Growth Differentiation Factor 15 as a Novel Biomarker for Metformin

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OBJECTIVE

Metformin is a commonly used glucose-lowering drug. However, apart from glycemic measures, no biomarker for its presence or dose has been identified.

RESEARCH DESIGN AND METHODS

A total of 237 biomarkers were assayed in baseline serum from 8,401 participants (2,317 receiving metformin) in the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial. Regression models were used to identify biomarkers for metformin use.

RESULTS

Growth differentiation factor 15 (GDF15) was strongly linked to metformin, such that the odds of metformin use per SD increase in level varied from 3.73 (95% CI 3.40, 4.09) to 3.94 (95% CI 3.59, 4.33) depending on the other included variables. For the remaining 25 linked biomarkers, the odds ranged from 0.71 to 1.24. A 1.64 ng/mL higher GDF15 level predicted a 188-mg higher metformin dose ($P < 0.0001$).

CONCLUSIONS

GDF15 levels are a biomarker for the use of metformin in people with dysglycemia, and its concentration reflects the dose of metformin.

Metformin is currently the most widely used glucose-lowering agent in the world that effectively lowers glucose levels; reduces incident diabetes (1); modestly reduces weight; and may reduce the occurrence of ischemic heart disease, mortality, and some malignancies (2). Whereas its glucometabolic effects are partially due to activation of the AMP-activated protein kinase (3), some of its other effects may be mediated by novel pathways. To identify nonglycemic biomarkers for such pathways, we screened a large panel of 237 markers, covering major physiological pathways that were assayed in baseline serum samples collected in 8,401 participants (~28% of whom were receiving various doses of metformin) in the recently completed Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial.

RESEARCH DESIGN AND METHODS

The ORIGIN trial recruited 12,537 people with diabetes, impaired glucose tolerance, or impaired fasting glucose levels who had additional cardiovascular (CV) risk factors (4). Prior to randomization, 8,494 participants (68%) provided baseline blood samples that were spun, separated, aliquoted, frozen (within 2 h of collection), and transported to the Population Health Research Institute Biobank in Hamilton, Ontario, Canada, where they were stored in nitrogen vapor–cooled tanks at −160°C. A

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subset of these participants (5,078) provided additional consent for the storage and analysis of genetic material from buffy coat samples.

After the ORIGIN trial was completed, a 1-mL serum aliquot per participant was transported to Myriad RBM Inc. (Austin, TX) for the application of a multiplex platform (Luminex) to quantify 284 biomarkers that prior research had suggested were related to CV disease, diabetes, or other diseases. As previously reported (5), after masked review of the reported results, 237 biomarkers from 8,401 participants were deemed suitable for further analysis.

Genetic Analyses

A subset of 5,078 of these individuals were genotyped on the HumanCore Exome chip (Illumina). Single nucleotide polymorphisms (SNPs) were excluded due to nonascertainment in ≥99% of participants, deviation from Hardy-Weinberg equilibrium ($P < 10^{-6}$), and rare variants (i.e., minor allele frequency <1%). People were excluded who were genetically related, whose genetic and reported sex or ethnicity did not match, and whose ethnicity was poorly represented ($n < 500$). After quality control, the sample comprised 4,147 participants and 284,024 SNPs from two ethnic groups (European and Latin American), representing 82% of participants with genetic data. Quality control steps were performed using PLINK and GCTA (Genome-wide Complex Trait Analysis) (6). Imputation to predict unobserved genotypes using IMPUTE2 Software with 1000 Genomes Project data (7) as the reference panel identified >30 million SNPs, allowing for comprehensive coverage of known genetic variants. SNPs imputed with low certainty were removed (INFO <0.3, as defined by IMPUTE2 Software) (8).

SNPs within 300 kb of the *GDF15* gene that were also available within the publicly available CARDIoGRAM database (www.cardiogramplusc4d.org) were then identified. This database includes summary associations from a meta-analysis of 48 genome-wide association studies conducted mainly in European individuals, and comprises 123,504 control subjects and 60,801 coronary artery disease (CAD) case patients, defined as having myocardial infarction, acute coronary syndrome, chronic stable angina, or coronary stenosis >50%.

The relationship between genetic determinants of growth differentiation factor 15 (GDF15) levels and CAD was then analyzed. β-Coefficients relating each of the GDF15 SNPs with GDF15 levels in the ORIGIN participants were determined using linear regression models with GDF15 levels as the dependent variable (i.e., one model per SNP). These coefficients were estimated by models adjusted for age, sex, metformin use, and the first five principal components that were run in each self-reported ethnic group separately. Next, SNPs with a β-coefficient *P* value $<5 \times 10^{-8}$ were pruned for linkage disequilibrium at a threshold of $r^2 < 0.1$ using the 1000 Genomes Project data (for European individuals) to identify a nonredundant set. Finally, β-coefficients relating each of the identified GDF15 SNPs with CAD were estimated using CARDIoGRAM.

Statistical Analyses

Statistical analyses were conducted using SAS version 9.2 for UNIX (SAS Institute Inc., Cary, NC) or R (version 3.0.1). As reported previously (5), all analyses were done after log transformation of biomarkers that were not normally distributed, and standardization of biomarkers to a mean of 0 and an SD of 1 for those analyzed as continuous variables, and after creating five incremental categories for those analyzed as ordinal variables. The GDF15 biomarker was identified by scrutinizing five different regression models that forced in age, sex, and clinical characteristics linked to the propensity to prescribe metformin (i.e., weight; prior CV disease; prior diabetes; and levels of creatinine, HbA_{1c}, and fasting plasma glucose). These models used forward selection to identify independent biomarker determinants of metformin use from all 237 biomarkers based on a Bonferroni-corrected *P* value for significance of 237/0.05 (i.e., $P = 0.00021$). After GDF15 was identified as a strong determinant of metformin use, these models were then rerun to determine whether the inclusion of the other biomarkers attenuated or amplified the GDF15-metformin relationship. The mean concentration of GDF15 in people receiving different doses of metformin was calculated, and the relationship between metformin dose and the GDF15 level was estimated after accounting for the independent variables noted above.

RESULTS

Clinical characteristics of the 2,317 of 8,401 participants (27.6%) with measured biomarkers who were receiving metformin therapy and the 6,084 participants

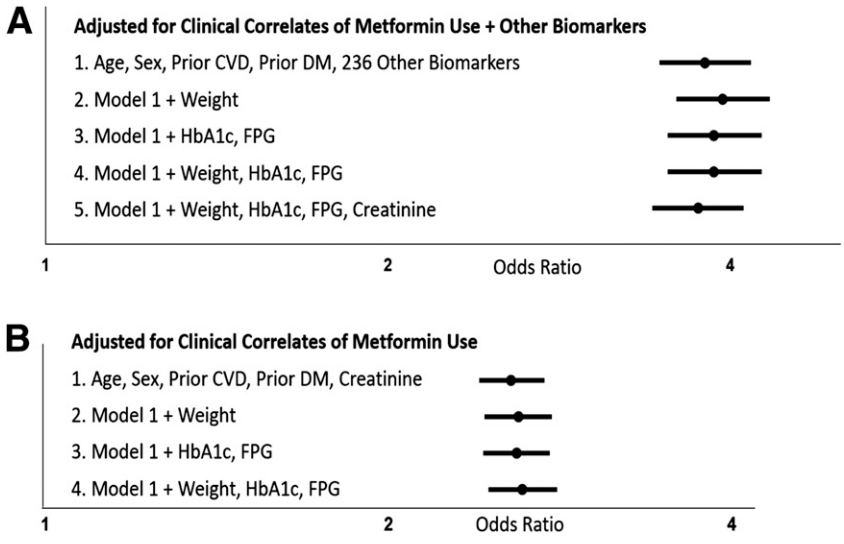

Figure 1—Relationship between GDF15 levels and metformin use. Models 1–5 in panel *A* display the odds of receiving metformin for every 1 SD higher natural logarithm–transformed GDF15 level, after accounting for the clinical factors associated with metformin use plus all of the 236 available biomarkers. Models 1–4 in panel *B* display the odds of receiving metformin for every 1 SD higher natural logarithm–transformed GDF15 level, after accounting for just the clinical factors associated with metformin use. CVD, CV disease; DM, diabetes; FPG, fasting plasma glucose.

(72.4%) who were not receiving metformin therapy at the time that the blood was drawn are reported in Supplementary Table 1. After adjustment, 26 biomarkers were independently associated with metformin use (*P* value for each <0.00021) (Table 1). Except for GDF15, the odds of metformin use for every 1 SD or one-level (for ordinal biomarkers) increase in these biomarker levels ranged from 0.71 to 1.24 (Table 1). Conversely, the odds of metformin use for every 1 SD increase in GDF15 level varied from 3.73 (95% CI 3.40, 4.09) to 3.94 (95% CI 3.59, 4.33), depending on the included variables (Fig. 1*A*). Logistic regression models that included only GDF15 (i.e., none of the other biomarkers) and the clinical variables consistently showed a smaller odds ratio of ~2.6 (Fig. 1*B*).

Mean GDF15 concentrations rose with metformin dose, and mean metformin dose rose with GDF15 concentrations (Supplementary Fig. 1). After accounting for the factors noted above, every 1 SD higher natural logarithm-transformed GDF15 level (equivalent to a back-transformed increment of 1.64 ng/mL) predicted a 188-mg higher metformin dose ($P < 0.0001$). Notably, there was no relationship between GDF15 levels and sulfonylurea use (i.e., the other commonly used glucose-lowering drug).

Adjusted analyses identified 2 SNPs (rs62122430 and rs62122429) shown on the regional plot (Supplementary Fig. 2) that were each independently associated with GDF15 levels in ORIGIN participants ($P < 5 \times 10^{-8}$). One of these SNPs (rs62122429) was inversely related to CAD, and one SNP (rs62122430) had a neutral effect when assessed using data from the CARDIoGRAM database (Supplementary Fig. 3).

Table 1—Independent biomarker determinants of metformin use at baseline

Biomarker[a]	Odds ratio	Lower CI	Upper CI	*P* value
GDF15	3.75	3.42	4.11	<0.01E-19
Epithelial-derived neutrophil-activating protein 78	1.24	1.16	1.33	5.94E-10
Vascular endothelial growth factor receptor 2	1.22	1.13	1.30	4.23E-08
Galectin-3	1.20	1.12	1.29	6.40E-07
Apolipoprotein A-IV	1.2	1.12	1.28	4.87E-08
CD163	1.18	1.10	1.26	5.33E-06
Glucagon-like peptide 1, total[b]	1.17	1.11	1.23	5.57E-10
Peptide YY[b]	1.15	1.09	1.21	4.57E-08
α-2-Macroglobulin	0.89	0.82	0.96	2.21E-03
Insulin	0.88	0.81	0.96	3.01E-03
Visceral adipose tissue–derived serpin A12	0.88	0.83	0.94	1.11E-04
Myoglobin	0.87	0.81	0.94	1.46E-04
α_1-Antitrypsin	0.86	0.80	0.92	1.49E-05
Apolipoprotein D	0.85	0.80	0.91	4.10E-06
Prostasin	0.85	0.79	0.91	9.28E-06
Collagen IV	0.85	0.79	0.90	9.31E-07
Tissue type plasminogen activator	0.84	0.78	0.91	2.38E-06
Neuronal cell adhesion molecule	0.84	0.78	0.90	2.55E-06
Urokinase-type plasminogen activator	0.83	0.77	0.89	7.16E-08
Angiopoietin-2	0.81	0.76	0.87	5.63E-09
Tenascin-C	0.79	0.74	0.85	6.97E-12
Proinsulin, intact[b]	0.78	0.73	0.82	1.97E-19
Fas ligand[b]	0.77	0.68	0.86	2.34E-06
Ferritin	0.76	0.71	0.81	1.67E-16
Osteocalcin	0.75	0.7	0.81	9.18E-15
Tumor necrosis factor receptor 2	0.71	0.665	0.78	5.06E-15

[a]Identified using logistic regression with forward selection after controlling for age; sex; weight; prior CV event; a prior diagnosis of diabetes; and levels of serum creatinine, HbA_{1c}, and fasting plasma glucose. [b]Values are reported per one increment for these biomarkers that were analyzed as ordinal variables and per SD for the other biomarkers that were analyzed as continuous variables.

CONCLUSIONS

This analysis of 237 serum biomarkers has identified GDF15 as a novel biomarker for the use and dose of metformin that may provide important clues about its mechanism of action and effect on health outcomes. The strength of this relationship (i.e., with an odds ratio of ~4) and the stringent statistical criteria used to identify the biomarker ($P < 0.00021$) suggest that it is robust and unlikely to be due to confounding or chance. The fact that the metformin-GDF15 relationship was not attenuated after adjustment for glucose and HbA_{1c} levels (Fig. 1), and was also independent of proinsulin and insulin (Table 1), suggests that the relationship reflects a nonglycemic effect of metformin.

The *GDF15* gene (also called nonsteroidal anti-inflammatory drug–activated gene-1 or NAG-1) is on chromosome 19p12–13.1. GDF15 (also called macrophage inhibitory cytokine-1 or MIC-1, placental bone morphogenetic protein B, and placental transforming growth factor-β) is secreted as a 40-kDa propeptide that is cleaved in the endoplasmic reticulum to release a 25-kDa active circulating dimeric protein (9). Whereas factors controlling GDF15 secretion are unclear, our observed strong link with metformin suggests that metformin activates secretory and/or reduces inhibitory pathways.

Human studies have reported a direct relationship between GDF15 levels and CV outcomes, diabetes, and impaired kidney function. GDF15 levels were a strong independent and validated predictor of death in a Swedish population registry, and GDF15 levels predicted CV outcomes and death in the ORIGIN trial (5). Such a relationship would occur if GDF15 secretion either caused CV outcomes or was secreted in response to CV damage in an attempt to mitigate these outcomes. The latter possibility is analogous to the relationship between leptin and obesity, in which leptin levels are higher in obese patients but leptin suppresses appetite (10), and is supported by our analysis showing that neither of the two independent genetic determinants of higher GDF15 levels are linked to greater CV outcomes, and one is linked to fewer outcomes; the fact that metformin is linked to both higher GDF15 levels and lower CV outcomes

(11); evidence that murine deletion of the *GDF15* gene increases atherosclerosis (12), renal damage (13), and body weight (14); and evidence that the expression of GDF15 suppresses inflammation (12,13).

The availability of GDF15 levels at only one point in time precludes prospective analyses of changes in metformin dose and changes in GDF15 levels, and the relationship between these changes and outcomes. Nevertheless, these GDF15-related observations highlight uncertainty regarding the mechanism of action of metformin and its effect on serious diseases. This is clearly the focus of ongoing research, and the availability of a novel, strong biomarker for metformin provides a very important new tool in this quest.

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Author Contributions. H.C.G. conceived of and analyzed the Outcome Reduction with Initial Glargine Intervention (ORIGIN) biomarker substudies, and wrote the first draft of the manuscript. G.P. conceived of and analyzed the ORIGIN biomarker substudies, and performed the genetic analyses. S.H. conceived of and analyzed the ORIGIN biomarker substudies, and suggested including growth differentiation factor 15 (GDF15) in the biomarker panel. R.J.F. and G.R.S. provided critical insights into the role of GDF15. J.S. and K.R. performed the genetic analyses. M.M. and H.H. conceived of and analyzed the ORIGIN biomarker substudies. S.L. conceived of and analyzed the ORIGIN biomarker substudies, and conducted the biomarker statistical analyses. All authors reviewed and edited the manuscript. H.C.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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