Genomic approach to therapeutic target validation identifies a glucose-lowering GLP1R variant protective for coronary heart disease

All authors with their affiliations appear at the end of this paper.

Author list


1Medical Research Council Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Biomedical Campus, Cambridge, UK.
Department of Public Health and Primary Care, Strangeways Research Laboratory, University of Cambridge, Cambridge, UK.

The Wellcome Trust Sanger Institute, Cambridge, UK.

Statistical Genetics, Projects, Clinical Platforms & Sciences, GlaxoSmithKline, Research Triangle Park, NC, USA.

Division of Preventive Medicine, Brigham and Women's Hospital, Boston MA, USA.

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

Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland, Kuopio, Finland.

Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA.

Department of Clinical Sciences, Genetic and Molecular Epidemiology Unit, Lund University, Malmö, Sweden.

Genetics of Complex Traits, University of Exeter Medical School, University of Exeter, Exeter, UK.

Genetic Epidemiology Unit, Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands.

Fairbanks School of Public Health, Department of Epidemiology, Indianapolis, IN, US.

Indiana University School of Medicine, Department of Medicine, Indianapolis, IN, USA.

Division of General Internal Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, Baltimore, Maryland.

Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland.

Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Strangeways Laboratory, Worts Causeway, Cambridge, UK.

Department of Epidemiology, Erasmus University Medical Center, Rotterdam, Netherlands.

University of Lille, Institut national de la santé et de la recherche médicale (Inserm), Centre Hospitalier Régional Universitaire de Lille, Institut Pasteur de Lille, UMR1167 - RID-AGE, F-59000 Lille, France.

Public Health Division of Gipuzkoa, San Sebastian, Spain.

Instituto BIO-Donostia, Basque Government, San Sebastian, Spain.

CIBER Epidemiología y Salud Pública (CIBERESP), Spain.
23 Department of Epidemiology and Public Health (EA3430), University of Strasbourg, Strasbourg, France.
24 Sobell Department of Motor Neuroscience, UCL Institute of Neurology, London, UK.
25 Inserm, Centre de recherche en épidémiologie et santé des populations (CESP), Villejuif, France.
26 Univ Paris-Sud, Villejuif, France.
27 Navarre Public Health Institute (ISPN), Pamplona, Spain.
28 University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, Cambridge, UK.
29 Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, USA.
30 Department of General and Interventional Cardiology, University Heart Center Hamburg, Germany.
31 Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA.
32 German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany.
33 Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston, Houston, TX, USA.
34 Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA.
35 Department of Genetics, Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO, USA.
36 Cancer Research UK Clinical Trials Unit, Institute for Cancer Studies, The University of Birmingham, Edgbaston, Birmingham, UK.
37 Cancer Research UK Cambridge Institute and Department of Oncology, Li Ka Shing Centre, University of Cambridge, Cambridge, UK.
38 University of Glasgow, Glasgow, UK.
39 National Heart, Lung, and Blood Institute (NHLBI) Framingham Heart Study, Framingham, MA, USA.
40 Dep. of Psychiatry, Washington University, School of Medicine, St. Louis, MO, US.
41 Division of Statistical Genomics, Department of Genetics and Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, MO, USA.
42 Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden.
43 Warwick Clinical Trials Unit, University of Warwick, Gibbet Hill Road, Coventry, UK.
University of Cambridge and National Institute of Health Research Cambridge Biomedical Research Centre at Cambridge University Hospitals National Health Service Foundation Trust, Cambridge, UK.

McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA.

Consiglio Nazionale delle Richerche (CNR) Institute of Clinical Physiology, Pisa, Italy.

Department of Epidemiology, UMR 1027- INSERM, Centre Hospitalier Universitaire (CHU) de Toulouse, Toulouse, France.

Department of Clinical and Experimental Medicine, Research Centre in Epidemiology and Preventive Medicine, University of Insubria, Varese, Italy.

Department of Epidemiology and Prevention, Istituti di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Italy.

Catalan Institute of Oncology (ICO), Barcelona, Spain.

Epidemiology and Prevention Unit, Milan, Italy.

Research Unit, Skellefteå, Sweden.

Department of Public Health & Clinical Medicine, Umeå University, Umeå, Sweden.

Steno Diabetes Center, Gentofte, Denmark.

National Institute of Public Health, Southern Denmark University, Denmark.

Leiden University Medical Center, Leiden, Netherlands.

German Cancer Research Centre (DKFZ), Heidelberg, Germany.

UK Clinical Research Collaboration (UKCRC) Centre of Excellence for Public Health, Queens University Belfast, Northern Ireland, UK.

University of Oxford, UK.

National Institute for Health and Welfare, Helsinki, Finland.

The Institute of Cancer Research, London, UK.

Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland, Kuopio, Finland.

Kuopio University Hospital, Kuopio, Finland.

Research Centre for Prevention and Health, Capital region, Copenhagen, Denmark.

Department of Clinical Experimental Research, Rigshospitalet, Glostrup, Denmark.

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

Framingham Heart Study, Population Sciences Branch, NHLBI/NIH, Bethesda, MD, USA.
Department of Genetics, University of North Carolina, Chapel Hill, NC, USA.

Department of Biostatistics, University of Liverpool, Liverpool, UK.

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

The University of Manchester, Centre for Epidemiology, Institute of Population Health, Oxford Road, Manchester, UK.

University of Warwick, Coventry, UK.

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

Department of Medicine I, Ludwig-Maximilians-University Munich, Munich, Germany.

DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany.

Clinical Pharmacology, William Harvey Research Institute, Barts and The London, Queen Mary University of London, London, UK.

Department of Epidemiology, Murcia Regional Health Council-IMIB Arrixaca, Murcia, Spain.

Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark.

Lund University, Malmö, Sweden.

Cancer Research and Prevention Institute (ISPO), Florence, Italy.

Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy.

Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA.

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

Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA.

Institute of Molecular Medicine Finland (FIMM), University of Helsinki.

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

Department of Medical Oncology, Arden Cancer Centre, University Hospital Coventry and Warwickshire.

Public Health Directorate, Asturias, Spain.

Umeå University, Umeå, Sweden.

Unit of Cancer Epidemiology, Citta’ della Salute e della Scienza Hospital-University of Turin.
91 Center for Cancer Prevention (CPO), Torino, Italy.
92 Human Genetics Foundation (HuGeF), Torino, Italy.
94 Institute of Psychological Medicine and Clinical Neuroscience, MRC Centre, Cardiff University, UK.
95 International Agency for Research on Cancer, Lyon, France.
96 Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, USA.
97 Cancer Registry and Histopathology Unit, "Civic M.P.Arezzo" Hospital, ASP Ragusa (Italy).
99 University Medical Center Utrecht, Utrecht, The Netherlands.
100 Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK.
101 Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Strangeways Laboratory, Worts Causeway, Cambridge, UK.
102 Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.
103 Genetics, PCPS, GlaxoSmithKline, RTP, NC, USA.
104 Royal Marsden NHS Foundation Trust, Fulham and Sutton, London and Surrey, UK.
105 Institut für Integrative und Experimentelle Genomik, Universität zu Lübeck, Lübeck, Germany.
106 Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, MA, USA.
107 Research Centre for Prevention and Health, Capital region, Denmark.
108 Department of Public Health, Institute of Health Science, University of Copenhagen, Denmark.
109 Faculty of Medicine, Aalborg University, Denmark.
110 National Heart and Lung Institute, Imperial College London, London, UK.
111 Imperial College Healthcare NHS Trust, London, UK.
112 Ealing Hospital NHS Trust, Middlesex, UK.
113 Department of Medicine, University of Kuopio, Kuopio, Finland.
114 Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), University of Oxford, UK.
115 Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, USA.
School of Public Health, Imperial College London, UK.

Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-University of California, Los Angeles (UCLA) Medical Center, Torrance, CA, USA.

Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, USA.

Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, UK.

National Institute for Health Research (NIHR) Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester, UK.

Deutsches Herzzentrum München, Technische Universität München, Munich, Germany.

Department of Internal Medicine, BH10-462, Internal Medicine, Lausanne University Hospital (CHUV), Lausanne, Switzerland.

MRC Metabolic Diseases Unit, Cambridge, UK.

National Institute of Health Research Cambridge Biomedical Research Centre, Cambridge, UK.

William Harvey Research Institute, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, UK.

Division of Endocrinology, Diabetes and Metabolism, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

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

Cardiology Division, Center for Human Genetic Research, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

Division of General Internal Medicine, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA.

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

Genetics, Projects, Clinical Platforms & Sciences, GlaxoSmithKline, Philadelphia, PA, USA.

† These authors contributed equally to the work

† Data used in the preparation of this article were obtained from the Genetic and Environmental Risk for Alzheimer’s Disease_Exome Chip (GERAD_EC) Consortium and Alzheimer's Disease Genetics Consortium (ADGC). As such, the investigators within the GERAD_EC Consortium and ADGC contributed to the design and implementation of GERAD_EC and ADGC and/or provided data but did not participate in analysis or writing of this report.

§ Current affiliation: PAREXEL, Durham, NC

*Correspondence can be addressed to:
One Sentence Summary: A missense variant in GLP1R associated with lower fasting glucose levels and protective against T2D is associated with lower risk of coronary heart disease, suggesting that GLP1R agonists are not associated with an unacceptable increase in cardiovascular risk.

Abstract

Regulatory authorities have indicated that new drugs to treat type 2 diabetes (T2D) should not be associated with an unacceptable increase in cardiovascular risk. Human genetics may be able to inform development of antidiabetic therapies by predicting cardiovascular and other health endpoints. We therefore investigated the association of variants in 6 genes that encode drug targets for obesity or T2D with a range of metabolic traits in up to 11,806 individuals by targeted exome sequencing, and follow-up in 39,979 individuals by targeted genotyping, with additional in silico follow up in consortia. We used these data to first compare associations of variants in genes encoding drug targets with the effects of pharmacological manipulation of those targets in clinical trials. We then tested the association those variants with disease outcomes, including coronary heart disease, to predict cardiovascular safety of these agents. A low-frequency missense variant (Ala316Thr;rs10305492) in the gene encoding glucagon-like peptide-1 receptor (GLP1R), the target of GLP1R agonists, was associated with lower fasting glucose and lower T2D risk, consistent with GLP1R agonist therapies. The minor allele was also associated with protection against heart disease, thus providing evidence that GLP1R agonists are not likely to be associated with an unacceptable increase in cardiovascular risk. Our results provide an encouraging signal that these agents may be associated with benefit, a question currently being addressed in randomised controlled trials. Genetic variants associated with metabolic traits and multiple disease outcomes can be used to validate therapeutic targets at an early stage in the drug development process.

Introduction

In 2008, the US Food and Drug Administration issued guidance for industry on new therapies to treat type 2 diabetes (T2D), recommending that sponsors should demonstrate that these treatments are “not associated with an unacceptable increase in cardiovascular risk” (1). This mandate challenges drug developers to prove safety during clinical trials, which is an expensive and late-phase strategy for the identification of such concerns. Instead, genetic approaches may aid in the identification of possible drug side-effects much earlier in the drug development process. Genetic variants can inform the treatment and prevention of human disease (2, 3), either reducing the prioritisation of potential targets (4, 5) or implicating new targets (6, 7). Functional exonic variants can be useful surrogates for
drug effects, when, for example, a loss-of-function (LoF) variant may be a useful tool to understand the consequences of pharmacological inhibition of a particular target protein (7). Recent sequencing efforts have identified a large number of potentially functional low-frequency and rare exonic variants in human populations, even among genes under purifying selection (8–12). Although such variants may influence susceptibility to disease, the high cost of these sequencing approaches has previously meant that they have not been performed in the sample sizes required to allow routine investigation of their association with complex disease and related traits.

A recent targeted exome sequencing study of 202 genes encoding potential drug targets identified an abundance of potentially functional exonic variants (8). Among these 202 genes, six genes encoding drug targets licensed or in development by GlaxoSmithKline (GSK) for treatment of obesity and/or T2D were included. Recognizing that these data could be used to test for genetic variants mimicking pharmacological manipulation of the encoded protein (drug target), we investigated six genes encoding targets of relevance to obesity and T2D. These variants could then serve as tools to aid the broader evaluation of drug-related risk for adverse events mediated via on-target effects.

As a proof of concept for use of genetic variants to evaluate the cardiovascular safety of anti-diabetic agents, we evaluated the widely used glucose-lowering glucagon-like peptide-1 receptor (GLP1R) agonists (13). These agents are long-acting mimetics of the incretin hormone GLP1, which increases insulin secretion after oral consumption of glucose but not after glucose administered intravenously. There are uncertainties over the role of these agents in the aetiology of rare, adverse pancreatic events that have been reported following their usage (14). These therapies have been associated with weight loss (15) and reduced cardiovascular risk factors, and while a recent trial reported non-inferiority of GLP1R-agonists in cardiovascular safety (16), multiple trials evaluating cardiovascular safety have not yet been completed (17). We used a genetic variant in GLP1R that is associated with variation in fasting glucose levels and with T2D risk (18) to evaluate the cardiovascular safety of GLP1R agonists. The low-frequency variant protective for T2D was also
protective for coronary heart disease (CHD). These findings support the notion that GLP1R agonists will not confer an increased cardiovascular risk in people. This study also demonstrates how genetic target validation approaches can be employed early in the drug development process to evaluate efficacy and safety.

Results

Association of genetic variants in genes encoding T2D and obesity drug targets

The study design consisted of initial discovery of variants with suggestive associations, to targeted genotyping and in silico follow-up analyses (Fig. 1). We investigated the association of 121 variants in six genes encoding therapeutic targets in use or in development for T2D or obesity (CNR2, DPP4, GLP1R, SLC5A1, HTR2C, MCHR1)—drawn from a recent targeted exome sequencing study of 202 genes encoding drug targets (8)—with variation in the following traits: T2D, obesity, body-mass index (BMI), waist circumference, fasting glucose, fasting insulin, and 2-h glucose (Fig. 1). In the “Discovery Analysis”, we identified seven variants potentially associated with T2D- or obesity-related traits (where p<0.001, or which were in a target of interest to GSK and p<0.05) (Table 1). For these seven variants, “Follow-Up Analysis” was performed by targeted genotyping in up to 39,979 additional individuals of European ancestry. Where possible, in silico follow-up analysis was performed for traits and variants available in large-scale genetic consortia data.

Initial discovery analyses included 1331 tests of association, with the threshold specified to reach significance in combined analyses being p<3.8x10^{-5}. In a combined analysis of results from the different phases, we identified a low-frequency (~1% minor allele frequency (MAF)) missense variant Ala316Thr; rs10305492 in the GLP1R gene to be associated with fasting glucose (Fig. 2A). The variant was in Hardy-Weinberg equilibrium in all genotyped samples (p > 0.2). The effect size (i.e. the difference per allele) of 0.09 mM was larger than most common variants previously reported for fasting glucose (Fig. 2B), and was recently found to be associated with fasting glucose in non-overlapping samples from large-scale analyses of coding variant associations with glycaemic traits.
The combined analysis of the six other variants in Table 1 did not show evidence of association (p > 3.8x10^{-5}, by linear or logistic regression) with the suggestively associated trait in the discovery analysis (“Follow-up” p-values > 0.05; “Combined” p-values > 0.005; Table 1).

The GLP1R gene encodes the GLP1 receptor, the target for GLP-1, a hormone that mediates the augmented response to insulin secretion following oral glucose administration. This receptor is the target for the GLP1R-agonist class of T2D therapeutics and the association of this variant with fasting glucose mimicked a major effect of this class of agents. To further corroborate the utility of this variant as a surrogate indicator of pharmacological modulation of the receptor, we investigated its association with T2D and found that the minor allele was associated with lower risk of T2D [odds ratio (OR) = 0.83 [0.76, 0.91]; P = 9.4x10^{-5}; in a fixed effect meta-analysis of log-odds ratios from studies and consortia listed in table S1 and in Supplementary Materials “Studies contributing to follow-up analyses of type-2 diabetes and obesity related traits”; n_{cases} = 25,868, n_{controls} = 122,393]. However, we saw no association of this GLP1R variant (Ala316Thr; rs10305492) with fasting insulin, nor with 2-h glucose (Fig. 2A).

Although there were no individuals carrying putative LoF variants in GLP1R in the targeted sequencing study, a single individual in the cohort-arm of the UK10K study had a LoF allele (W297*) but did not have an extreme glycaemic phenotype. This individual’s fasting glucose and insulin concentrations were within the range of 95% of the values for this population. Nine high-confidence LoF variants in GLP1R were observed in the ExAC database (19). Eight were singletons and the most common had a frequency of less than 1/10,000, highlighting the difficulty in restricting analyses to individual LoF variants.

**Association of GLP1R variant with quantitative traits and comparison with effects observed in clinical trials of GLP1R agonists**

To further characterise the extent to which the GLP1R variant associations mirrored the effects of GLP1R-agonist therapy, we compared genetic associations to the metabolic effects observed in...
previously reported clinical trials (Fig. 3, table S2). GLP1R agonist therapy can result in lower fasting and post-challenge glucose, weight loss, a reduction in systolic blood pressure, reduced total- and LDL-cholesterol and an increase in resting heart rate. The effects of GLP1R-agonists on glycaemic measures (fasting glucose and 2-h glucose) were stronger than those on non-glycaemic factors (Fig. 3), which have been detectable only in some meta-analyses of clinical trials (20–23).

Using fasting glucose as the benchmark, the per-allele association of the genetic variant with glucose (-0.15 SDs [-0.20,-0.11], from Fig. 2) was 3.3-fold weaker than the effect observed for GLP1R-agonist treatment (-0.49 [-0.60,-0.37], from Fig. 3). We therefore rescaled the genetic associations to account for this difference, by multiplying the magnitude of all observed genetic associations by 3.3 (Fig. 3), and demonstrated that there was little difference between the magnitude of association of the GLP1R variant and effects observed in clinical trials beyond that expected by chance (α=0.0025).

An exception to this observation was the impact of GLP1R agonist therapy on weight in non-diabetic individuals when compared to the observed association between the variant and BMI (p=2.6x10^-4, Cochrane’s Q test) (table S2). The genetic variant was not associated with BMI (Fig. 3), whereas the agonist therapy caused a reduction in body mass in non-diabetic individuals but not in individuals with T2D (fig. S1, table S2). However, five of the six trials in non-diabetic individuals were performed in obese participants (table S3), whose higher starting weight may have enabled a greater weight loss.

GLP1R agonists appeared to have a greater effect on 2-h glucose than the magnitude of association observed for the variant (p=2.1x10^-12, Cochrane’s Q test) (Fig. 3; fig. S2; table S2). The difference was most pronounced in comparison to trials in individuals with T2D, among whom we observed heterogeneity in the effect of GLP1R agonists on 2-h glucose, even within drug class (I²=97%) (fig. S2B). There was no significant difference between the magnitude of genetic association and the impact of GLP1R agonist therapy on 2-h glucose in non-diabetic individuals (Fig. 3; table S2), although the number of people included in such trials was much smaller than in trials including individuals with T2D (table S3).
Association of GLP1R variant with disease outcomes

Our final aim was to describe the association of the GLP1R variant with CHD and other outcomes. In a large-scale international collaboration, we studied 61,846 individuals with CHD and 163,728 controls, and found that the fasting glucose–lowering allele of GLP1R was associated with protection against CHD (Fig. 4). The association with CHD is greater than the 1% reduction in risk that would be predicted based on the association of this variant with fasting glucose alone (24) (see Supplementary Methods on “Calculating the reduction in coronary heart disease risk attributable to lower fasting glucose levels”), suggesting that lowering of fasting glucose alone is unlikely to explain the observed association between the GLP1R variant and lower risk of CHD. Although not significant, carriage of the minor allele was associated with lower LDL cholesterol, triglycerides, systolic blood pressure, and higher HDL cholesterol.

Using data from international consortia, we found no evidence for association of the GLP1R variant with pancreatic cancer, although the confidence intervals were wide owing to the comparatively small sample size (4987 cases and 8627 controls) and low frequency of the allele (Fig. 4). There was no evidence of association with breast, ovarian, or prostate cancer risk. Given the interest in GLP1R agonist therapy for neurological disease, including Parkinson’s (25) and Alzheimer’s (26), we also investigated the association of the GLP1R variant with those diseases, but found no evidence of association (Fig. 4).

Discussion

Anticipating the side effects of drugs prior to phase III clinical trials could support drug discovery and development, reducing attrition rates and saving considerable time and money. The promise of human genetics in this endeavour (2, 3, 7, 27) depends on the availability of genetic variants that mimic pharmaceutical interventions. We undertook a systematic study to identify such genetic
variants in the context of diabetes and obesity, and identified an association between fasting glucose and T2D with a missense variant in \textit{GLP1R}, the gene encoding the GLP-1 receptor—the target of the GLP1R agonist class of T2D therapies. Regulatory authorities require evidence that therapies for T2D are not associated with unacceptable increases in cardiovascular risk. The reduced risk associated with the glucose-lowering genetic variant in \textit{GLP1R} provides evidence that not only will GLP1R agonists meet this regulatory hurdle, but they may also reduce CHD events. Ongoing trials of GLP1R agonists are designed to resolve this uncertainty and will also augment the evidence on the broader validity of genetic approaches in drug-target validation.

A key consideration in assessing whether genetic variants can be used to understand therapeutic effects is how well the genetic variant mirrors the effects of pharmacological intervention at the same target. Genetic association data, here and reported previously (18), suggest that lifelong carriage of the minor \textit{GLP1R} allele (at rs10305492) is associated with lower fasting glucose and lower risk of T2D, although not with 2-h glucose. Clinical trial data from individuals with T2D, who may have a diminished incretin effect, show that GLP1R agonists lower 2-h glucose considerably (28), whereas the effect on 2-h glucose is smaller in individuals without T2D (29), presumably because non-diabetic individuals are less likely to have an impaired incretin effect requiring therapeutic correction. Similarly, GLP1R agonists were associated with greater weight loss in obese individuals than in non-obese. Such a phenomenon has previously been suggested for the effects of GLP1R-agonism on blood pressure, where GLP1R-agonist therapy appears to lower blood pressure in individuals with high blood pressure but not in non-hypertensive individuals (30, 31). This highlights a limitation in the use of genetic variants in target validation: that the association of genetic variants is often tested in individuals of “normal” physiology, whereas clinical trials are generally performed in individuals with prevalent disease.

An important step in evaluating the utility of genomics in target validation is to understand the functional consequences of variants. For potential novel targets, whether the variant confers gain or loss of function informs the development of either an agonist or antagonist therapy. For
example, LoF variants have been used to understand the consequences of antagonism of a novel drug target (7, 32). However, researchers have gained insights using variants validated as instruments when their phenotypic associations mirrored pharmacological action, even in the absence of strong functional insights into the mechanism of those variants (33). GLP1R-agonist therapy reduces fasting glucose in humans, as does administration of GLP1, regardless of the duration or severity of T2D (34). In mice, the loss of GLP1R leads to fasting hyperglycaemia (35, 36). Together, these findings in humans and in mice suggest that the glucose-lowering minor allele at rs10305492 confers gain of function. However, differences in basal activity of the human and murine GLP1R (37) limit our ability to extrapolate findings from GLP1R knockout mice to humans (15, 32). Previous attempts to characterise the effect of this variant in cellular models have been inconclusive (38, 39). The rarity of putative LoF alleles in the GLP1R impaired our ability to restrict analyses to such variants. Although the absence of definitive functional characterisation is a limitation of this study, our observation that the minor allele is strongly associated with lower fasting glucose levels and is protective against T2D supports the validity of the variant as a genetic instrument for GLP1R-agonist therapy. Future integration of large-scale human genetic data with functional characterisation in appropriate cell models will allow broader application of variants, other than those characterised as LoF, in target validation.

Although the GLP1R variant was not associated with any of the other non-glycaemic or quantitative cardiovascular parameters, there was insufficient evidence to suggest the genetic associations and pharmacological effects were different. Power calculations indicated that, to detect the expected association with systolic blood pressure or resting heart rate, a sample size of more than 250,000 individuals would be required. This is considerably larger than most current genetic consortia, although this limitation could soon be overcome as larger studies become available (40), further strengthening the promise of genomics in target validation. Although we did not observe overall evidence for association of variants other than the GLP1R variant, the discovery phase, from which we selected variants for follow-up, was relatively small in comparison to the overall sample
and there remains a possibility of type II error in the discovery phase. As larger resources of genetic data become available, these potential concerns will also be reduced.

The detection of rare adverse effects of a drug remains a challenge. Pharmacoepidemiological approaches using routine database analysis may identify rare adverse outcomes associated with treatment, but the approach is rarely conclusive because of confounding, particularly by indication. Our demonstration that the GLP1R variant is not associated with pancreatic, breast, prostate, or ovarian cancer, or with Parkinson’s or Alzheimer’s disease is limited by the upper bounds of the confidence intervals, which are too high to allow strong inference about the likely long-term safety of GLP1R agonists with regard to these outcomes. Although these data represent the largest resources available globally, the accumulation of studies with greater numbers of individuals with genetic data and robust disease outcome classification will considerably enhance the potential of this type of investigation. The comparisons of other traits and disease outcomes, beyond the primary indications, makes the assumption that pharmacological effects are mediated via “on-target” effects and not “off-target” effects (i.e. those mediated by effects of the agent on other non-specific targets). Although our results offer insight into the effects of GLP1R agonists, they do not necessarily apply to other agents targeting the incretin pathway through different mechanisms, such as by DPP-4 inhibition (41).

In conclusion, through a targeted exome sequencing approach, we identified that a low-frequency missense variant in GLP1R was associated with lower fasting glucose and risk of T2D, similar to the effects of GLP1R agonist therapy. This variant was also associated with lower risk of CHD, thus providing supportive evidence that these agents are not likely to be associated with an unacceptable increase in cardiovascular risk and may indeed be associated with benefit, a question currently being addressed in randomised controlled trials. We propose that future drug development and investment decisions could be informed by genomic data much earlier in the development process, providing insight into both efficacy and side-effects.
Methods

Study design

We studied six genes encoding therapeutic targets licensed or in development for obesity or T2D (CNR2, DPP4, GLP1R, SLC5A1, HTR2C, MCHR1), drawn from a recent targeted exome sequencing study of 202 genes encoding drug targets (8), which represented approximately 1% of the coding genome and 7% of all genes considered current or potential drug targets (8). In the “Discovery Analysis”, we investigated the association of common and rare variants in these six genes with seven T2D and obesity-related traits (Fig. 1). We analysed all variants which had i) MAF ≥ 0.5% or well imputed (R² > 0.5) in CoLaus; ii) MAF ≥ 0.5% in GEMS; or iii) MAF ≥ 0.1% in BMI (given the larger sample size) in the CoLaus study (42), the GEMS study (43), or all individuals with BMI measurements. We examined 121 variants for association with six traits in the CoLaus study (6*121 = 726 tests); 4 traits in GEMS (4*121 = 484 tests); and 1 trait in the BMI study, comprising a total of 1331 tests of association. First, we analysed a subset of the population-based CoLaus study (n=2086) for T2D, obesity, waist circumference, fasting glucose, fasting insulin, and 2-h glucose traits. Second, in the GEMs dyslipidaemic case and normolipidaemic control study (n_cases=787, n_controls=792), we analysed obesity, waist circumference, fasting glucose, and fasting insulin traits. We performed discovery analyses in the CoLaus and GEMS studies separately due to the different study designs and traits analysed in attempt to maximise sensitivity to detect associations that might be masked by context-dependent associations. Third, BMI measures were available in a larger sample size from 11 studies (Fig. 1), and were analysed together. We provide the sample sizes for the discovery analyses in Figure 1 and trait-specific sample sizes in Table 1 (n = 505 – 11,806). We augmented the sequence data for the CoLaus study with imputed data in the remainder of the study (n=3539) where variants were imputable (R²>0.5) using a custom imputation process on individuals genotyped on the Affymetrix 500K chip but not included in the targeted sequencing experiment (Supplementary Materials).
Using results from the discovery analyses, we identified variants that were associated with T2D or obesity-related traits at the p<0.001 level or were located in genes encoding targets of strategic interest to GSK, including GLP1R, DPP4, CNR2, and HTR2C with a p value threshold of <0.05. To maximise sensitivity to detect associations in these genes of highest interest, we took forward to follow up those variants reaching p<0.05 in the discovery analyses. However, this did not affect the threshold for statistical significance or overall alpha value (3.8x10^-5), for which we accounted for all association tests performed in the discovery analyses (N=1331). The principal reason for prioritising specific genes was to ensure a balance between sensitivity for targets of high priority to GSK and to maintain specificity: given that initial replication was performed by de novo large-scale targeted genotyping, we were practically unable to follow up vast numbers of variants. This does not bias the variants selected for follow-up, nor raise the risk of type I error. Indeed, the only variant we determined to be mimicking pharmacological manipulation was well beyond “genome-wide significance” even if all possible low-frequency and common variants in the genome had been tested.

We then genotyped seven variants in six genes in up to 39,979 follow-up participants of European ancestry drawn from multiple studies (Fig. 1): CoLaus (when GEMS was the discovery sample), GEMS (when CoLaus was the discovery set), Ely (44) (n=1722), EPIC-Norfolk (45) (n=25313), Fenland (46) (n=6379), and LOLIPOP (47) (n=6565) studies. The follow-up analysis of T2D included participants from the Norfolk Diabetes Study (Ncases=5587 and Ncontrols=19012), GenOA study (Ncases=129 and Ncontrols=1501), and individuals with T2D from the ADDITION study (48) (Ncases=816) who were combined with additional cases from the Ely study (Ncases=116) and compared to non-diabetic controls from the Ely study (Ncontrols=1487).

We also sought further in silico follow-up Analysis to further evaluate associations in collaborative studies utilizing results from the MAGIC and CHARGE consortia. Five of the seven variants were available for in silico analysis (table 1). Further details on each of the studies and consortia are provided in the Supplementary Materials and table S1 and S4.
**Statistical analyses**

We carried out genetic association analyses on variants identified via targeted sequencing using an additive genetic model by linear or logistic regression, adjusting for age and sex and other study-specific covariates. We combined study-specific estimates using fixed effect meta-analysis. We performed analyses on standardised variables (mean=0 and SD =1) and, as such, expressed effect sizes as standard deviations (SDs) for quantitative traits. In total, we analysed 121 single nucleotide variants (SNVs). Overall, we performed 1,331 tests of association in the discovery analyses and, as such, associations that were \( \alpha < 3.8 \times 10^{-5} \) in the combined analysis were deemed to be statistically significant.

We performed targeted genotyping of selected variants from discovery analyses using Sequenom for the Ely, EPIC-Norfolk, Fenland, and ADDITION studies and KASPar for the LOLLIPOP study. Imputed data were also available in the GenOA study using reference haplotypes from participants in the previous sequencing study (8). We carried out genetic association analyses in each study under an additive genetic model using linear or logistic regression, again adjusting for age, sex and study-specific covariates. We sought further *in silico* follow-up from summary association results from MAGIC and CHARGE consortia (table 1). We converted summary association result effect sizes to SDs using the SD of fasting glucose from the population-based Fenland study (SD = 0.65 mM) (46). We meta-analyzed results from the discovery analysis, follow-up analysis and *in silico* follow-up analysis using a fixed effect, inverse-variance weighted approach. The discovery analysis of the CoLaus study included association results from the sequence variants and imputed variants (table 1). In the entire CoLaus study, we later directly genotyped (KASPar technology) variants which had been imputed in the unsequenced CoLaus participants study as part of the original follow-up analysis. The combined analysis results in table 1 therefore represent those from the directly genotyped data.
For variants that showed statistically significant associations in the combined analysis ($p<3.8 \times 10^{-5}$), we investigated their association with a range of anthropometric, metabolic, and cardiovascular risk factors and disease outcomes in the studies described previously, as well as in additional studies described in table S1 and S4 and in Supplementary Materials. We also investigated the association of variants reaching statistical significance after follow up ($\alpha<3.8 \times 10^{-5}$) with coronary heart disease (CHD) through targeted genotyping and collaboration with large-scale Exome chip consortia (table S1). For these variants, we also investigated association with a range of other disease outcomes (table S1), with a particular focus on diseases previously suggested as potential opportunities for repositioning (i.e. where existing drugs might be used for alternative indications). However, as the variant reaching statistical significance was not well covered on existing GWAS arrays or in HapMap, we were limited to those disease outcomes for which we could obtain association data. For genes that contained variants with $p<3.8 \times 10^{-5}$ in the combined analysis, we investigated the presence of putative LoF alleles in individuals in whom we had performed targeted sequencing (8) and in individuals with whole-genome sequencing from the UK10K study (www.uk10k.org).

**Comparison of clinical trial effects and genetic associations**

Randomized clinical trials of GLP1R-agonists were identified through previous systematic reviews and by performing a supplementary literature search, as detailed in the Supplementary Materials. Only trials with placebo or no-drug comparison groups (i.e. no trials with active comparison groups), with $\geq 4$ weeks drug treatment (i.e. no single dose studies) and $\geq 10$ participants per trial arm were included. Treatment effects were expressed in SDs before pooling across trials using random effects meta-analysis (see table S3 for details of clinical trials included). P-values derived from Cochrane’s Q test were used as a guide to assess whether there were pairwise differences between the rescaled genetic and trial estimates.
Table 1. Discovery, follow-up, and combined results for variants taken forward to follow-up. Seven variants in six genes reached p<0.001 (or p<0.05 in target of interest to GSK) in sequence-based discovery analyses (Fig. 1) and were taken forward to follow-up in additional samples, by targeted genotyping and by in silico lookup from existing consortia. Data and P values are from fixed effect meta-analysis of linear regression for quantitative traits or logistic regression for binary disease status.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Chr</th>
<th>Position (NCBI b37 genome alignment)</th>
<th>Consequence</th>
<th>Trait</th>
<th>Effect allele</th>
<th>Other allele</th>
<th>MAF</th>
<th>Stage</th>
<th>Study</th>
<th>n (case/control for binary trait)</th>
<th>Beta (Odds ratio for binary trait)</th>
<th>se [CI for OR]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP1R</td>
<td>rs10305492</td>
<td>6</td>
<td>39046794</td>
<td>A316T</td>
<td>Fasting glucose</td>
<td>A</td>
<td>G</td>
<td>0.015</td>
<td>Discovery</td>
<td>Sequenced CoLaus*</td>
<td>1869</td>
<td>-0.28</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Targeted follow-up</td>
<td></td>
<td></td>
<td></td>
<td>Additional CoLaus,ELY,LOLIPOP, GEMS MAGIC (29)</td>
<td>18,937</td>
<td>-0.13</td>
<td>0.04</td>
<td>1.5x10⁻³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In silico follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20,077</td>
<td>-0.16</td>
<td>0.03</td>
<td>1.1x10⁻⁷</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40,883</td>
<td>-0.15</td>
<td>0.02</td>
<td>2.6x10⁻¹⁰</td>
<td></td>
</tr>
<tr>
<td>DPP4</td>
<td>rs56179129</td>
<td>2</td>
<td>162890142</td>
<td>V266I</td>
<td>Fasting glucose</td>
<td>T</td>
<td>C</td>
<td>0.008</td>
<td>Discovery</td>
<td>GEMS</td>
<td>1416</td>
<td>0.61</td>
<td>0.21</td>
<td>3.6x10⁻³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Targeted follow-up</td>
<td></td>
<td></td>
<td></td>
<td>In silico follow-up</td>
<td>CoLaus,ELY,LOLIPOP</td>
<td>12934</td>
<td>0.00</td>
<td>0.07</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49838</td>
<td>0.00</td>
<td>0.03</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64188</td>
<td>0.01</td>
<td>0.03</td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC5A1</td>
<td>rs200410750</td>
<td>22</td>
<td>32439209</td>
<td>5’ UTR</td>
<td>Fasting Glucose</td>
<td>T</td>
<td>C</td>
<td>0.001</td>
<td>Discovery</td>
<td>Sequenced and imputed CoLaus</td>
<td>5210</td>
<td>1.44</td>
<td>0.33</td>
<td>1.7x10⁻⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Targeted follow-up</td>
<td></td>
<td></td>
<td></td>
<td>In silico follow-up</td>
<td>ELY,Fenland,LOLIPOP</td>
<td>12707</td>
<td>-0.16</td>
<td>0.27</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n/a</td>
<td>0.01</td>
<td>0.03</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>CNR2</td>
<td>rs4649124</td>
<td>1</td>
<td>24201357</td>
<td>Synonymous</td>
<td>2 hour glucose</td>
<td>A</td>
<td>G</td>
<td>0.420</td>
<td>Discovery</td>
<td>Sequenced and imputed CoLaus</td>
<td>505</td>
<td>0.18</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Targeted follow-up</td>
<td></td>
<td></td>
<td></td>
<td>In silico follow-up</td>
<td>ELY,Fenland</td>
<td>6377</td>
<td>0.00</td>
<td>0.02</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15234</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22106</td>
<td>0.00</td>
<td>0.01</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNR2</td>
<td>rs2229579</td>
<td>1</td>
<td>24201162</td>
<td>H316Y</td>
<td>T2D</td>
<td>T</td>
<td>C</td>
<td>0.110</td>
<td>Discovery</td>
<td>Sequenced and imputed CoLaus</td>
<td>385/5241</td>
<td>0.73</td>
<td>[0.55,0.97]</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Targeted follow-up</td>
<td></td>
<td></td>
<td></td>
<td>In silico follow-up</td>
<td>ADDITION-Ely, NDS,LOLIPOP,GenOA</td>
<td>7141/27096</td>
<td>1.06</td>
<td>[0.99, 1.14]</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9524/60718</td>
<td>0.96</td>
<td>[0.90, 1.01]</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17047/93225</td>
<td>0.99</td>
<td>[0.95, 1.04]</td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>rs ID</td>
<td>Position</td>
<td>Trait</td>
<td>Effect</td>
<td>p Value</td>
<td>Sample Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>----------</td>
<td>-------</td>
<td>--------</td>
<td>----------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTR2C</td>
<td>rs56372597</td>
<td>X 113951968 Intronic</td>
<td>BMI</td>
<td>A &gt; G</td>
<td>0.150</td>
<td>Discovery: 10798</td>
<td>Targeted follow-up: 36983</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Additional CoLaus, ELY, EPIC, Fenland, LOUPOP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In silico follow-up: n/a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined: 47781</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHARGE adiposity Exome chip working group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCHR1</td>
<td>rs117372135</td>
<td>22 41075523 T25M</td>
<td>BMI</td>
<td>T &gt; C</td>
<td>0.002</td>
<td>Discovery: 10952</td>
<td>Targeted follow-up: 37240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Additional CoLaus, ELY, EPIC, Fenland, LOUPOP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In silico follow-up: 68978</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined: 117170</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Analyzed in sequenced CoLaus participants only owing to low imputation quality (R² < 0.5) in additional CoLaus participants at the discovery stage. #Not analyzed in GEMS due to low number of carriers (< 5 minor alleles)
Figure legends

Figure 1. Overall study design, participating studies, and consortia. Discovery analyses were performed using targeted exome sequencing of variation in six genes tested for association with seven traits. Variants were taken forward to follow-up by targeted genotyping. Additional *in silico* results were obtained using available association results. Combined results were obtained by fixed-effect meta-analysis of estimates from linear or logistic regression, as appropriate. Based on the 1331 statistical tests performed in discovery analyses, \( p < 3.8 \times 10^{-5} \) was used as the threshold for statistical significance. In targeted genotyping, (g) refers to studies that were directly genotyped for relevant markers, whereas (i) indicates those in which relevant variants were captured by imputation.

Figure 2. Association of *GLP1R* variant (rs10305492) with glycaemic traits. (A) Genetic variant association with glycaemic traits. Data are standard deviations per minor allele at rs10305492. Fasting glucose results are from the combined analysis (Table 1). Individual studies contributing to the associations for fasting insulin and 2-h glucose are in table S4. All results reflect point estimates and 95% confidence intervals (CI) from a fixed-effect meta-analysis of linear regression estimates. (B) Effect size of the *GLP1R* variant (in red) and loci previously reported to be associated with fasting glucose. Effect sizes are reported from discovery analyses of available MAGIC results (50), and from the combined estimate for the *GLP1R* variant in (A).

Figure 3. Comparison of *GLP1R* variant (rs10305492) associations with effects observed in clinical trials of GLP1R agonists in non-diabetic individuals and in individuals with T2D. Genetic associations are all scaled to match the effects of GLP1R-agonists on fasting glucose (i.e. per 3.3 copies of the minor (A) allele). Genetic variant results are beta estimates and 95% confidence intervals from fixed effect meta-analysis of linear regression results. Trial results are estimates from fixed-effect meta-analyses of standardised mean differences between treatment and comparison groups of the individual trials listed in table S3. *Trials reported effects on body mass, whereas genetic associations were only available for BMI.

Figure 4. Association of *GLP1R* variant (rs10305492) with disease outcomes. Association with disease outcomes are reported per-minor allele at rs10305492. Data show odds ratios and 95% confidence intervals from logistic regression models.

Supplementary materials

Figure S1. Effects of GLP1R agonists on body weight.
Figure S2. Effects of GLP1R-agonists on 2h glucose.
Table S1. Study characteristics for disease traits.
Table S2. Comparison of heterogeneity between trial and rescaled genetic estimates.
Table S3. Details of randomized trials contributing to analyses of GLP1R-agonist effects included in Fig. 2.
Table S4. Study characteristics for quantitative traits.
References and notes


35. L. A. Scrocchi, T. J. Brown, N. MacLusky, P. L. Brubaker, A. B. Auerbach, A. L. Joyner, D. J. Drucker,


95. J. B. Buse, R. M. Bergenstal, L. C. Glass, C. R. Heilmann, M. S. Lewis, A. Y. M. Kwan, B. J. Hoogwerf,


Acknowledgments: We are grateful for the contributions of the studies and consortia which provided summary results. For a complete list of Acknowledgements, see Supplementary Materials.

Funding: The work described in this manuscript was funded, in part, by GSK, and, in part, by the Medical Research Council (MC_UU_12015/1). CHARGE AD analyses were funded by R01 AG033193 (PI Seshadri). ADGC AD analyses were funded by U01AG032984 (PI Schellenberg). Additional funding sources are outlined in the Supplementary Materials. Author contributions: RAS, MGE, NJW, DMW conceived and designed the study. RAS, DFF, LL, AYC, PS, RY, NG, AS, YC, TVV, HY, JAL, JHZ, SMW, JW, SW, NM, KM, AP, SlvdL, CG, AAAO, PA, LA, DA, IAO, BB, AB, IB, SBG, JCB, SB, MB, HB, EB, IBB, JBI, SB, CC, MC, LAC, CC, JC, MdH, JAD, HME, GBE, EF, JF, TF, IF, NGF, FG, CG, SG, LH, JHJ, MEJ, JWJ, RK, FK, NDK, TJK, JK, ZKJ, ATK, KK, JK, AL, CL, GM, KLM, APM, KM, MMN, PBM, CN, SFN, PMN, BGN, CJP, DP, SP, GMP, MP, AP, CJP, JRE, OR, CS, VS, MJ, NS, SJS, RS, NS, JAS, DJT, ST, RT, Dlvda, YTvdS, JV, MW, KW, JEA, LTA, MLA, ASB, JC, JD, DFE, RA, JE, PWF, TMF, TH, JMMH, TJ, JK, ML, CL, MIM, JSP, OP, BR, ER, JIR, DS, NJS, HS, PV, SOR, PD, JD, MOG, SK, JBM, MGE, NJW, DMW participated in the acquisition and/or analysis of data. LL, PS, JAL, JHZ, SJS and JMMH performed analyses and/or provided statistical guidance. IB, SB, MB, HB, EB, IBB, LAC, KLM, PBM, SJS, MW, LTA, JD, DFE, ML, CL, MIM, JSP, PV, SOR, PD, JD, MOG, SK, JBM, MGE, NJW, DMW supervised analyses. RAS, DFF, LL, MGE, NJW, DMW wrote the manuscript, with input from all authors. Competing interests: J.L. A, M.G. E, L. L, and D.M. W are GSK stockholders. All named authors are solely responsible for the content of the manuscript. The current manuscript was a collaborative effort between academic and GSK scientists and analyses focused on a subset of genes included in the original sequencing paper (8), which focused on target genes of interest to GlaxoSmithKline. Since October 2015, D.F. has been a full time employee of Bayer AG, Germany. Data and materials availability: Data on glycemic traits were contributed by MAGIC investigators and were downloaded from www.magicinvestigators.org. Associations with T2D in a GWAS of Europeans were obtained from The DIAGRAM (DIAbetes Genetics Replication And Meta-analysis) investigators (http://diagram-consortium.org/downloads.html). The authors would like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at http://exac.broadinstitute.org/about. Additional materials can be obtained by request to the corresponding authors and may be subject to MTA requirements.