Common Genetic Variation in *GLP1R* and Insulin Secretion in Response to Exogenous GLP-1 in Nondiabetic Subjects

A pilot study

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OBJECTIVE — Glucagon-like peptide (GLP)-1 receptor is encoded by *GLP1R*. The effect of genetic variation at this locus on the response to GLP-1 is unknown. This study assessed the effect of *GLP1R* polymorphisms on insulin secretion in response to hyperglycemia and to infused GLP-1 in nondiabetic subjects.

RESEARCH DESIGN AND METHODS — Eighty-eight healthy individuals (aged 26.3 ± 0.6 years, fasting glucose 4.83 ± 0.04 mmol/l) were studied using a hyperglycemic clamp. GLP-1 was infused for the last 2 h of the study (0.75 pmol/kg/min over 121–180 min, 1.5 pmol/kg/min over 181–240 min). β-Cell responsivity (Φ_{Total}) was measured using a C-peptide minimal model. The effect of 21 tag single nucleotide polymorphisms (SNPs) in *GLP1R* on Φ_{Total} was examined.

RESULTS — Two SNPs (rs6923761 and rs3765467) were nominally associated with altered β-cell responsivity in response to GLP-1 infusion.

CONCLUSIONS — Variation in *GLP1R* may alter insulin secretion in response to exogenous GLP-1. Future studies will determine whether such variation accounts for interindividual differences in response to GLP-1–based therapy.

Diabetes Care 33:2074–2076, 2010

Expression of a nonsynonymous single nucleotide polymorphism (SNP), which results in substitution of methionine for threonine at position 149 of *GLP1R* in cell systems, decreases binding affinity for glucagon-like peptide (GLP)-1 and intracellular signaling after hormone-receptor binding (1). These functional effects suggest that genetic variation in *GLP1R* may alter responsiveness to GLP-1 in vivo. To examine this hypothesis, we used a hyperglycemic clamp, together with GLP-1–amide (7,36) infusion, and measured insulin secretion using a modification of the C-peptide minimal model to determine β-cell responsivity (Φ_{Total}) to GLP-1 in vivo.

RESEARCH DESIGN AND METHODS

— After approval from the Mayo Institutional Review Board, 88 healthy, nondiabetic subjects gave informed written consent to participate (online appendix, available at http://care.diabetesjournals.org/cgi/content/full/dc10-0200/DC1). The study was registered at www.clinicaltrials.gov (NCT00588380).

After an overnight fast, at 0700 (0 min) a primed (0.1 g/kg over 4 min), continuous infusion of 50% dextrose maintained peripheral glucose concentrations at ~8.5 mmol/l. At 0900 (120 min), GLP-1–amide (7,36) (Bachem, San Diego, CA) was infused (1.5 pmol/kg over 10 min, subsequently 0.75 pmol/kg/min). At 1000 (180 min), the infusion rate was increased to 1.5 pmol/kg/min.

Measurement of insulin secretion: C-peptide minimal model

The model used to describe β-cell secretion is a modification of the C-peptide minimal model that assumes a nonlinear and derivative action of GLP-1 on both the static (Φ_s) and dynamic (Φ_d) components of total insulin secretion (Φ_{Total}) (2,3).

Selection and genotyping of tag SNPs for *GLP1R*

Twenty-one tag SNPs were genotyped (online appendix).

Statistical analysis

All data are presented as means ± SEM. Using the Kruskal-Wallis (general allelic model), we assessed univariate associations of genotype with Φ_{Total} in the presence of either glucose alone (mean values at 110–120 min), or glucose and 0.75 pmol/kg/min GLP-1 (170–180 min), or glucose and 1.5 pmol/kg/min GLP-1 (230–240 min), and with peak values of Φ_{Total} observed in the presence of GLP-1. If the *P* value for the overall univariate test of association was <0.1, then the associations for specific genotype pairs (e.g., 1,1 vs. 1,2 or 2,2 vs. 1,1) were also examined using a Mann-Whitney rank sum test. The SAS package was used for analyses, and a *P* value <0.05 was considered to be statistically significant.

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Received 1 February 2010 and accepted 12 May 2010.
C.D.M. and F.M. contributed equally to this work.
DOI: 10.2337/dc10-0200. Clinical trial reg. no. NCT00588380, clinicaltrials.gov.

See accompanying editorial, p. 2123.

RESULTS

Effect of rs6923761 and rs3765467 genotype on β-cell responsivity

Fig. 1*A* shows univariate association of rs6923761 genotype with Φ_{Total} assuming a general genetic model. At 120 min, in the presence of glucose alone, no significant associations with Φ_{Total} were detected (34 ± 3 vs. 35 ± 4 vs. 29 ± 2 min^{-1}, $P = 0.84$) in the 1,1 ($n = 39$) versus the 1,2 ($n = 34$) and 2,2 ($n = 14$) groups, respectively. At 180 min (low-dose GLP-1), the associations with Φ_{Total} also were not statistically significant (104 ± 9 vs. 94 ± 11 vs. 81 ± 8 min^{-1}, $P = 0.11$). The associations with Φ_{Total} at 240 min (high-dose GLP-1) were not significant (152 ± 12 vs. 133 ± 15 vs. 112 ± 10 min^{-1}, $P = 0.10$). There was no association with peak values of Φ_{Total} (160 ± 12 vs. 143 ± 17 vs. 119 ± 10 min^{-1}, $P = 0.09$).

When the effect of rs6923761 genotype on Φ_{Total} was examined (Fig. 1*B*) using a recessive model (i.e., 1,1 vs. individuals with one or more copies of the minor allele), the associations at 240 min (152 ± 12 vs. 127 ± 11 min^{-1}, $P = 0.03$) and at peak Φ_{Total} (160 ± 12 vs. 136 ± 12 min^{-1}, $P = 0.03$) were nominally significant. Differences in Φ_{Total} at 180 min (104 ± 9 vs. 90 ± 8) were not significant ($P = 0.09$).

The three heterozygotes for the minor allele of rs3765467 (Fig. 1*C*) exhibited differences in Φ_{Total} at 120 min prior to GLP-1 infusion (32 ± 2 vs. 73 ± 14 min^{-1}, $P = 0.006$), as well as in response to GLP-1 at 180 min (92 ± 6 vs. 219 ± 35 min^{-1}, $P = 0.005$) and at 240 min (132 ± 7 vs. 325 ± 44 min^{-1}, $P = 0.004$). Nominally significant associations at peak values of Φ_{Total} were also observed (140 ± 8 vs. 332 ± 40 min^{-1}, $P = 0.005$). An ANCOVA adjusting for sex, BMI, and fasting glucose strengthened the association of rs3765467 with peak and 240 min Φ_{Total} ($P = 0.0021$, $P = 0.0026$, respectively). None of the reported *P* values were corrected for multiple testing; applying a Benjamini-Hochberg approach to correct for 21 SNPs and 3 measurements, a *P* value of <0.0024 would be significant (4).

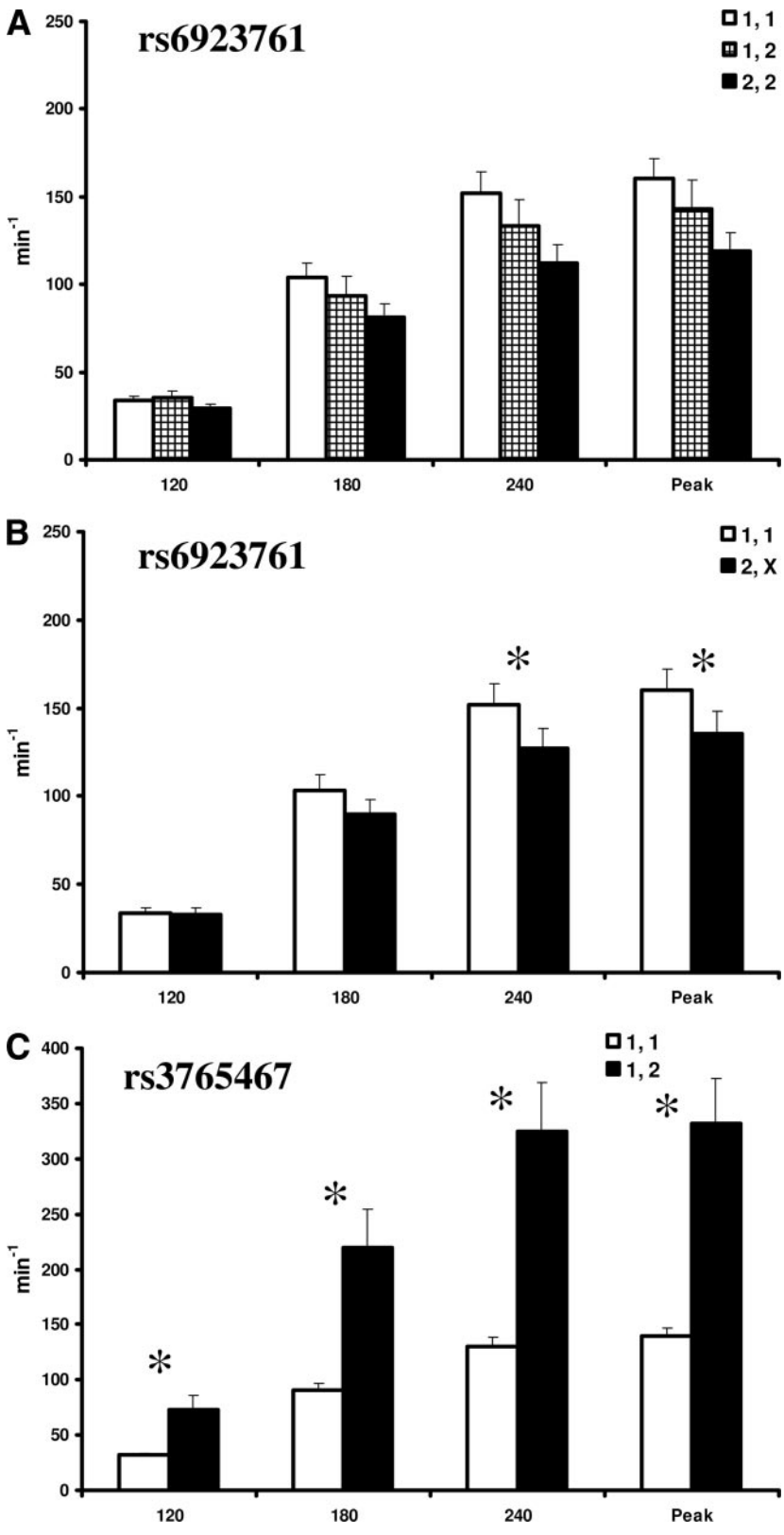

Figure 1—*Effect of rs6923761, analyzed with the general model* (A)*, and with the recessive model* (B)*, and of rs3765467* (C) *on* Φ_{Total} *in the presence and absence of GLP-1. *P < 0.05.*

CONCLUSIONS— In this pilot study, we show that in the presence of hyperglycemia, two nonsynonymous SNPs in *GLP1R* are nominally associated with altered insulin secretory response to infused GLP-1. One of these nonsynonymous SNPs, rs6923761 (which has a minor allele frequency of ~29% in Caucasians), results in the substitution of serine for glycine at position 168 and may decrease responsiveness to infused GLP-1. Homozygotes for the major allele of rs6923761 exhibited a ~15% increase

in mean Φ_{Total} compared with heterozygotes or homozygotes for the minor allele.

The other nonsynonymous SNP, rs3765467, results in substitution of glutamine for arginine at position 131. Heterozygotes for the minor allele of rs3765467 exhibited >100% increase in Φ_{Total} compared with homozygotes for the major allele. However, the observed increase in Φ_{Total} in response to hyperglycemia alone suggests that these observations may not be solely due to altered responsiveness to endogenous GLP-1.

The actions of GLP-1 (primarily stimulation of insulin secretion and suppression of glucagon secretion) are mediated by binding to its cognate receptor. Exenatide, a GLP-1 receptor agonist, binds to the GLP-1 receptor with greater affinity than its natural ligand due to a nine amino-acid COOH-terminal sequence that is absent in native GLP-1 (5). Substitution of glycine for alanine at position eight of native GLP-1 decreases affinity for the receptor (6), suggesting that both N- and COOH-terminal ends of GLP-1 bind the receptor.

A consequence of the constant glucose concentrations during the experiment was that Φ_d was a small component of Φ_{Total}; prior studies have suggested that incretins alter both static and dynamic components of β-cell responses (7,8). GLP-1 also inhibits gastrointestinal motility and may alter glucose delivery to the small intestine (9). By design, our experiment could not test for any potential effects of variation in *GLP1R* on these parameters. At present, variation in *GLP1R* has not been associated with type 2 diabetes (10); together with the data in our study, this suggests that genetic differences in GLP-1 responsiveness attributable to variation in *GLP1R* likely occurs at supraphysiologic GLP-1 concentrations. Given the exploratory nature of this experiment, the results should be interpreted cautiously prior to replication in other cohorts with an increased frequency of the minor allele of rs3765467.

Acknowledgments— This study was supported by Mayo Clinic Center for Translational Science Activities Grant RR24150 and Minnesota Obesity Center Grant DK50456. A.S. was supported in part by a grant from the Endocrine Fellows Foundation. M.C. is supported by Grant DK 67071; R.A.R. is supported by Grant DK29953; and A.V. is supported by Grant DK78646.

Genotyping and assay costs were defrayed by an investigator-initiated grant funded by Merck to A.V. A.V. also has received research grants from Merck and has consulted for sanofi-aventis and Daiichi Sankyo. R.A.R. is a member of the advisory boards of Merck, Novo Nordisk, Takeda, Mankind, and Eli Lilly and is a consultant for Abbott and Eli Lilly. No other potential conflicts of interest relevant to this article were reported.

A.S., C.D.M., F.M., A.R.Z., P.D.G., J.M.L., and A.V. researched the data. A.S., C.D.M., F.M., A.R.Z., M.C., G.T., R.A.R., C.C., and A.V. contributed to the discussion. A.V. wrote the manuscript. A.S., A.R.Z., M.C., R.A.R., and A.V. reviewed and edited the manuscript.

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