, Wayne H-H Sheu^{266,267,268}, Xueling Sim^{20,269}, Andrew J Slater^{270,271}, Kerrin S Small²⁷², Albert Vernon Smith^{145,146}, Lorraine Southam^{15,17}, Timothy D Spector²⁷², Elizabeth K Speliotes^{183,184,185}, John M Starr^{94,273}, Kari Stefansson^{145,274}, Valgerdur Steinthorsdottir²⁷⁴, Kathleen E Stirrups^{22,25}, Konstantin Strauch^{157,275}, Heather M Stringham²⁰, Michael Stumvoll^{61,62}, Liang Sun^{153,154}, Praveen Surendran⁷⁷, Amy J Swift⁹², Hayato Tada^{240,276}, Katherine E Tansey^{113,277}, Jean-Claude Tardif^{1,116}, Kent D Taylor¹⁴, Alexander Teumer²⁷⁸, Deborah J Thompson¹¹¹, Gudmar Thorleifsson²⁷⁴, Unnur Thorsteinsdottir^{145,274}, Betina H Thuesen¹⁸⁰, Anke Tönjes²⁷⁹, Gerard Tromp^{81,280}, Stella Trompet^{181,281}, Emmanouil Tsafantakis²⁸², Jaakko Tuomilehto^{191,283,284,285}, Anne Tybjaerg-Hansen^{57,133}, Jonathan P Tyrer¹¹⁷, Rudolf Uher²⁸⁶, André G Uitterlinden^{31,32}, Matti Uusitupa²⁸⁷, Sander W van der Laan¹¹⁰, Cornelia M van Duijn³¹, Nienke van Leeuwen^{288,289}, Jessica van Setten⁴⁷, Mauno Vanhala^{201,290}, Anette Varbo^{56,57}, Tibor V Varga¹²⁹, Rohit Varma²⁹¹, Digna R Velez Edwards²⁹², Sita H Vermeulen⁴⁰, Giovanni Veronesi²⁹³, Henrik Vestergaard^{67,178}, Veronique Vitart¹⁵², Thomas F Vogt²⁹⁴, Uwe Völker^{295,296}, Dragana Vuckovic^{75,135}, Lynne E Wagenknecht²²⁰, Mark Walker²⁹⁷, Lars Wallentin²⁹⁸, Feijie Wang¹⁶⁸, Carol A Wang²⁴⁴, Shuai Wang²¹⁸, Yiqin Wang¹⁶⁸, Erin B Ware^{60,299}, Nicholas J Wareham³⁷, Helen R Warren^{22,82}, Dawn M Waterworth³⁰⁰, Jennifer Wessel³⁰¹, Harvey D White³⁰², Cristen J Willer^{183,184,303}, James G Wilson³⁰⁴, Daniel R Witte^{305,306}, Andrew R Wood²⁶¹, Ying Wu²⁰⁶, Hanieh Yaghootkar²⁶¹, Jie Yao¹⁴, Pang Yao¹⁶⁸, Laura M Yerges-Armstrong^{239,307}, Robin Young^{77,127}, Eleftheria Zeggini¹⁵, Xiaowei Zhan³⁰⁸, Weihua Zhang^{83,84}, Jing Hua Zhao³⁷, Wei Zhao²⁶², Wei Zhao⁶⁰, Wei Zhou^{183,184}, Krina T Zondervan^{17,309}, CHD

Exome+ Consortium, EPIC-CVD Consortium, ExomeBP Consortium, Global Lipids Genetic Consortium, GoT2D Genes Consortium, InterAct, INTERVAL Study, ReproGen Consortium, T2D-Genes Consortium, The MAGIC Investigators, Understanding Society Scientific Group, Jerome I Rotter¹⁴, John A Pospisilik¹⁹, Fernando Rivadeneira^{31,32}, Ingrid B Borecki²⁶, Panos Deloukas^{22,42}, Timothy M Frayling²⁶¹, Guillaume Lettre^{1,116,§}, Kari E North^{310,§}, Cecilia M Lindgren^{17,311,§}, Joel N Hirschhorn^{7,9,312,§}, and Ruth JF Loos^{3,4,313,§}

Affiliations

¹Montreal Heart Institute, Universite de Montreal, Montreal, Quebec, H1T 1C8, Canada ²Division of Epidemiology, Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN, 37203, USA ³The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA ⁴The Genetics of Obesity and Related Metabolic Traits Program, Icahn School of Medicine at Mount Sinai, New York, NY, 10069, USA ⁵Department of Epidemiology, University of North Carolina, Chapel Hill, NC, 27514, USA ⁶Human Genetics Center, The University of Texas School of Public Health, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, The University of Texas Health Science Center at Houston, Houston, TX, 77030, USA ⁷Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA ⁸Department of Genetics, Harvard Medical School, Boston, MA, 02115, USA ⁹Division of Endocrinology and Center for Basic and Translational Obesity Research, Boston Children's Hospital, Boston, MA, 02115, USA ¹⁰Center for Applied Genomics, Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA ¹¹Quantinuum Research LLC, San Diego CA, 92101, USA ¹²Estonian Genome Center, University of Tartu, Tartu, 51010, Estonia ¹³Division of Epidemiology, Department of Medicine, Institute for Medicine and Public Health, Vanderbilt Genetics Institute, Vanderbilt University, Nashville, TN, 37203, USA ¹⁴Institute for Translational Genomics and Population Sciences, LABioMed at Harbor-UCLA Medical Center, Torrance, CA, 90502, USA ¹⁵Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, UK ¹⁶Department of Mathematical and Statistical Sciences, University of Colorado, Denver, CO, 80204, USA ¹⁷Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK ¹⁸Department of Biological Sciences, Faculty of Arts and Sciences, Eastern Mediterranean University, Famagusta, Cyprus ¹⁹Max Planck Institute of Immunobiology and Epigenetics, Freiburg, 79108, Germany ²⁰Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, 48109, USA ²¹McDonnell Genome Institute, Washington University School of Medicine, Saint Louis, MO, 63108, USA ²²William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, EC1M 6BQ, UK ²³Department of Vascular Medicine, AMC, Amsterdam, 1105 AZ, The Netherlands ²⁴Massachusetts General Hospital, Boston, MA, 02114, USA ²⁵Department of Haematology, University of Cambridge, Cambridge, CB2 0PT, UK ²⁶Division of Statistical Genomics, Department of

Genetics, Washington University School of Medicine, St. Louis, MO, 63108, USA ²⁷Department of Cardiovascular Sciences, Univeristy of Leicester, Glenfield Hospital, Leicester, LE3 9QP, UK ²⁸NIHR Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, LE3 9QP, UK ²⁹Department of Medicine, Harvard University Medical School, Boston, MA, 02115, USA ³⁰Medical and Population Genetics Program, Broad Institute, Cambridge, MA, 02141, USA ³¹Department of Epidemiology, Erasmus Medical Center, Rotterdam, 3015 GE, The Netherlands ³²Department of Internal Medicine, Erasmus Medical Center, Rotterdam, 3015 GE, The Netherlands ³³Center for Diabetes Research, Wake Forest School of Medicine, Winston-Salem, NC, 27157, USA ³⁴Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, NC, 27157, USA ³⁵Department of Epidemiology, University of Washington, Seattle, WA, 98195, USA ³⁶Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle WA, 98109, USA ³⁷MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge, CB2 0QQ, UK ³⁸Department of Genetic Epidemiology, University of Regensburg, Regensburg, D-93051, Germany ³⁹Netherlands Comprehensive Cancer Organisation, Utrecht, 3501 DB, The Netherlands ⁴⁰Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, 6500 HB, The Netherlands ⁴¹School of Kinesiology and Health Science, Faculty of Health, York University, Toronto ⁴²Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders (PACER-HD), King Abdulaziz University, Jeddah, 21589, Saudi Arabia ⁴³Department of Family Medicine & Public Health, University of California, San Diego, La Jolla, CA, 92093, USA ⁴⁴INSERM U1167, Lille, F-59019, France ⁴⁵Institut Pasteur de Lille, U1167, Lille, F-59019, France ⁴⁶Universite de Lille, U1167 - RID-AGE - Risk factors and molecular determinants of aging-related diseases, Lille, F-59019, France ⁴⁷Department of Cardiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands ⁴⁸Durrer Center for Cardiogenetic Research, ICIN-Netherlands Heart Institute, Utrecht, The Netherlands ⁴⁹Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, London, UK ⁵⁰Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI, 53201, USA ⁵¹INSERM U1018, Centre de recherche en Épidémiologie et Santé des Populations (CESP), Villejuif, France ⁵²Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, 2100, Denmark ⁵³Metabolic Research Laboratories, University of Cambridge, Cambridge, CB2 0QQ, UK ⁵⁴NIHR Cambridge Biomedical Research Centre, Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK ⁵⁵Department of Biomedical Informatics, Vanderbilt University, Nashville, TN, 37203, USA ⁵⁶Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, 2730, Denmark ⁵⁷Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, 2200, Denmark ⁵⁸Department of Computational Biology, University of Lausanne, Lausanne, 1011, Switzerland ⁵⁹Swiss Institute of Bioinformatics, Lausanne, 1015, Switzerland

⁶⁰Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, 48109, USA ⁶¹IFB Adiposity Diseases, University of Leipzig, Leipzig, 04103, Germany ⁶²University of Leipzig, Department of Medicine, Leipzig, 04103, Germany ⁶³Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, 14558, Germany ⁶⁴School of Public Health, Human Genetics Center, The University of Texas Health Science Center at Houston, Houston, TX, 77030, USA ⁶⁵Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, 77030 USA ⁶⁶Department of Nephrology, University Hospital Regensburg, Regensburg, 93042, Germany ⁶⁷The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, 2100, Denmark ⁶⁸Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands ⁶⁹Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA ⁷⁰Department of Clinical Biochemistry, Lillebaelt Hospital, Vejle, 7100, Denmark ⁷¹Institute of Regional Health Research, University of Southern Denmark, Odense, 5000, Denmark ⁷²MRC Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, SE5 8AF, UK ⁷³NIHR Biomedical Research Centre for Mental Health, South London and Maudsley Hospital, London, BR3 3BX, UK ⁷⁴Marshfield Clinic Research Institute, Marshfield, WI, 54449, USA ⁷⁵Department of Medical Sciences, University of Trieste, Trieste, 34137, Italy ⁷⁶Department of Medicine, University of Washington, Seattle, WA, 98195, USA ⁷⁷MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, CB1 8RN, UK ⁷⁸NIHR Blood and Transplant Research Unit in Donor Health and Genomics, Department of Public Health and Primary Care, University of Cambridge, Cambridge CB1 8RN, UK ⁷⁹American Cancer Society, Epidemiology Research Program, Atlanta, GA, 30303, USA ⁸⁰Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste, 34137, Italy ⁸¹Weis Center for Research, Geisinger Health System, Danville, PA 17822 ⁸²NIHR Barts Cardiovascular Research Unit, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, London, EC1M 6BQ, UK ⁸³Department of Cardiology, London North West Healthcare NHS Trust, Ealing Hospital, Middlesex, UB1 3HW, UK ⁸⁴Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, W2 1PG, UK ⁸⁵Imperial College Healthcare NHS Trust, London, W12 0HS, UK ⁸⁶Division of Genetics, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, 02115, USA ⁸⁷Division of Preventive Medicine, Brigham and Women's and Harvard Medical School, Boston, MA, 02215, USA ⁸⁸Harvard Medical School, Boston, MA, 02115, USA ⁸⁹Medical department, Lillebaelt Hospital, Vejle, 7100, Denmark ⁹⁰NHLBI Framingham Heart Study, Framingham, MA, 01702, USA ⁹¹Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, 34100, Italy ⁹²Medical Genomics and Metabolic Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, 20892, USA ⁹³Department of Biostatistics, University of Liverpool, Liverpool, L69 3GL, UK ⁹⁴Centre for Cognitive

Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, EH8 9JZ, UK ⁹⁵Department of Psychology, University of Edinburgh, Edinburgh, EH8 9JZ, UK ⁹⁶Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, 6500 HB, The Netherlands ⁹⁷Department of Ophthalmology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, 6500 HB, The Netherlands ⁹⁸Menzies Health Institute Queensland, Griffith University, Southport, QLD, Australia ⁹⁹Department of Biomedical Informatics and Medical Education, University of Washington, Seattle, WA, 98195, USA ¹⁰⁰Diamantina Institute, University of Queensland, Brisbane, Queensland, 4072, Australia ¹⁰¹QIMR Berghofer Medical Research Institute, Brisbane, Queensland, 4006, Australia ¹⁰²British Heart Foundation Cambridge Centre of Excellence, Department of Medicine, University of Cambridge, Cambridge, CB2 0QQ, UK ¹⁰³Department of Genetics, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, 3584 CX, The Netherlands ¹⁰⁴Department of Clinical Chemistry and Haematology, Division of Laboratory and Pharmacy, University Medical Center Utrecht, Utrecht, 3508 GA, The Netherlands ¹⁰⁵Utrecht Institute for Pharmaceutical Sciences, Division Pharmacoepidemiology & Clinical Pharmacology, Utrecht University, Utrecht, 3508 TB, The Netherlands ¹⁰⁶Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, 2300RC, The Netherlands ¹⁰⁷Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, Athens, 17671, Greece ¹⁰⁸Division of Epidemiology & Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, 55454, USA ¹⁰⁹VU University Medical Center, Department of Internal Medicine, Amsterdam, 1007 MB, The Netherlands ¹¹⁰Laboratory of Experimental Cardiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, 3584 CX, The Netherlands ¹¹¹Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, CB1 8RN, UK ¹¹²Institute of Cardiovascular Science, University College London, London, WC1E 6JF, UK ¹¹³MRC Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol, Bristol, BS8 2BN, UK ¹¹⁴Fred Hutchinson Cancer Research Center, Public Health Sciences Division, Seattle, WA, 98109, USA ¹¹⁵Memorial Sloan Kettering Cancer Center, Department of Epidemiology and Biostatistics, New York, NY, 10017, USA ¹¹⁶Department of Medicine, Faculty of Medicine, Université de Montréal, Montréal, Québec, H3T 1J4, Canada ¹¹⁷Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, CB1 8RN, UK ¹¹⁸Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany ¹¹⁹Department of Epidemiology and Biostatistics, MRC-PHE Centre for Environment and Health, School of Public Health, Imperial College London, London, W2 1PG, UK ¹²⁰Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, 45110, Greece ¹²¹Department of Health Sciences, University of Leicester, Leicester, LE1 7RH, UK ¹²²Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor, MI, 48104, USA ¹²³Department of

Ophthalmology, University of Cologne, Cologne, 50937, Germany ¹²⁴CNR Institute of Clinical Physiology, Pisa, Italy ¹²⁵Department of Clinical & Experimental Medicine, University of Pisa, Italy ¹²⁶Toulouse University School of Medicine, Toulouse, TSA 50032 31059, France ¹²⁷University of Glasgow, Glasgow, G12 8QQ, UK ¹²⁸Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, TX, 77030, USA ¹²⁹Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University, Malmö, SE-20502, Sweden ¹³⁰Department of Nutrition, Harvard School of Public Health, Boston, MA, 02115, USA ¹³¹Department of Public Health and Clinical Medicine, Unit of Medicine, Umeå University, Umeå, 901 87, Sweden ¹³²Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, 17475, Germany ¹³³Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, 2100, Denmark ¹³⁴Ilaria Gandin, Research Unit, AREA Science Park, Trieste, 34149, Italy ¹³⁵Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste, Italy ¹³⁶Centre for Biological Sciences, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton, SO17 1BJ, UK ¹³⁷Geriatrics, Department of Public Health, Uppsala University, Uppsala, 751 85, Sweden ¹³⁸Carolina Population Center, University of North Carolina, Chapel Hill, NC, 27514, USA ¹³⁹Department of Nutrition, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, 27514, USA ¹⁴⁰Department of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, 17475, Germany ¹⁴¹German Center for Neurodegenerative Diseases (DZNE), Rostock/Greifswald, Greifswald, 17475, Germany ¹⁴²Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA ¹⁴³Division of Endocrinology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, 19104, USA ¹⁴⁴Vision Sciences, Clinical Neurosciences Research Group, Faculty of Medicine, University of Southampton, Southampton, SO16 6YD, UK ¹⁴⁵Faculty of Medicine, University of Iceland, Reykjavik, 101, Iceland ¹⁴⁶Icelandic Heart Association, Kopavogur, 201, Iceland ¹⁴⁷Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, 751 41, Sweden ¹⁴⁸Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Amsterdam Neuroscience, VU University Amsterdam, Amsterdam, 1081 HV, The Netherlands ¹⁴⁹Department of Sociology, University of North Carolina, Chapel Hill, NC, 27514, USA ¹⁵⁰Laboratory of Epidemiology and Population Sciences, National Institute on Aging, Intramural Research Program, National Institutes of Health, Bethesda, MD, 20892, USA ¹⁵¹University of Exeter Medical School, University of Exeter, Exeter, EX2 5DW, UK ¹⁵²MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, EH4 2XU, UK ¹⁵³Biodemography of Aging Research Unit, Social Science Research Institute, Duke University, Durham, NC, 27708, USA ¹⁵⁴Department of Public Health, University of Helsinki, Helsinki, FI-00014, Finland ¹⁵⁵Department of Neurology, Boston University School of Medicine, Boston, MA, 02118, USA ¹⁵⁶Department of Psychiatry, Washington University, Saint Louis, MO, 63110, USA ¹⁵⁷Institute of Genetic

Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, 85764, Germany ¹⁵⁸Department of Pediatrics, Haukeland University Hospital, Bergen, 5021, Norway ¹⁵⁹KG Jebsen Center for Diabetes Research, Department of Clinical Science, University of Bergen, Bergen, 5020, Norway ¹⁶⁰Department of Cardiology, Heart Center, Tampere University Hospital, and Faculty of Medicine and Life Sciences, University of Tampere, Tampere, 33521, Finland ¹⁶¹Department of Clinical Chemistry, Fimlab Laboratories, Tampere, 33521, Finland ¹⁶²Department of Clinical Chemistry, Finnish Cardiovascular Research Center - Tampere, Faculty of Medicine and Life Sciences, University of Tampere, Tampere 33014, Finland ¹⁶³Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, Melbourne, Victoria, 3002, Australia ¹⁶⁴Centre for Ophthalmology and Vision Science, Lions Eye Institute, University of Western Australia, Perth, Western Australia, 6009, Australia ¹⁶⁵Menzies Research Institute Tasmania, University of Tasmania, Hobart, Tasmania, 7000, Australia ¹⁶⁶K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health, NTNU, Norwegian University of Science and Technology, Trondheim, 7600, Norway ¹⁶⁷AMC, Department of Vascular Medicine, Amsterdam, 1105 AZ, The Netherlands ¹⁶⁸Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, University of the Chinese Academy of Sciences, Shanghai, People's Republic of China, Shanghai, 200031, China ¹⁶⁹Department of Neurology, Erasmus Medical Center, Rotterdam, 3015 GE, The Netherlands ¹⁷⁰Department of Radiology, Erasmus Medical Center, Rotterdam, 3015 GE, The Netherlands ¹⁷¹Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA, 943 05, USA ¹⁷²Department of Public Health & Clinical Medicine, Umeå University, Umeå, SE-90185, Sweden ¹⁷³Research Unit Skellefteå, Skellefteå, SE-93141, Sweden ¹⁷⁴Department of Genome Sciences, University of Washington, Seattle, WA, 98195, USA ¹⁷⁵The Copenhagen City Heart Study, Frederiksberg Hospital, Frederiksberg, 2000, Denmark ¹⁷⁶Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, 5021, Norway ¹⁷⁷National Institute of Public Health, University of Southern Denmark, Copenhagen, 1353, Denmark ¹⁷⁸Steno Diabetes Center Copenhagen, Gentofte, 2800, Denmark ¹⁷⁹Faculty of medicine, Aalborg University, Aalborg, DK-9000, Denmark ¹⁸⁰Research Center for Prevention and Health, Capital Region of Denmark, Glostrup, DK-2600, Denmark ¹⁸¹Department of Cardiology, Leiden University Medical Center, Leiden, 2333, The Netherlands ¹⁸²The Interuniversity Cardiology Institute of the Netherlands, Utrecht, 2333, The Netherlands ¹⁸³Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, 48109, USA ¹⁸⁴Department of Internal Medicine, University of Michigan, Ann Arbor, MI, 48109, USA ¹⁸⁵Division of Gastroenterology, University of Michigan, Ann Arbor, MI, 48109, USA ¹⁸⁶Centre for Brain Research, Indian Institute of Science, Bangalore 560012, India ¹⁸⁷Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, 3584 CG, The Netherlands ¹⁸⁸Department of Clinical Physiology, Faculty of

Medicine and Life Sciences, University of Tampere, Tampere, 33014, Finland
¹⁸⁹Department of Clinical Physiology, Tampere University Hospital, Tampere 33521, Finland
¹⁹⁰Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, FI-00014, Finland
¹⁹¹National Institute for Health and Welfare, Helsinki, FI-00271, Finland
¹⁹²Echinos Medical Centre, Echinos, Greece
¹⁹³Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, OX3 7LE, UK
¹⁹⁴Oxford NIHR Biomedical Research Centre, Oxford University Hospitals Trust, Oxford, OX3 7LE, UK
¹⁹⁵UKCRC Centre of Excellence for Public Health Research, Queens University Belfast, Belfast, UK, BT12 6BJ, UK
¹⁹⁶Foundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, 70100, Finland
¹⁹⁷Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio Campus, 70210, Finland
¹⁹⁸Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland
¹⁹⁹National Heart and Lung Institute, Imperial College London, Hammersmith Hospital Campus, London, W12 0NN, UK
²⁰⁰MRC-PHE Centre for Environment and Health, Imperial College London, London, W2 1PG, UK
²⁰¹University of Eastern Finland, Kuopio, 70210, Finland
²⁰²University of Helsinki, Helsinki, 00100, Finland
²⁰³Department of Psychiatry, and Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Western Cape, 7505, South Africa
²⁰⁴Institute of Social and Preventive Medicine, Lausanne University Hospital, Lausanne, 1010, Switzerland
²⁰⁵Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, 70210, Finland
²⁰⁶Department of Genetics, University of North Carolina, Chapel Hill, NC, 27599, USA
²⁰⁷Kaiser Permanente Washington Health Research Institute Seattle WA 98101
²⁰⁸Department of Health Services, University of Washington, Seattle WA 98101
²⁰⁹Department of Anthropology, Sociology, and History, University of San Carlos, Cebu City, 6000, Philippines
²¹⁰USC-Office of Population Studies Foundation, Inc., University of San Carlos, Cebu City, 6000, Philippines
²¹¹Division of Preventive Medicine University of Alabama at Birmingham, Birmingham, AL 35205, USA
²¹²Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Palo Alto, CA, 94304, USA
²¹³Department of Medicine, Boston University School of Medicine, Boston, MA, 02118, USA
²¹⁴Department of Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan 407, Taiwan
²¹⁵Uppsala University, Uppsala, 75185, Sweden
²¹⁶Department of Clinical Experimental Research, Rigshospitalet, Copenhagen, DK-2200, Denmark
²¹⁷Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, 2200, Denmark
²¹⁸Department of Biostatistics, Boston University School of Public Health, Boston, MA, 02118, USA
²¹⁹Department of Public Health Sciences, Institute for Personalized Medicine, the Pennsylvania State University College of Medicine, Hershey, PA, 17033, USA
²²⁰Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC, 27157, USA
²²¹Division of Health Sciences, Warwick Medical School, Warwick University, Coventry, CV4 7AL, UK

²²²Department of Preventive Medicine, Keck School of Medicine of the University of California, Los Angeles, CA, 90089, USA ²²³Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, 14558, Germany ²²⁴German Center for Diabetes Research, München-Neuherberg, 85764, Germany ²²⁵Westmead Millennium Institute of Medical Research, Centre for Vision Research and Department of Ophthalmology, University of Sydney, Sydney, New South Wales, 2022, Australia ²²⁶Department of Medicine, Kuopio University Hospital, Kuopio, 70210, Finland ²²⁷Department of Epidemiology and Public Health, University of Strasbourg, Strasbourg, F-67085, France ²²⁸Department of Public Health, University Hospital of Strasbourg, Strasbourg, F-67081, France ²²⁹Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, 4072, Australia ²³⁰Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, 2300RC, The Netherlands ²³¹INTERVAL Coordinating Centre, Department of Public Health and Primary Care, University of Cambridge, Cambridge, CB1 8RN, UK ²³²School of Medicine and Pharmacology, The University of Western Australia, Perth, Western Australia, 6009, Australia ²³³Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, EH8 9AG, UK ²³⁴Department of Medicine I, University Hospital Grosshadern, Ludwig-Maximilians-Universitat, Munich, 81377, Germany ²³⁵DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, 80802, Germany ²³⁶Laboratory of Neurogenetics, National Institute on Aging, NIH, Bethesda, MD, 20892, USA ²³⁷data tecnica international, Glen Echo, MD, USA ²³⁸Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, 4059, Australia ²³⁹Program for Personalized and Genomic Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, 21201, US ²⁴⁰Division of Cardiovascular Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, 02115, USA ²⁴¹Center for Neurobehavioral Genetics, UCLA, Los Angeles, CA, 90095, USA ²⁴²Pat Macpherson Centre for Pharmacogenetics and Pharmacogenomics, Medical Research Institute, Ninewells Hospital and Medical School, Dundee, DD1 9SY, UK ²⁴³Laboratory of Clinical Chemistry and Hematology, Division Laboratories and Pharmacy, University Medical Center Utrecht, Utrecht, 3584 CX, The Netherlands ²⁴⁴School of Women's and Infants' Health, The University of Western Australia, Perth, Western Australia, 6009, Australia ²⁴⁵University of Helsinki, Institute for Molecular Medicine (FIMM) and Diabetes and Obesity Research Program, Helsinki, FI00014, Finland ²⁴⁶University of Tartu, Estonian Genome Center, Tartu, Estonia, Tartu, 51010, Estonia ²⁴⁷Department of Epidemiology Research, Statens Serum Institut, Copenhagen, 2200, Denmark ²⁴⁸Institute of Epidemiology II, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, 85764, Germany ²⁴⁹Department of Clinical Immunology and Biochemistry, Lillebaelt Hospital, Vejle, 7100, Denmark ²⁵⁰School of Medicine, University of Split, Split, 21000, Croatia ²⁵¹South Ostrobothnia Central Hospital, Seinajoki, 60220, Finland ²⁵²Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, 20521,

Finland ²⁵³Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, 20520, Finland ²⁵⁴Centre for Non-Communicable Diseases, Karachi, Pakistan ²⁵⁵Merck, Sharp & Dohme, Genetics and Pharmacogenomics, Boston, MA, 02115, USA ²⁵⁶Department of Biobank Research, Umeå University, Umeå, SE-90187, Sweden ²⁵⁷Nuffield Department of Clinical Medicine, Oxford, OX37 BN, UK ²⁵⁸NHS Blood and Transplant - Oxford Centre, Oxford, OX3 9BQ, UK ²⁵⁹BRC Haematology Theme and Radcliffe Department of Medicine, University of Oxford, Oxford, OX3 9DU, UK ²⁶⁰Department of Public Health and Clinical Medicine, Unit of Family Medicine, Umeå University, Umeå, 90185, Sweden ²⁶¹Genetics of Complex Traits, University of Exeter Medical School, University of Exeter, Exeter, EX2 5DW, UK ²⁶²Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA ²⁶³University of Pittsburgh Medical Center, Departments of Medicine and Epidemiology, Pittsburgh, PA, 15213, USA ²⁶⁴Division of Epidemiology & Community Health University of Minnesota, Minneapolis, MN, 55454, USA ²⁶⁵Duke Molecular Physiology Institute, Duke University, Durham NC, 27701 ²⁶⁶Division of Endocrinology and Metabolism, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan 407, Taiwan ²⁶⁷School of Medicine, National Defense Medical Center, Taipei, Taiwan 114, Taiwan ²⁶⁸School of Medicine, National Yang-Ming University, Taipei, Taiwan ²⁶⁹Saw Swee Hock School of Public Health, National University Health System, National University of Singapore, Singapore 117549, Singapore ²⁷⁰Genetics, Target Sciences, GlaxoSmithKline, Research Triangle Park, NC, 27709, US ²⁷¹OmicSoft a QIAGEN Company, Cary, NC, 27513, US ²⁷²Department of Twin Research and Genetic Epidemiology, King's College London, London, SE1 7EH, UK ²⁷³Alzheimer Scotland Dementia Research Centre, University of Edinburgh, Edinburgh, EH8 9JZ, UK ²⁷⁴deCODE Genetics/Amgen inc., Reykjavik, 101, Iceland ²⁷⁵Chair of Genetic Epidemiology, IBE, Faculty of Medicine, LMU Munich, 81377, Germany ²⁷⁶Kanazawa University, Kanazawa, 920-8641, Japan ²⁷⁷College of Biomedical and Life Sciences, Cardiff University, Cardiff, CF14 4EP, UK ²⁷⁸Institute for Community Medicine, University Medicine Greifswald, Greifswald, 17475, Germany ²⁷⁹Center for Pediatric Research, Department for Women's and Child Health, University of Leipzig, Leipzig, 04103, Germany ²⁸⁰Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, Western Cape, 7505, South Africa ²⁸¹Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, 2333, The Netherlands ²⁸²Anogia Medical Centre, Anogia, Greece ²⁸³Centre for Vascular Prevention, Danube-University Krems, Krems, 3500, Austria ²⁸⁴Dasman Diabetes Institute, Dasman, 15462, Kuwait ²⁸⁵Diabetes Research Group, King Abdulaziz University, Jeddah, 21589, Saudi Arabia ²⁸⁶Department of Psychiatry, Dalhousie University, Halifax, B3H 4R2, Canada ²⁸⁷Department of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, 70210, Finland ²⁸⁸Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, 1081BT, The Netherlands ²⁸⁹Department of Molecular Cell Biology,

Leiden University Medical Center, Leiden, 2333ZC, The Netherlands ²⁹⁰Central Finland Central Hospital, Jyväskylä, 40620, Finland ²⁹¹USC Roski Eye Institute, Department of Ophthalmology, Keck School of Medicine of the University of Southern California, Los Angeles, CA, 90033, USA ²⁹²Department of Obstetrics and Gynecology, Institute for Medicine and Public Health, Vanderbilt Genetics Institute, Vanderbilt University, Nashville, TN, 37203, USA ²⁹³Research Center on Epidemiology and Preventive Medicine, Department of Medicine and Surgery, University of Insubria, Varese, 21100, Italy ²⁹⁴Merck, Sharp & Dohme, Cardiometabolic Disease, Kenilworth, NJ, 07033, USA ²⁹⁵DZHK (German Centre for Cardiovascular Research), partner site Greifswald, Greifswald, 17475, Germany ²⁹⁶Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, 17475, Germany ²⁹⁷Institute of Cellular Medicine, The Medical School, Newcastle University, Newcastle, NE2 4HH, UK ²⁹⁸Department of Medical Sciences, Cardiology, Uppsala Clinical Research Center, Uppsala University, Uppsala, 752 37, Sweden ²⁹⁹Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor, MI, 48104, USA ³⁰⁰Genetics, Target Sciences, GlaxoSmithKline, King of Prussia, PA, US ³⁰¹Departments of Epidemiology & Medicine, Diabetes Translational Research Center, Fairbanks School of Public Health & School of Medicine, Indiana University, Indiana, IN, 46202, USA ³⁰²Green Lane Cardiovascular Service, Auckland City Hospital and University of Auckland, Auckland, New Zealand ³⁰³Department of Human Genetics, University of Michigan, Ann Arbor, MI, 48109, USA ³⁰⁴Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, 39216, USA ³⁰⁵Danish Diabetes Academy, Odense, 5000, Denmark ³⁰⁶Department of Public Health, Aarhus University, Aarhus, 8000, Denmark ³⁰⁷GlaxoSmithKline, King of Prussia, PA, 19406, USA ³⁰⁸Department of Clinical Sciences, Quantitative Biomedical Research Center, Center for the Genetics of Host Defense, University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA ³⁰⁹Endometriosis CaRe Centre, Nuffield Department of Obstetrics & Gynaecology, University of Oxford, Oxford, OX3 9DU, UK ³¹⁰Department of Epidemiology and Carolina Center of Genome Sciences, Chapel Hill, NC, 27514, USA ³¹¹Li Ka Shing Centre for Health Information and Discovery, The Big Data Institute, University of Oxford, Oxford, OX3 7BN, UK ³¹²Departments of Pediatrics and Genetics, Harvard Medical School, Boston, MA, 02115, USA ³¹³The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY, 10069, USA

Acknowledgments

Alex Reiners was supported by R01DK089256. Alex Hewitt is supported by an NHMRC Practitioner Fellowship (APP1103329). Alisa Manning received funding from NIH/NIDDK K01 DK107836. Andrew Hattersley is a Wellcome Trust Senior Investigator (WT098395); and a NIH Research Senior Investigator. Andrew Morris is a Wellcome Trust Senior Fellow in Basic Biomedical Science (WT098017). Andrew Wood is supported by the European Research Council (SZ-245 50371-GLUCOSEGENES-FP7-IDEAS-ERC). Anne Jackson is supported by the American Heart Association (13POST16500011) and NIH (R01DK089256, R01DK101855, K99HL130580). Bratati Kahali and Elizabeth Speliotes were supported by the Doris Duke Medical Foundation, NIH (R01DK106621), the University of Michigan Internal Medicine Department, Division of Gastroenterology, the University of Michigan Biological Sciences Scholars Program and The Central Society for Clinical Research.

Cristen Willer is supported by NIH (HL094535, HL109946). Daniel Liu is supported by R01HG008983 and R21DA040177. Daniel Witte is supported by the Danish Diabetes Academy, which is funded by the Novo Nordisk Foundation. Veiko Salomaa has been supported by the Finnish Foundation for Cardiovascular Research. Folkert Asselbergs is supported by a Dekker scholarship-Junior Staff Member 2014T001 Netherlands Heart Foundation and UCL Hospitals NIHR Biomedical Research Centre. Fotios Drenos is supported by the UK MRC (MC_UU_12013/1-9). Gabriela Partida received scholarship support from the University of Queensland and QIMR Berghofer. Guillaume Lettre is funded by the Montreal Heart Institute Foundation and the Canada Research Chair program. Hanieh Yaghootkar and Tim Frayling are supported by the European Research Council (323195; SZ-245 50371-GLUCOSEGENES-FP7-IDEAS-ERC). Iris Heid is supported by BMBF (01ER1206) and BMBF (01ER1507m), NIH and Max Planck Society. Jeff Haessler was supported by NHLBI R21HL121422. Joel Hirschhorn is supported by NIH R01DK075787. Kari North was supported by NIH (R01DK089256; R01HD057194; U01HG007416; R01DK101855), and AHA (13GRNT16490017). Manuel Rivas is supported by Nuffield Department of Clinical Medicine Award, Clarendon Scholarship. Mark McCarthy is a Wellcome Trust Senior Investigator (WT098381); and a NIH Research Senior Investigator. Mengmeng Du is supported by the NCI (R25CA94880, P30CA008748). Pal Njolstad is supported by the European Research Council (AdG; 293574), Research Council of Norway, University of Bergen, KG Jebsen Foundation, Helse Vest, Norwegian Diabetes Association. Patrick Ellinor is supported by the NIH (1R01HL092577, R01HL128914, K24HL105780), an Established Investigator Award from the American Heart Association (13EIA14220013) and by the Foundation Leducq (14CVD01). Paul Auer was supported by NHLBI R21HL121422 and R01DK089256. Paul Huang is supported by NIH (NS33335, HL57818). Rebecca Fine is supported by NIH (T32GM096911). Ruth loos is supported by the NIH (R01DK110113, U01HG007417, R01DK101855, R01DK107786). Steven Lubitz is supported by NIH (K23HL114724) and a Doris Duke Charitable Foundation Clinical Scientist Development Award. Timothy Spector holds an ERC Advanced Principal Investigator award. Trevor Mori is supported by an Australian National Health and Medical Research Fellowship (APP1042255). Tune Pers received a Lundbeck Foundation and Benzon Foundation support. Valerie Turcot is supported by a postdoctoral fellowship from the Canadian Institutes of Health Research (CIHR). Zoltan Kutalik is supported by the Leenaards Foundation, Swiss National Science Foundation (31003A-143914) and SystemsX.ch (51RTP0_151019). Part of this work was conducted using the UK Biobank resource (Project Numbers 1251, 9072). A full list of acknowledgments appears in the Supplementary Note.

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BOX 1**Brief description of the 13 genes (alphabetical) identified**

ACHE (acetylcholinesterase). *ACHE* is mainly expressed in brain and muscle⁷⁶. Its encoded protein hydrolyzes acetylcholine (Ach) at brain cholinergic synapses and neuromuscular junctions, and thus terminates signal transmission⁶⁷. Knockout mice showed a reduction in expression of muscarinic Ach receptors in brain regions associated with learning and memory and showed lower ability to initiate the signaling cascade⁷⁷. This gene has fewer missense variants than expected and is highly intolerant to loss of function (LoF) mutations⁵².

ENTPD6 (ectonucleoside triphosphate diphosphohydrolase 6). Previously known as *Interleukin 6 Signal Transducer-2*, this gene is similar to E-type nucleotidases that participate in purine and pyrimidine metabolism, calcium ion binding, hydrolase activity, magnesium ion binding and nucleoside-diphosphatase activity⁷⁸. It is widely expressed in many different tissues, in particular in the brain⁷⁶.

GIPR (gastric inhibitory polypeptide receptor). *GIPR* encodes a G-protein coupled receptor for gastric inhibitory polypeptide that is secreted by intestinal K-cells after food ingestion⁵⁹. *GIPR* activation stimulates insulin secretion from pancreatic β -cells and mediates fat deposition by increasing lipoprotein lipase activity, lipogenesis, fatty acid and glucose uptake in adipocytes. *GIPR* is mostly expressed in EBV-transformed lymphocytes, stomach and visceral adipose tissue⁷⁶.

HIP1R (huntingtin interacting protein 1 related). *HIP1R* is a multi-domain protein that promotes actin binding and cell survival and interacts with *CLTB* and *HIP1* (GeneCards). *HIP1* and *HIP1R* appear to play central roles in clathrin-coated vesicle formation and intracellular membrane trafficking by promoting transient interaction between actin filaments and the endocytic machinery^{79,80}. *HIP1R* is most expressed in the stomach tissue, brain (substantia nigra, spinal cord, hippocampus), and sun-exposed skin⁷⁶.

KSR2 (kinase suppressor of ras 2). *KSR2* is an intracellular protein that functions as a molecular scaffold to regulate MAP kinases *ERK1/2* and determine cell fates. *KSR2* also regulates *AMPK* activity controlling cellular thermogenesis, fat oxidation, and glucose metabolism^{18,61,62}. Knockout mouse models and human mutations have been linked to obesity risk⁶². *KSR2* is almost exclusively expressed in the brain. It has fewer missense variants than expected and is highly intolerant to LoF mutations⁵².

MC4R (melanocortin 4 receptor). *MC4R* is a seven-transmembrane G-protein coupled receptor, predominantly expressed in the brain⁷⁶. *MC4R* has been known to play a key role in body weight regulation for more than 20 years. Activation of *MC4R* by α -MSH, a POMC-derived peptide, suppresses food intake; *MC4R* antagonists increase food intake and *MC4R* deficiency in human and rodent models results in hyperphagia and severe and early-onset obesity⁸¹. More than 150 *MC4R* mutations have been identified in individuals with severe, early-onset obesity⁸¹, many of which lead to a complete or partial loss of function^{82,83}. Up to 6% of individuals with severe, early-onset obesity carry pathogenic

mutations in *MC4R*, making *MC4R* deficiency the most common form of monogenic obesity^{82,84}.

PRKAG1 (protein kinase AMP-activated non-catalytic subunit gamma 1). The protein encoded by *PRKAG1* is one of the gamma regulatory subunits of the AMP-activated protein kinase (AMPK), which is an important energy-sensing enzyme that monitors cellular energy status³⁴. AMPK and PRKAG1 are ubiquitously expressed⁷⁶. In the hypothalamus, AMPK influences food intake, energy expenditure and glucose homeostasis³⁶. Muscle-specific overexpression of AMPK γ 1 subunit in mice results in increased food intake, but does not affect body weight, presumably through a compensatory increased energy expenditure⁸⁵.

RAB21 (member RAS oncogene family). RAB21 belongs to the Rab family of monomeric GTPases involved in the control of cellular membrane traffic. The encoded protein is widely expressed⁷⁶ and plays a role in the targeted trafficking of integrins, and is involved in the regulation of cell adhesion and migration⁸⁶. *RAB21* is thought to be intolerant to LoF mutations⁵².

RAPGEF3 (rap guanine nucleotide exchange factor 3; also EPAC1). *RAPGEF3* encodes the exchange protein directly activated by cAMP isoform 1 (EPAC1), one of two cAMP sensors that are involved in numerous intracellular cAMP-mediated functions⁸⁷. EPAC1 is ubiquitously expressed⁷⁶, and insights from mouse knockout models suggest a role in energy homeostasis and the development of obesity and diabetes through the regulation of leptin and insulin signaling^{31,87}.

ZFR2 (zinc finger RNA binding protein 2). The biological function of the gene product is as yet undetermined. GO annotations related to this gene include *nucleic acid binding*. It may have a role in dendritic branching and axon guidance^{88,89}. *ZFR2* is predominantly expressed in the brain⁷⁶.

ZBTB7B (zinc finger and BTB domain containing 7B, also ThPOK). *ZBTB7B* is a transcription factor regulating T-cell fate in the thymus, particularly as the master regulator of CD4⁺ lineage commitment⁹⁰. It is a repressor of type 1 collagen gene expression⁹¹. This gene is mainly expressed in T-cell lineages, skin and gastrointestinal tissues. *ZBTB7B* is thought to be intolerant to LoF mutations⁵².

ZFHX3 (zinc finger homeobox 3). *ZFHX3* encodes a transcription factor with multiple homeodomains and zinc finger motifs and plays a role in cell-cycle, myogenic and neuronal differentiation. This gene is a tumor suppressor⁹² that influences circadian rhythms^{93,94} and sleep⁹⁴. It may also contribute to the genesis of atrial fibrillation⁹⁵. *ZFHX3* is highly expressed in arterial tissue and also other tissues⁷⁶. The *ZFHX3* gene is highly intolerant to LoF mutations⁵².

ZNF169 (zinc finger protein 169). The biological function of the gene product is as yet unclear. GO annotations suggest that *ZNF169* is involved in *nucleic acid binding and transcriptional regulation*. This gene is ubiquitously expressed⁷⁶.

More details and references in Supplementary Table 24.

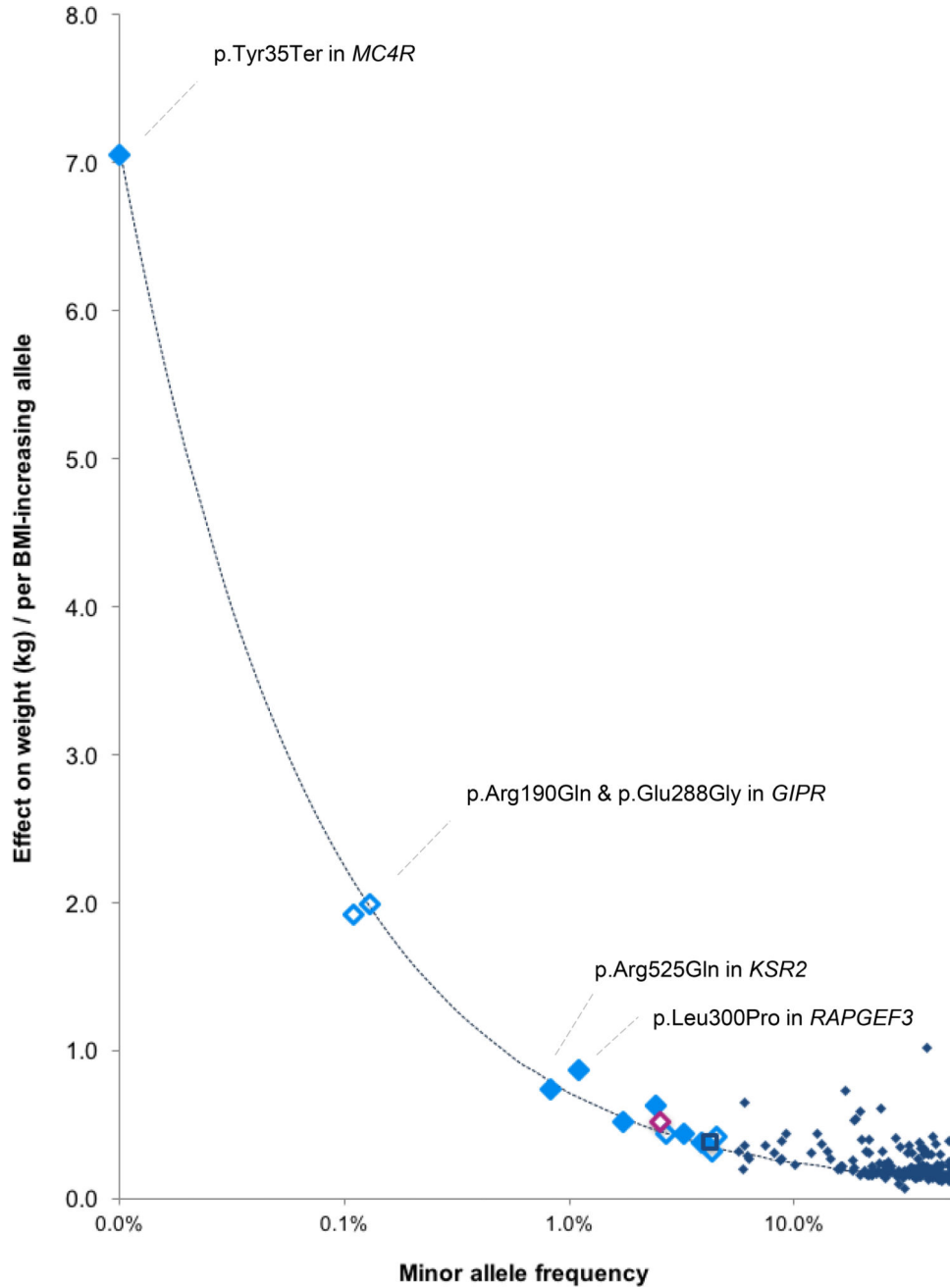


Figure 1. Effect sizes (y-axis) of the 14 BMI-associated R/LF coding variants by their minor allele frequency
 Effect sizes are expressed in body weight (kg) per allele, assuming a SD of 4.5 kg and an average-sized person of 1.7m tall. Solid markers indicate that the minor allele is associated with higher BMI, and clear markers indicate that the minor allele is associated with lower BMI. Variants were identified in all-ancestry analyses (light blue diamonds), the European ancestry analyses (dark blue square) and women-only analyses (pink diamond). Effect sizes for previously identified GWAS loci are shown in navy blue diamonds. The dotted line represents 80% power, assuming $\alpha = 2 \times 10^{-7}$ and $N = 525,000$ (discovery sample size).

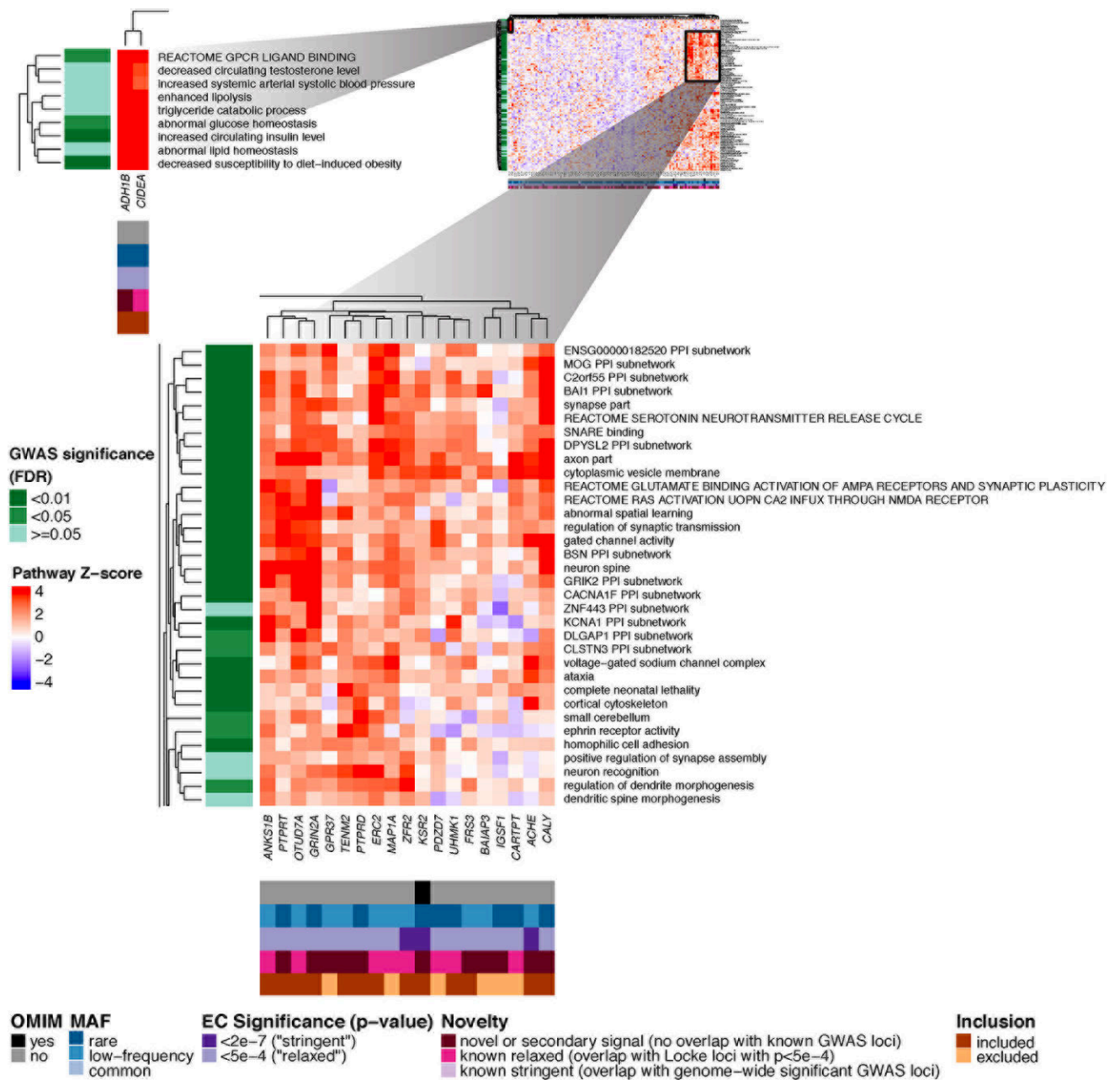


Figure 2. Heatmap showing DEPICT gene set enrichment results for suggestive and significant rare and low-frequency coding SNVs

For any given square, the color indicates how strongly the corresponding gene (x-axis) is predicted to belong to the reconstituted gene set (y-axis), based on the gene's Z-score for gene set inclusion in DEPICT's reconstituted gene sets (red indicates a higher, blue a lower Z-score). To visually reduce redundancy and increase clarity, we chose one representative "meta-gene set" for each group of highly correlated gene sets based on affinity propagation clustering (Online Methods, Supplementary Note). Heatmap intensity and DEPICT *P*-values (Supplementary Table 17) correspond to the most significantly enriched gene set within the meta-gene set. Annotations for genes indicate (1) whether it has an OMIM annotation as

underlying a monogenic obesity disorder (black/grey), (2) the MAF of the significant ExomeChip (EC) variant (blue), (3) whether the variant's P -value reached array-wide significance ($<2 \times 10^{-7}$) or suggestive significance ($<5 \times 10^{-4}$) (purple), (4) whether the variant was novel, overlapping "relaxed" GWAS signals from Locke *et al.*⁵ (GWAS $P < 5 \times 10^{-4}$), or overlapping "stringent" GWAS hits (GWAS $P < 5 \times 10^{-8}$) (pink), and (5) whether the gene was included in the gene set enrichment analysis or excluded by filters (orange/brown) (Online Methods, Supplementary Note). Annotations for gene sets indicate if the meta-gene set was significant (green; FDR < 0.01 , < 0.05 , or not significant) in the DEPICT analysis of GWAS results⁵. Here, two regions of particularly strong gene set membership are shown (see full heat map in Supplementary Figure 10a).

Table 1

Rare and low-frequency coding variants significantly associated with BMI

Chr:position	Variant	Coding locus	Allele	Amino acid change	EAF (%)	β (SD/allele)	SE	P-value	N	Explained variance (%)	
											Effect
All-ancestries additive											
1:154987704	rs141845046	<i>ZBTB7B</i> *	T	C	p.Pro190Ser	2.44%	0.048	0.006	7.73E-18	718,628	0.011%
7:100490797	rs1799805	<i>ACHE</i> *	T	G	p.His353Asn	3.90%	0.029	0.005	2.82E-10	707,448	0.006%
12:48143315	rs145878042	<i>RAPGEF3</i> *	G	A	p.Leu300Pro	1.10%	0.066	0.008	1.56E-15	700,852	0.010%
12:49399132	rs1126930	<i>PRKAG1</i>	C	G	p.Thr38Ser	3.22%	0.034	0.005	3.98E-12	712,354	0.007%
12:72179446	rs61754230	<i>RAB21</i> *	T	C	p.Ser224Phe	1.74%	0.040	0.007	1.33E-09	693,373	0.005%
12:117977550	rs56214831	<i>KSR2</i>	T	C	p.Arg525Gln	0.82%	0.057	0.010	1.08E-08	655,049	0.005%
12:123345509	rs34149579	<i>HPIR</i>	T	G	p.Cys938Phe	4.54%	-0.032	0.004	2.00E-14	716,253	0.009%
16:72830539	rs62051555	<i>ZFX3</i> *	G	C	p.Gln1100His	4.34%	-0.024	0.004	4.01E-08	690,637	0.005%
18:58039478	rs13447324	<i>MC4R</i>	T	G	p.Tyr35Ter	0.01%	0.542	0.086	2.26E-10	631,683	0.006%
19:46178020	rs139215588	<i>GIPR</i>	A	G	p.Arg190Gln	0.11%	-0.148	0.028	1.25E-07	695,800	0.005%
19:46180976	rs143430880	<i>GIPR</i>	G	A	p.Glu288Gly	0.13%	-0.153	0.028	2.96E-08	599,574	0.006%
20:251955509	rs6050446	<i>ENTPD6</i> *	A	G	p.Lys185Glu	2.71%	-0.034	0.005	2.40E-10	717,084	0.006%
All-ancestries sex-specific additive (women only)											
19:3813906	rs45465594	<i>ZFR2</i> *	C	A	p.Ile718Met	2.55%	-0.040	0.008	1.94E-07	373,848	0.008%
European Ancestry additive											
9:97062981	rs12236219	<i>ZNF169</i> *	T	C	p.Arg381Cys	4.23%	-0.029	0.005	8.78E-10	612,396	0.007%

Array-wide significant is defined as $P < 2 \times 10^{-7}$.

Variant positions are reported according to Build 37 and their alleles are coded based on the positive strand.

Alleles (effect/other), effect allele frequency (EAF), beta (b), standard error (SE) and P values are based on the meta-analysis of Discovery Stage (GIANT) and Validations stage (deCODE, UKBiobank) studies. Effect allele is always the minor allele. Effects (b) are expressed in SD, assuming mean=0 and SD=1.

The amino acid change from the most abundant coding transcript is shown in this table (see Supplementary Table 25 for more details on protein annotation based on VEP tool and transcript abundance from GTEx database).

* Novel gene; i.e. not previously implicated in human obesity

Table 2
Genes significantly associated with BMI in a gene-based meta-analysis, aggregating R/LF coding SNVs

Gene	Location longest coding transcript	Test ^d	N variants	P-value	Conditioned P-value ^a	Single variant	P-value
						Top variant	P-value
All-ancestries sex-combined							
<i>SLC6A17</i>	chr1:110693132–110744823	SKAT	13	2.73E-07	0.13	rs41313405	4.45E-07
<i>RAPGEF3</i>	chr12:48128453–48152889	SKAT	19	8.91E-15	0.20	rs145878042	5.16E-14
<i>PRKAG1</i>	chr12:49396055–49412629	SKAT	4	2.75E-12	0.53	rs1126930	2.63E-12
<i>RAB21</i>	chr12:72148643–72187256	SKAT	5	4.81E-08	0.27	rs61754230	4.96E-08
<i>KSR2</i>	chr12:117890817–118406028	SKAT	7	7.15E-09	0.19	rs56214831	4.59E-08
<i>MAP1A</i>	chr15:43809806–43823818	SKAT	25	9.42E-07	0.16	rs55707100	1.01E-06
<i>MC4R</i>	chr18:58038564–58040001	VT	4	3.72E-09	0.01	rs13447325	2.97E-11
<i>GIPR</i>	chr19:46171502–46186982	VT	10	8.24E-09	1.12E-04	rs143430880	5.76E-06
All-ancestries sex-specific							
<i>ALDH3A1</i> (men only)	chr17:19641298–19651746	SKAT	15	3.24E-07	0.003	rs142078447	8.62E-06
<i>ZFR2</i> (women only)	chr19:3804022–3869027	SKAT	19	1.81E-07	0.82	rs45465594	3.64E-07
European sex-combined							
<i>ACHE</i>	chr7:100487615–100493592	SKAT	6	3.30E-10	0.12	rs386545548	7.22E-10
European sex-specific							
<i>ANGPTL7</i> (men only)	chr1:11249346–11256038	VT	3	2.50E-06	0.008	rs202182115	2.56E-05
<i>ZNF169</i> (women only)	chr9:97021548–97064111	SKAT	9	1.89E-07	0.24	rs12236219	1.06E-06

Array-wide significant gene-based association is defined as $P < 2.5 \times 10^{-6}$. P-values are based on the meta-analysis of Discovery Stage studies.

Gene-based analyses were performed with SKAT and VT; results shown are from the test (SKAT or VT) for which the significance exceeded $P < 2.5 \times 10^{-6}$. Only results using the "broad" SNV inclusion criteria reached array-wide significance.

Transcript positions are reported according to Build 37 for the longest coding transcript supported by RefSeq (as displayed in USCS Genome Browser).

^aP-value after conditioning on the most significant (top) single variant aggregated in the gene-based test.