Pharmacogenomics of GLP-1 receptor agonists: a genome-wide analysis of observational data and large randomised controlled trials

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Summary

Background In the treatment of type 2 diabetes, GLP-1 receptor agonists lower blood glucose concentrations, body weight, and have cardiovascular benefits. The efficacy and side effects of GLP-1 receptor agonists vary between people. Human pharmacogenomic studies of this inter-individual variation can provide both biological insight into drug action and provide biomarkers to inform clinical decision making. We therefore aimed to identify genetic variants associated with glycaemic response to GLP-1 receptor agonist treatment.

Methods In this genome-wide analysis we included adults (aged ≥18 years) with type 2 diabetes treated with GLP-1 receptor agonists with baseline HbA_{1c} of 7% or more (53 mmol/mol) from four prospective observational cohorts (DIRECT, PRIBA, PROMASTER, and GoDARTS) and two randomised clinical trials (HARMONY phase 3 and AWARD). The primary endpoint was HbA_{1c} reduction at 6 months after starting GLP-1 receptor agonists. We evaluated variants in *GLP1R*, then did a genome-wide association study and gene-based burden tests.

Findings 4571 adults were included in our analysis, of these, 3339 (73%) were White European, 449 (10%) Hispanic, 312 (7%) American Indian or Alaskan Native, and 471 (10%) were other, and around 2140 (47%) of the participants were women. Variation in HbA_{1c} reduction with GLP-1 receptor agonists treatment was associated with rs6923761G→A (Gly168Ser) in the *GLP1R* (0·08% [95% CI 0·04–0·12] or 0·9 mmol/mol lower reduction in HbA_{1c} per serine, $p=6·0\times10^{-5}$) and low frequency variants in *ARRB1* (optimal sequence kernel association test $p=6·7\times10^{-8}$), largely driven by rs140226575G→A (Thr370Met; 0·25% [SE 0·06] or 2·7 mmol/mol [SE 0·7] greater HbA_{1c} reduction per methionine, $p=5·2\times10^{-6}$). A similar effect size for the *ARRB1* Thr370Met was seen in Hispanic and American Indian or Alaska Native populations who have a higher frequency of this variant (6–11%) than in White European populations. Combining these two genes identified 4% of the population who had a 30% greater reduction in HbA_{1c} than the 9% of the population with the worse response.

Interpretation This genome-wide pharmacogenomic study of GLP-1 receptor agonists provides novel biological and clinical insights. Clinically, when genotype is routinely available at the point of prescribing, individuals with *ARRB1* variants might benefit from earlier initiation of GLP-1 receptor agonists.

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Introduction

In the American Diabetes Association–European Association for the Study of Diabetes guidelines, GLP-1 receptor agonists are recommended as a second-line and third-line therapy option in people with type 2 diabetes across the treatment continuum. These include those at high risk of or with established atherosclerotic cardiovascular disease, chronic kidney disease, or heart failure and in patients without these comorbidities who have a compelling need to reduce the risk of hypoglycaemia, minimise weight gain, or promote weight loss.[1] There is considerable heterogeneity in how well patients respond to GLP-1 receptor agonists treatment, with some individuals having a marked response and others having no improvement. However, the mechanisms for this variation are uncertain.

Glycaemic response to metformin and sulphonylureas treatment has been established to be heritable, with 34–37% of the variance explained by common genetic variants.[2,3] Pharmacogenomic studies have the potential to reveal novel insights into drug action, reflecting variation in underlying pharmacokinetics (eg, *SLCO1B1* or *SLCO1B3* variation for sulphonylureas),[2] pharmacodynamic variation due to differences in the drug target and downstream mechanisms, or more generally due to variation in the cause of diabetes (eg, *ATM*, *NPAT*, or *SLC2A2* variation for metformin).[4,5] We hypothesise that pharmacogenomic studies of GLP-1 receptor agonists would provide similar insights into their mechanism of action and have the potential to identify variants that could be clinically informative for treatment decisions.

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Research in context

Evidence before this study

Treatment with GLP-1 receptor agonists are preferred in people with type 2 diabetes who are at high risk of cardiovascular and kidney disease. It is also used in people who do not have these comorbidities but have a compelling need to reduce the risk of hypoglycaemia, minimise weight gain, or promote weight loss. However, there is considerable heterogeneity in how well patients respond to GLP-1 receptor agonist treatment, with some individuals having a marked response and others achieving no improvement. Additionally, the biological mechanisms for this variation are uncertain. We searched PubMed for genome-wide studies assessing efficacy of GLP-1 receptor agonists with the search terms "GLP-1RA", "Exenatide", "Liraglutide", "Dulaglutide", "Albiglutide", "Lixisenatide", "Semaglutide", "genome wide association", "GWAS", "HbA1c", and "glucose" for articles published from database inception up to Jan 1, 2022, in English. We found no genome-wide studies undertaken on the pharmacogenomics of GLP-1 receptor agonists. In this study, we used a three step approach (candidate gene, GWAS, and burden test) to identify pharmacogenetic associations using large, randomised trials and prospective observational cohorts.

Added value of this study

This study advances our mechanistic understanding of the genetics of heterogeneity in glycaemic response to GLP-1 receptor agonists. This analysis identified loci in the *GLP-1R* and *ARRB1* that were significantly associated with HbA_{1c} reduction. These identified loci were GLP-1 receptor agonists specific, with no association with HbA_{1c} reduction to other glucose lowering drugs. A combined genotype derived from these two genes identified 4% of the population who had a 30% greater reduction in HbA_{1c} than the 9% of the population with the worst response.

Implications of all the available evidence

We identified known and novel regions that are associated with glycaemic response to GLP-1 receptor agonists at a genome-wide scale for the first time. These findings establish a novel key role for ß-Arrestin1 mechanistically in mediating glucose reduction by GLP-1 receptor agonists. Clinically, when genotype is routinely available at the point of prescribing, individuals with ARRB1 variants might benefit from earlier initiation of GLP-1 receptor agonists.

We therefore studied the effect of common, low frequency, and rare genetic variants on glycaemic response to GLP-1 receptor agonists in 4571 patients with type 2 diabetes as part of the Innovative Medicines Initiative funded Diabetes Research on Patient Stratification (DIRECT) Consortium, including genome-wide data from two large randomised controlled trials.

Methods

Study design and participants

DIRECT is a pan-European consortium established with the overarching aim to identify and validate biomarkers that address current bottlenecks in diabetes drug development and to develop a stratified medicines approach to the treatment of type 2 diabetes with either existing or novel therapies.[6] In this genome-wide analysis, data were taken from participants identified from National Health Service primary, secondary care, or research databases across 26 centres in the UK on the use of GLP-1 receptor agonists and this data was augmented with clinical trial datasets. Accordingly, we included four prospective observational studies consisting of 1238 participants with type 2 diabetes of European ancestry (DIRECT, Predicting Response to Incretin Based Agents in Type 2 Diabetes [PRIBA], Prospective Cohort MRC ABPI Stratification and Extreme Response Mechanism in Diabetes [PROMASTER], and Genetics of Diabetes Audit and Research in Tayside Scotland [GoDARTS]), and two clinical trial datasets from the HARMONY phase 3 trials (randomised controlled trials [RCTs] of albiglutide; appendix p 5) and AWARD trials (RCTs of dulaglutide; appendix p 7) consisting of 3333 participants (1562 from AWARD and 1771 from HARMONY) randomly assigned to the GLP-1 receptor agonists group. Finally, the GLP-1 receptor agonists group of the HARMONY outcomes data consisting of 3748 participants was included for replication and ethnicity stratified analysis. Eligible participants were adults (aged ≥18 years) with a clinical diagnosis of type 2 diabetes, a baseline HbA_{1c} of 7% or more (53 mmol/mol) and on stable treatment for at least 3 months. Detailed information on each cohort, the inclusion and exclusion criteria and the analysis workflow are provided in the appendix (pp 2–4, 19). All human research was approved by the relevant institutional review boards, and all participants provided written informed consent.

See Online for appendix

Genotyping and imputation

A full description of each genotype dataset and the quality control procedures for each study are available in the appendix (pp 8–10). In short, each dataset underwent initial quality control and imputation. Standard quality control procedures were applied to all the data sets to filter poorly performing genetic markers and samples before imputation. Each study performed imputation to the 1000 Genomes reference panel. Post-imputation, variants with poor imputation quality (<0·3 for common variants and <0·6 for low frequency and rare variants) and monomorphic variants were excluded.

Assessment of glycaemic response to GLP-1 receptor agonists

Glycaemic response to GLP-1 receptor agonists was assessed as the quantitative phenotype of HbA_{1c} reduction between baseline HbA_{1c} and treatment HbA_{1c} while the

patients were maintained on stable treatment. The baseline HbA_{1c} was the closest HbA_{1c} measure within 3 months before starting GLP-1 receptor agonist treatment, and treatment HbA_{1c} was taken as the closest HbA_{1c} measure to 6 months and within 3–9 months after treatment initiation.

Statistical analyses

We first performed a candidate variant analysis for two variants of *GLP1R* (rs6923761G→A [Gly168Ser] and rs10305420C→T [Pro7Leu]). We then performed two genome-wide studies, a genome-wide association study (GWAS) on common variants (minor allele frequency [MAF] ≥5%) and gene-based burden tests on low frequency and rare coding variants (MAF <5%). For all analyses, we used a linear regression model with HbA_{1c} reduction as the dependent variable, adjusted for baseline HbA_{1c}, sex, duration of diabetes, principal components of ancestry, and other study specific covariates (appendix p 11). Baseline HbA_{1c} has been shown as the strongest predictor of glycaemic response in pharmacogenomic studies in diabetes and our aim was to discover variants that altered treatment HbA_{1c} independent of baseline HbA_{1c} and therefore baseline HbA_{1c} was included as a covariate in all the cohorts. Missing data was <5% in our study population and individuals with missing data were therefore exclude.

Candidate variant analyses

Given the role of the GLP1R as the target for GLP-1 receptor agonists, we examined two non-synonymous variants in the *GLP1R* gene, rs6923761G→A (Gly168Ser) and rs10305420C→T (Pro7Leu), which have previously been reported to potentially alter response to GLP-1 receptor agonists.[7–10] The two GLP1R single-nucleotide polymorphisms (SNPs) were extracted from the respective GWAS data of contributing cohorts from DIRECT, PRIBA, PROMASTER, HARMONY phase 3 and AWARD. Both SNPs were either directly genotyped or imputed with imputation score of more than 0·95 and Hardy-Weinberg Equilibrium p>0·05. In this study, we assumed an additive genetic model. Study specific estimates were combined with random-effect meta-analysis using the metaphor package (version 3.8-1) in R.[11] Accounting for the two tests, p≤0·025 was considered as statistically significant. With a mean baseline HbA_{1c} of 8% (64 mmol/mol) and SD of 1% (10 mmol/mol), a sample size of 4571 provides 80% power to detect a genetic variant of 27% MAF responsible for a change in 0·07% (0·8 mmol/mol) at a significance threshold of 0·025.

GWAS

For the meta-GWAS, each respective cohort (DIRECT, PRIBA, PROMASTER, HARMONY phase 3, and AWARD) conducted GWAS under an additive genetic model to assess the role of common variants (MAF≥5%) in glycaemic response to GLP-1 receptor agonists. Each variant was tested for association with GLP-1 receptor agonists related HbA_{1c} reduction within each cohort. A linear regression model was undertaken after adjusting for the covariates mentioned in the appendix (p 11); post-regression quality control of the summary statistics was performed using EasyQC R package (version 23.8).[12] Postregression, variants with an imputation quality score of less than 0·3 or absolute β or standard error of more than 10 were removed. All datasets were meta-analysed using an inverse variance weighted random effect model, implemented in GWAMA (version 2.2.2). Post meta-analysis, variants available in fewer than four studies were excluded. We used the commonly accepted threshold of $5{\cdot}0\times10^{-8}$ for joint p values to determine statistical significance. Cochran's heterogeneity statistic's p value was reported. The CMplot package (version 4.2.0)[13] in R was used to generate Manhattan and quantile-quantile plots. Power calculations are shown in the appendix (p 10).

For more on **GWAMA** see https://github.com/Kyoko-wtnb/mvGWAMA

Rare variant analysis

To understand the impact of less common and rare coding variants on glycaemic response to GLP-1 receptor agonists, we performed gene-based burden and variance-component association tests (sequence kernel association test [SKAT] and optimal SKAT [SKAT-O]). These were designed to improve power for a mixed set of risk and protective variants of lower frequency and rare protein coding variants (MAF<5%) across the DIRECT, PRIBA, HARMONY, and AWARD cohorts. The R package seqMeta (v1.6.7) was used for these analyses. seqMeta computes necessary information to meta analyse gene-based tests for genetic variants in individual studies. The burden test collapsed genetic variants for each gene by means of a count statistic, which is defined by the number of rare alleles in the gene region, and variance-component tests are more powerful when a small percentage of variants are causal, or the effects have mixed directions. Weights of individual variant-score test statistics for SKAT were chosen as proposed by Wu and colleagues.[14] Burden and SKAT tests were performed using a quantitative measure of HbA_{1c} reduction in the linear regression model after adjusting for similar covariates as the common variant analyses. To correct for multiple testing, a $p<3{\cdot}7\times10^{-6}$ (corresponding to 0·05 of 13 654 tested genes) was considered statistically significant. Replication in the *ARRB1* gene was performed using data from the HARMONY outcomes trial. Finally, ethnicity stratified analyses of the association between significant variants and drug response were performed.

For more on **seqMeta** see https://github.com/DavisBrian/seqMeta

Sensitivity analyses

Genetic variants that are significantly associated with HbA_{1c} reduction were also tested for their association with baseline BMI, treated BMI, and BMI reduction

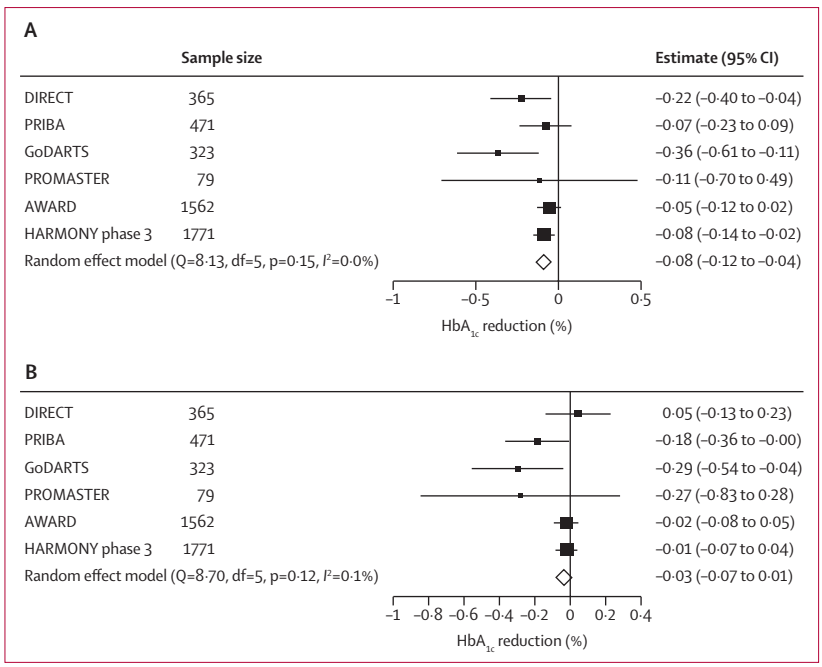

Figure 1: **Forest plot representing meta-analysis of the association between *GLP1R*, Gly168Ser (A) and Pro7Leu (B) and HbA_{1c} reduction observed across DIRECT, PRIBA, GoDARTS, PROMASTER, AWARD, and HARMONY studies**

Effect estimates represent HbA_{1c} reduction (Diabetes Control and Complications Trial) per minor allele. Study sample size is shown.

after 6 months of treatment with GLP-1 receptor agonists using data from the HARMONY phase 3 trials, DIRECT, PRIBA, and PROMASTER (n=3009). Association between identified variants and treatment response to metformin and sulphonylureas has also been investigated using our previously published data from the MetGen and MetGen Plus consortia.[2,5] Additionally, we investigated association between identified variants and HbA_{1c} reduction using data from other comparator groups in the HARMONY trials. Finally, we combined variants in the *GLP1R* and *ARRB1* and this was tested against HbA_{1c} reduction after adjusting for the same covariates as in the GWAS.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

In our analysis we included 4571 individuals with type 2 diabetes with complete data for HbA_{1c} reduction at 6 months and other covariates. Of these, 3339 (73%) were White European, 449 (10%) Hispanic, 312 (7%) American Indian or Alaska Native, and 471 (10%) were of other ethnicity. The baseline characteristics for the four observational studies and RCTs are provided in the appendix (p 12). 2140 (47%)of the participants were women, the mean age range across studies was 54–64 years. Baseline HbA_{1c} varied from a mean 8·14% (SD 1·04; 65·5 mmol/mol) in the AWARD studies to 9·85% (SD 1·60; 84·2 mmol/mol) in PROMASTER. The mean HbA_{1c} reduction at 6 months ranges from 0·75% (1·71; 8·11 mmol/mol) in GoDARTS to 1·38% (1·55; 15·1 mmol/mol) in PRIBA.

We investigated the association between two common missense variants, rs6923761G→A (Gly168Ser, MAF in these cohorts was 0·27) and rs10305420C→T (Pro7Leu, MAF in these cohorts was 0·32), and HbA_{1c} reduction after GLP-1 receptor agonists treatment. In a meta-analysis consisting of 4571 individuals, Gly168Ser was significantly associated with glycaemic response to GLP-1 receptor agonists. Each copy of the serine allele was associated with a smaller HbA_{1c} reduction of 0·08% (95% CI 0·04–0·12) or 0·9 mmol/mol; $p=6·0\times10^{-5}$, heterogeneity p=0·20). This association was consistent across the constituent datasets of the meta-analysis (figure 1). Stratified analysis by GLP-1 receptor agonists type revealed no difference of the variant with glycaemic response across the different drugs (appendix p 19). There was no association between Pro7Leu and HbA_{1c} reduction (β=–0·03 [–0·07 to 0·01] or 3·3mmol/mol, p=0·11, heterogeneity p=0·13).

The meta-GWAS across four prospective observational cohorts and two trials included 6 228 682 common autosomal variants from 4563 independent individuals treated with GLP-1 receptor agonists (appendix p 20). No inflation of variant test statistics was observed on quantile-quantile plots and estimates of the genomic inflation factor (λ=1·04; appendix p 20). There was no association for any common genetic variant with HbA_{1c} reduction with GLP-1 receptor agonists treatment at the genome-wide level of statistical significance ($p<5·0\times10^{-8}$). However, multiple implicated loci with lead variant and nearest gene at rs61800555 (*LMX1A*), rs2268640 (*GLP1R*), rs7687008 (*LINC00499*), and rs10224036 (*PTPN12*), reached a suggestive genome-wide significance level ($p<1·0\times10^{-5}$; appendix pp 13, 20). Given rs6923761 is in partial linkage disequilibrium with rs2268640 ($r^2=0·46$, D□=0·91), we carried out conditional analysis by including these SNPs in the model together using individual-level data from the DIRECT, PRIBA, PROMASTER, and HARMONY phase 3 studies (n=3009). Although the effect estimate of rs6923761 did not change after conditioning (β conditioned=–0·11 [–1·1 mmol/mol], $p=6·12\times10^{-4}$), the association between rs2268640 and glycaemic response to GLP-1 receptor agonists is diminished (β=–0·02 [–0·3 mmol/mol], p=0·031).

In the gene based meta-analysis there was one gene, β-arrestin1 (*ARRB1*), for which the burden of low frequency and rare coding genetic variants was associated with HbA_{1c} reduction (cumulative MAF=2·4%, burden $p=1·1\times10^{-7}$, SKAT-O $p=6·7\times10^{-8}$; inverse variance weighted meta-analysis $p<3·7\times10^{-6}$, a Bonferroni correction for 13 654 genes; figure 2; appendix p 14).

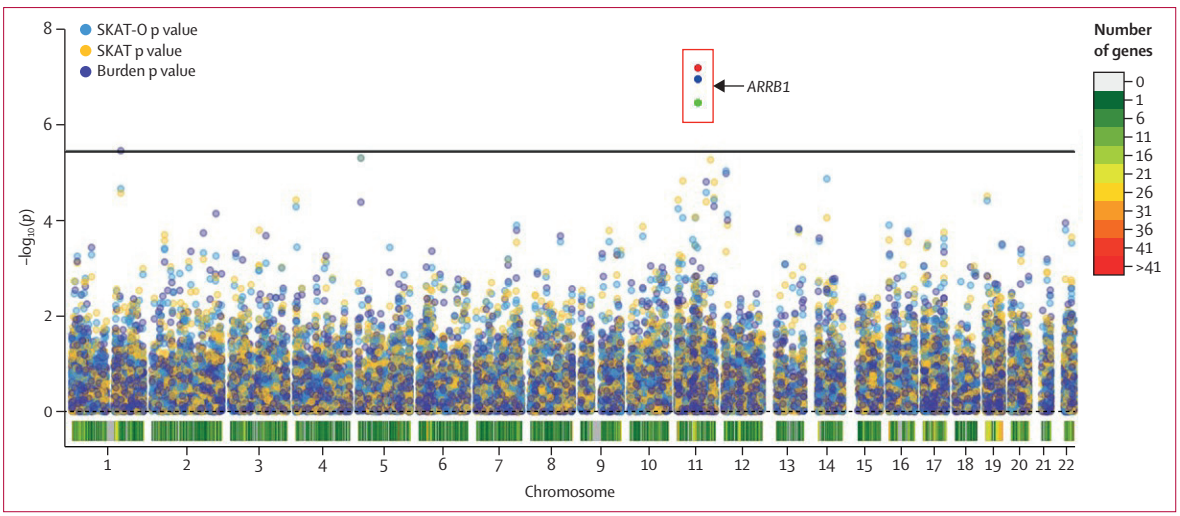

Figure 2: **Gene-level association of low frequency and rare coding variants (MAF<5%) using the burden, SKAT, and SKAT-O association tests**
Results of association with HbA_{1c} reduction are shown for 13 654 genes, adjusting for baseline HbA_{1c}, age, sex, baseline BMI, and first three principal components. Genes with $p<3{\cdot}66\times10^{-6}$ are annotated. MAF=minor allele frequency. SKAT=sequence kernel association test. SKAT-O=optimal sequence kernel association test.

Four variants, rs140226575G→A (Thr370Met, MAF=2·2%), rs78979036G→A (Thr275Ile, MAF=0·06%), rs58428187T→C (Ile158Val, MAF=0·03%), and rs78052828T→C (Gly411Ser, MAF=0·08%), contributed to the observed association. Compared with individuals without the rare alleles, carriers of one or more rare alleles showed greater reduction in HbA_{1c} (1·09% [SE 0·01] or 11·9 mmol/mol [SE 0·2] *vs* 1·29% [0·06] or 14·04 mmol/mol [0·7], p<0·0001; figure 3). This association was mainly driven by a low frequency coding variant, rs140226575 (Thr370Met), MAF=2·2%, β per allele=0·25% (0·06) or 2·7 mmol/mol (0·7), $p=5{\cdot}2\times10^{-6}$. This association is further replicated in the recently acquired data using the GLP-1 receptor agonists group data of the HARMONY outcomes trial data (β per allele=0·14% [0·04] or 1·5 mmol/mol [0·4], $p=2{\cdot}3\times10^{-4}$). This analysis was carried out to validate the finding in an independent dataset. The frequency of rs140226575G→A (Thr370Met) is high in Hispanics (MAF 6%), and American Indians or Alaska Natives (MAF 11%), compared with White Europeans (MAF 0·5%).[15] We therefore performed race stratified analysis and this revealed a consistent association between rs140226575G→A (Thr370Met) and glycaemic response to GLP-1 receptor agonists with per A allele effect size of 0·18% (0·05) or 2·0 mmol/mol (0·5) in White Europeans, 0·17% (0·05) or 1·8 mmol/mol (0·5) in Hispanics, and 0·11% (0·05) or 1·2 mmol/mol (0·5) in American Indians or Alaska Natives (appendix p 21).

To assess whether genetic variants in the *GLP1R* and *ARRB1* that are associated with glycaemic response to GLP-1 receptor agonists mark a general ability to respond to any other glucose lowering drugs, we examined association between the above variants and HbA_{1c} reduction after treatment with metformin (n=11 933) or sulphonylureas (n=5 479). Additionally, we analysed comparator arms from the HARMONY phase 3 trials for pioglitazone (n=191), placebo (n=315), glimepiride (n=207), or insulin (n=187). Neither the *GLP1R* nor *ARRB1* variants were associated with glycaemic response to any of the non GLP-1 receptor agonists treatment drugs including placebo (appendix p 15). These data suggests a specific role of these variants on glycaemic response to GLP-1 receptor agonists, rather than a general effect.

Given treatment with GLP-1 receptor agonists is associated with weight loss, we evaluated the association between variants in the *GLP1R* (Gly168Ser) and *ARRB1* with baseline BMI, treated BMI and BMI reduction after 6 months of treatment. None of the variants showed significant association with baseline BMI, treated BMI or weight loss however we saw a mean weight loss of 3·27 kg in our study population (appendix p 16).

To evaluate the clinical relevance of *GLP1R* and *ARRB1* in glycaemic response to GLP-1 receptor agonists treatment, we categorised individuals based on their genotype at *GLP1R* (Gly168Ser) and *ARRB1* (Thr370Met, Thr275Ile, Ile158Val, and Gly411Ser) by aligning the alleles with the same direction of effect together (figure 4). These groups were then compared using least-square means by taking the least responding group as a reference. The 4% of the study population who carry one or more variant alleles in *ARRB1* and no Ser allele at Gly168Ser had a mean HbA_{1c} reduction of 1·3% (standard error of the mean [SEM] 0·07; 14·2 mmol/mol [SEM 0.8]) in response to GLP-1 receptor agonists treatment. In contrast, 9% of the population who carry no variant allele at *ARRB1* and two serine alleles at position 168 of the *GLP1R* had a mean HbA_{1c} reduction of 1% (0·05; 11·0 mmol/mol [0·5]) a difference of 0·3% (3·2 mmol/mol; p<0·0001). Those who had no variant allele at *ARRB1* and no Ser allele at GLP1R (53%) had a mean HbA_{1c} reduction of 1·1% (0·02; 12·4 mmol/mol

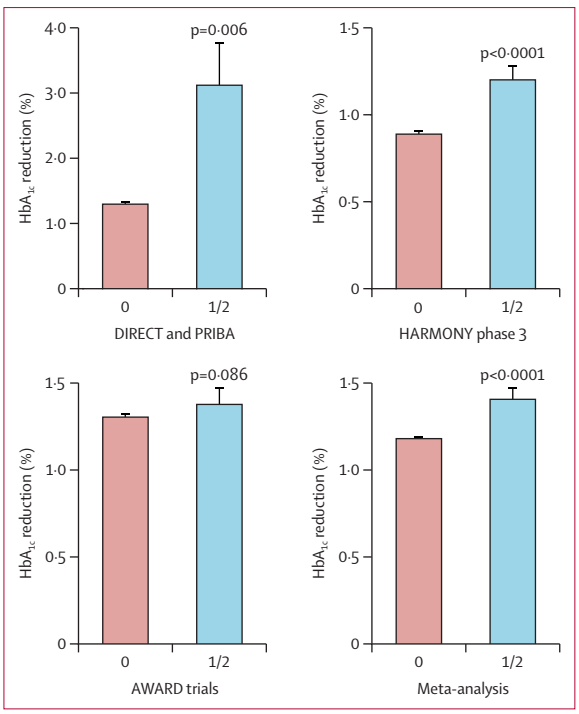

Figure 3: **Bar plot of HbA_{1c} post GLP-1 receptor agonists therapy stratified by *ARRB1* variants Thr370Met, Thr275Ile, Ile158Val, and Gly411Ser**
0 indicates wild type at all the single-nucleotide polymorphisms and 1/2 indicates those who carry one or more variant alleles at *ARRB1*.

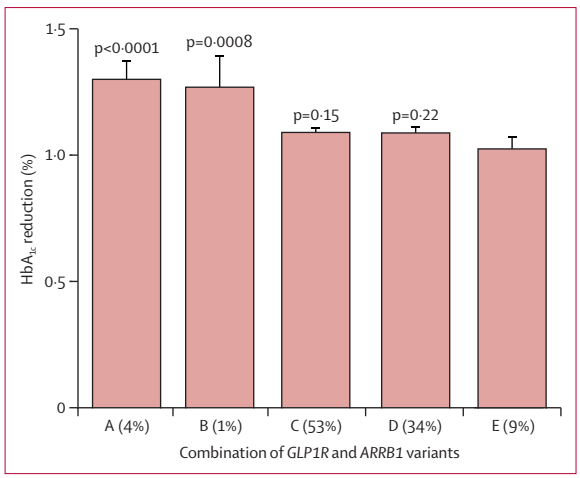

Figure 4: **Bar plot of HbA_{1c} with GLP-1 receptor agonists therapy stratified by the genetic combination from *GLP1R* (Gly168Ser) and *ARRB1* single-nucleotide polymorphisms (Thr370Met, Thr275Ile, Ile158Val, and Gly411Ser)**
A indicates no serine allele at Gly168Ser and more than one *ARRB1* variant allele. B indicates one serine allele at Gly168Ser and more than one *ARRB1* variant allele. C indicates wild type at both *GLP1R* and *ARRB1*. D indicates one serine allele at *GLP1R* and wild type at *ARRB1*. E indicates two variant alleles at *GLP1R* and wild type at *ARRB1*. Percentages next to A to E indicate the proportion of the study population that carries this combination. Bars represent mean HbA_{1c} reduction and error bars represent standard error around the mean. Comparisons are made by taking the least responding group (E) as a reference.

[0·2]; figure 4). The A allele at rs6932671 was shown to be associated with lower fasting glucose (adjusted for BMI; $p=3·3\times10^{-7}$; appendix p 17). We also investigated the genomic region around these two SNPs for possible function by looking at conservation and information about histone marks using data curated by Ensembl, release 106.[16] rs140226575 lies within conserved regions and rs6923761 lies on the boundary of regions that are significantly conserved across 91 mammalian species (appendix p 22), evidence that genetic variants in these regions can have functional consequences. Additionally, ChromHMM,[17] applied to chromatin immunoprecipitation followed by sequencing experiments in pancreas tissue, calls both regions as weak enhancers. This information is consistent with these variants having regulatory effects.

Discussion

We report an association of a common protein coding variant, rs6923761 (Gly168Ser), in the *GLP1R*, and low frequency and rare variants in the *ARRB1* with HbA_{1c} reduction after treatment with GLP-1 receptor agonists in 4571 individuals across prospective observational and clinical trial data sets.

Although we recognise that the traditional genome-wide significant threshold was not achieved, the candidate gene was selected because of previous strong evidence of *GLP1R* being involved in GLP-1 receptor agonist response. rs6923761, which has a minor allele frequency of 29% in White Europeans, results in the substitution of serine for glycine at position 168. Our findings for the Gly168Ser variant are supported by previous studies that resulted in this variant being selected as a candidate. This variant has previously been reported to be associated with glycaemic response to DPP4 inhibitors.[7] Additionally, Gly168Ser was shown to be nominally associated with GLP-1 induced insulin secretion in healthy individuals studied by hyperglycaemic clamp, with carriers of the serine allele showing 15% reduction in insulin secretion compared with homozygous carriers of the parent glycine allele.[10] It is interesting that a study found that the Gly168Ser variant does not alter the function of GLP-1R.[18] However, previous reports in transfected Chinese hamster cell lines show that association of the 168Ser variant with reduced cell surface expression of the GLP1R and reduced intracellular calcium mobilisation following GLP-1 stimulation.[19] The worse response seen with the Gly168Ser variant could also be due to reduced gene expression of GLP1R in the pancreas. The worse response seen with the Gly168Ser variant could also be due to reduced gene expression of GLP1R in the pancreas associated with the A allele.[20] In the GWAS, one of the variants suggestive of association was rs2268640, which is also a cis expression quantitative trait loci for the *GLP1R* in the pancreas.

Our finding that *ARRB1* variants are associated with greater glycaemic response to GLP-1 receptor agonists followed a hypothesis-free genome-wide analysis. The β-arrestins represent strong biological candidates for

GLP-1 receptor agonists response, as they have a key role in canonical G protein-coupled receptor (GPCR) internalisation, with β-arrestin recruitment targeting GPCR internalisation via clathrin mediated mechanisms.[21,22] The preference of subcellular localisation of β-arrestin1 might affect GLP1R signal transduction to the nucleus. Unfortunately, the protein structure of ARRB1 Thr370Met region is still unknown. In the most recent study on the C-tail structure of ARRB1, the Thr370Met mutation is shown to be in the proximal region of the C-terminus.[23] Clathrin and AP2, two adaptor proteins which can facilitate GLP1R internalisation were also reported to bind to the C-terminus of ARRB1.[24] Except for the direct and indirect interactions with GLP1R, ARRB1 also interacts with TRAF6 at the C-terminus (amino acids 318–418) and prevents TRAF6 autoubiquitination and oligomerisation.[25] Suppression of TRAF6 expression has been shown to attenuate insulin-mediated inhibition of gluconeogenesis in hepatocytes.[26] Further investigation is warranted to clarify whether the Thr370Met mutation affects TRAF6 binding to ARRB1.

β-arrestin recruitment to GLP1R during acute and sustained agonist exposure has been shown to have opposite effect on insulin secretion—although β-arrestins seemed to have a role for acute stimulation of insulin release, β-cell knockout enhances insulin secretion.[27,28] Our human pharmacogenetic studies resolve much of this uncertainty as they show convincingly that β-arrestin1 clearly has a role in glycaemic response to GLP-1 receptor agonists, but not weight response to GLP-1 receptor agonists pointing to a likely β-cell mechanism. We show the 370Met allele associated with greater HbA_{1c} reduction. Substitution of Thr370 by 370Met has been predicted to have possibly damaging effect on protein function.[29] A reduction in β-arrestin 1 recruitment was shown to retain GLP1R at the plasma membrane and thus produce greater long-term insulin release.[30]

We did find differences in HbA_{1c} reduction by *ARRB1* genotype across cohorts. *ARRB1* variants had greater HbA_{1c} reduction in users of exenatide and liraglutide (DIRECT and PRIBA) followed by albiglutide (HARMONY) and dulaglutide (AWARD). This finding might be due to the fact that different GLP-1R agonists engage different signalling pathways, otherwise termed as biased agonism. Current evidence of bias between cAMP and β-arrestin recruitment on approved GLP-1 receptor agonists is inconclusive.[31–33] Although this mechanism needs further investigation, G protein-biased GLP-1 receptor agonists appear to achieve enhanced insulin release and thus greater glucose lowering efficacy by avoiding GLP-1 receptor desensitisation and down-regulation, partly via reduced β-arrestin recruitment.[34]

In this study, none of the variants were significantly associated with weight loss consistent with the genetic variation altering pancreatic β-cell response to GLP-1 receptor agonists but not the gastric or hypothalamic actions of this drug class. Clinical trials and observational studies have shown a robust association of GLP-1 receptor agonists treatment with weight loss in participants with and without diabetes. Consistent with this finding, we observed a mean weight loss of 3·27 kg in our study population. However, interindividual variation in weight reduction to GLP-1 receptor agonists has been previously reported.[35] Gly168Ser and Pro7Leu have been investigated in relation to GLP-1 receptor agonists induced weight loss. Some but not all studies have reported association of the Gly168Ser variant in *GLP1R* with weight response to GLP-1 receptor agonists treatment in people with type 2 diabetes.[36] In a pilot study involving women with obesity with polycystic ovary syndrome, the Pro7Leu has also been associated with weight lowering potential of liraglutide.[8]

The frequency of the minor allele in rs140226575G→A (Thr370Met), a variant that largely drives the *ARRB1* signal, varies by ethnicity with a frequency of 0·05% in White Europeans, 6% in Hispanics, and 11% in American Indians or Alaska Natives, and is almost monomorphic in populations of African and Asian descent. However, the effect size for associations between rs140226575 and GLP-1 receptor agonists response was similar across ethnicities, which will result in greater population effect of this variant on GLP-1 receptor agonists response among Hispanics and American Indians or Alaska Natives. Given that this effect could have greater importance for clinical care among individuals of specific ancestry, there is a need to undertake well powered, diversity focused, multi-ethnic studies focusing on populations with higher frequency of *ARRB1* variants (eg, Hispanics or American Indians).

For treatment of common complex diseases like type 2 diabetes, it is highly improbable that genetic variants alone will be used to dictate treatment choice, which is in contrast to monogenic diabetes. We know that drug response is variable and influenced by many factors, and that treatment decisions are based on more factors than glucose levels. However, there is often treatment equipoise, with more than one treatment being suitable. We describe 4% of the population in our study who have low frequency variants in *ARRB1* respond 30% better (absolute benefit of 3·2 mmol/mol) to GLP-1 receptor agonists than the 9% of the population who have normal *ARRB1*, but two *GLP1R* variants. Given that the average rate of glycaemic deterioration is 1 mmol/mol per year[37] these 4% of the study population could have 3 years longer before treatment failure, with the potential to use GLP-1 receptor agonists earlier in their treatment regime. Clinically, GLP-1 receptor agonists have benefits beyond glucose lowering, in particular, reduction in cardiovascular morbidity and mortality. It would also be important to explore whether the genetic variants we have identified to alter glycaemic response to GLP-1 receptor agonists also impact on cardiovascular risk, and to undertake genome-wide studies on cardiovascular and renal outcomes with

GLP-1 receptor agonists. This study has limited statistical power to model these outcomes, especially for the rare variants we have identified in *ARRB1*. Moreover, it would be important to investigate if there is an interaction between dose and genotype in larger data sets. A further limitation of our study is the limited availability of data on non-white ethnicities, particularly Asian and African American populations. Given that we have used array based GWAS data and imputation, our study might be limited to capture some deleterious rare variants. Therefore, targeted sequencing of the *GLP-1R* and *ARRB1* genes would be of considerable interest.

In conclusion, to the best of our knowledge, we have undertaken the first large-scale candidate based and genome-wide analysis of glycaemic response to GLP-1 receptor agonists and have identified that variants in genes encoding the GLP-1 receptor and β-arrestin1 alter glycaemic response to this drug class. This analysis provides novel insight into GLP-1 signalling in the pancreatic β-cell. We established that a coding variant previously shown to reduce GLP-1R membrane expression and GLP-1 stimulated insulin secretion in β-cells reduces GLP-1 receptor agonists response. We also provided clarity that, in humans, β-arrestin1 does play a role in glycaemic response to GLP-1 receptor agonists. Finally, we suggest that the genetic effect sizes identified can add to clinical data to identify a subgroup of patients who respond particularly well to GLP-1 receptor agonists supporting early use of these drugs for those individuals, especially in Hispanic and American Indian populations.

Contributors

ERP, AYD, AGJ, TJM, LM'tH, AndM, IP, HR, and MW conceived of and designed the study. AYD, PB, LL, SW, KZ, M-GH, SS, XW, and AB did the data analysis. NRR, AnuM, IF, AGJ, and TJM did the data collection and genotyping. AYD and ERP wrote and prepared the manuscript. All authors were involved in reading and editing the manuscript. AYD and ERP are guarantors of this work. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. AYD and ERP have accessed and verified the data.

Declaration of interests

ERP has received honoraria for speaking from Lilly and Sanofi. AndM received consultancy fees and support from Eli Lilly to attend the International Conference on Advanced Technologies & Treatments for Diabetes, 2022. LL and SW worked for pharmaLex contracted by Eli Lilly. AnuM is an employee of Genentech and shareholder of Roche. HR is stock option holder from Sanofi. IM is employee and shareholder of Eli Lilly and co. PB is employee and shareholder of Eli Lilly. All other authors declare no competing interests.

Data sharing

Summary-level data that underlie the results reported in this article will be available upon request to the corresponding authors.

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