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

A pilot study

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, lasting 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_T) was measured using a C-peptide minimal model. The effect of 21 tag single nucleotide polymorphisms (SNPs) in GLP1R on Φ_T 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.
RESULTS

Effect of rs6923761 and rs3765467 genotype on β-cell responsivity

Fig. 1A shows univariate association of rs6923761 genotype with \( \Phi_{\text{Total}} \) assuming a general genetic model. At 120 min, in the presence of glucose alone, no significant associations with \( \Phi_{\text{Total}} \) 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 \( \Phi_{\text{Total}} \) also were not statistically significant (104 ± 9 vs. 94 ± 11 vs. 81 ± 8 min\(^{-1}\), \( P = 0.11 \)). The associations with \( \Phi_{\text{Total}} \) 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 \( \Phi_{\text{Total}} \) (160 ± 12 vs. 143 ± 17 vs. 119 ± 10 min\(^{-1}\), \( P = 0.09 \)).

When the effect of rs6923761 genotype on \( \Phi_{\text{Total}} \) was examined (Fig. 1B) 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 \( \Phi_{\text{Total}} \) (160 ± 12 vs. 136 ± 12 min\(^{-1}\), \( P = 0.03 \)) were nominally significant. Differences in \( \Phi_{\text{Total}} \) at 180 min (104 ± 9 vs. 90 ± 8) were not significant (\( P = 0.09 \)).

The three heterozygotes for the minor allele of rs3765467 (Fig. 1C) exhibited differences in \( \Phi_{\text{Total}} \) 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 \( \Phi_{\text{Total}} \) 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 \( \Phi_{\text{Total}} \) (\( P = 0.0021 \), \( P = 0.0026 \), 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).

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

Figure 1—Effect of rs6923761, analyzed with the general model (A), and with the recessive model (B), and of rs3765467 (C) on \( \Phi_{\text{Total}} \) in the presence and absence of GLP-1. *\( P < 0.05 \).
in mean $\Phi_{\text{Total}}$. 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 $\geq 100\%$ increase in $\Phi_{\text{Total}}$ compared with homozygotes for the major allele. However, the observed increase in $\Phi_{\text{Total}}$ 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 $\Phi_{\text{A}}$ was a small component of $\Phi_{\text{Total}}$. Prior studies have suggested that incretins alter both static and dynamic components of $\beta$-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.

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