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Genetic modifiers of response to glucose–insulin–potassium (GIK) infusion in acute coronary syndromes and associations with clinical outcomes in the IMMEDIATE trial

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Abstract

Modifiers of response to glucose, insulin and potassium (GIK) infusion may affect clinical outcomes in acute coronary syndromes (ACS). In an Immediate Myocardial Metabolic Enhancement During Initial Assessment And Treatment In Emergency Care (IMMEDIATE) trial's sub-study ($n = 318$), we explored effects of 132 634 genetic variants on plasma glucose and potassium response to 12-h GIK infusion. Associations between metabolite-associated variants and infarct size ($n = 84$) were assessed. The 'G' allele of rs12641551, near *ACSL1*, as well as the 'A' allele of *XP04* rs2585897 were associated with a differential glucose response (P for 2 degrees of freedom test, $P_{2df} = 4.75 \times 10^{-7}$) and infarct size with GIK ($P_{2df} < 0.05$). Variants within or near *TAS1R3*, *LCA5*, *DNAH5*, *PTPRG*, *MAG11*, *PTCSC3*, *STRADA*, *AKAP12*, *ARFGEF2*, *ADCYAP1*, *SETX*, *NDRG4* and *ABCB11* modified glucose response, and near *CSF1/AHCYL1* potassium response ($P_{2df} = 4.26 \times 10^{-7}$), but not outcomes. Gene variants may modify glucose and potassium response to GIK infusion, contributing to cardiovascular outcomes in ACS.

INTRODUCTION

Administration of intravenous glucose, insulin and potassium (GIK) in patients with acute coronary syndromes (ACS) is an investigational approach aimed at promoting a shift in

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CONFLICT OF INTEREST

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myocardial energy production from high-oxygen-consuming fatty acid oxidation to glycolysis. GIK can increase and sustain production of glycolytic ATP, which may be beneficial in protecting against myocardial ischemia and reducing the extent of myocardial necrosis. Animal studies have demonstrated that GIK therapy can also lead to a slower onset of irreversible myocardial damage, improved contractile function and greater recovery after reperfusion.¹⁻⁴ These benefits of GIK have been shown to be greatest when administered early in the course of cardiac ischemia, especially for prevention of cardiac arrest, for which the risk is highest during the first hour of ACS and acute myocardial infarction (MI).⁵ The Immediate Myocardial Metabolic Enhancement During Initial Assessment And Treatment In Emergency Care (IMMEDIATE) trial, which was designed to deliver metabolic support to the ischemic myocardium immediately upon the patient's presentation with ACS,⁶ demonstrated that out-of-hospital administration of GIK, compared with placebo, was associated with lower rates of cardiac arrest and in-hospital mortality, as well as a smaller infarct size at 30 days, even though no reduction in progression to MI was observed.⁶

Any potential beneficial effects of GIK, however, could be counterbalanced, enhanced or neutralized by the genetic variation in pathways involved in GIK response. For example, although GIK works by both increasing the availability of and facilitating the entry of glucose into the cardiomyocyte, genetic variation resulting in low glucose uptake and slow metabolism may cause hyperglycemia, an important independent factor associated with a poor outcome in the setting of MI.⁷ Genetic variation in insulin-related pathways could contribute to a failure to restore normoglycemia and prevent insulin's beneficial anti-inflammatory, antioxidant, vasodilatory and profibrinolytic effects. Similarly, genetic variation could mitigate the protective effects of exogenous potassium that prevents hypokalemia and electrically stabilizes the myocardial cell membrane to avert arrhythmias.

Although the role of pharmacogenetics in GIK response is largely unknown, a number of animal studies have reported variable responses following the administration of different combinations of glucose and insulin.^{8,9} Furthermore, in patients undergoing on-pump coronary artery bypass surgery, the hemodynamic benefits of GIK were associated with changes in left ventricular expression of adrenoceptor beta 1 (ADRB1), sarcoplasmic reticulum Ca-ATPase (SERCA2a) and phospholamban (PLB).¹⁰ Although these studies provide some mechanistic insight into the actions of GIK, these findings are unlikely to represent the only pathways associated with the beneficial effects of this metabolic therapy.

Our earlier findings suggested a link between changes in glucose and potassium levels in participants of the IMMEDIATE trial Genetics Ancillary Study and variation in genes previously implicated in glucose and insulin metabolism.¹¹ To expand our findings and determine whether further genetic variation has a role in GIK response, in this current study we have investigated the effects of a large number of additional genetic variants assayed on two comprehensive chip arrays, the MetaboChip and Exome chip. Most importantly, we have also extended on previous findings by assessing whether significantly associated genetic modifiers of GIK-related metabolites also influence cardiovascular outcomes. This is the first study to investigate a large array of potential genetic modifiers of GIK therapy, and may help to shed light on the biological mechanisms of GIK action.

MATERIALS AND METHODS

Study population

The IMMEDIATE trial was a double-blind randomized controlled clinical trial conducted in 13 cities across the United States that assessed the effectiveness of intravenous GIK administered early in the course of ACS for 12 h in 871 participants.¹² Inclusion and exclusion criteria for the IMMEDIATE trial have been previously described.¹² The aim of the IMMEDIATE Genetic Ancillary Study was to identify genetic modifiers of response to GIK therapy. At nine participating sites, a total of 321 participants were recruited during parent trial enrollment. Provided that consent for genetic analysis was obtained, all IMMEDIATE trial participants were eligible for inclusion. The Icahn School of Medicine at Mount Sinai's Institutional Review Board (IRB) as well as the IRB at each participating site approved this study.

Metabolite and infarct size measurements

Each study site hospital laboratory measured glucose and potassium levels upon arrival at the emergency department and after 6 and 12 h of study drug infusion, or alternatively at the point the drug was stopped prematurely. A subset of 117 Genetic Ancillary Study participants that was concurrently enrolled in the biological mechanisms cohort¹² had both glucose and insulin levels measured initially and at 6 and 12 h at the Tufts Clinical and Translational Science Institute core laboratory. At all three time points, the glucose levels generated by the hospital labs and the core lab were strongly correlated.¹¹ As a result of blood samples being obtained upon arrival at the emergency department, the metabolite levels were non-fasting. For biological mechanism cohort study participants, infarct size was measured by 99mTc-sestamibi single-photon emission computed tomographic perfusion imaging at 30 days after initial hospitalization, with central reading and infarct size determination carried out at the trial's core laboratory at Tufts Medical Center.^{6,12}

Genotyping and data quality control

Genomic DNA was extracted from either whole blood or saliva as described elsewhere.¹¹ Genotyping was carried out using the Illumina (San Diego, CA, USA) Cardio-MetaboChip¹³ and HumanExome BeadChip.¹⁴ Single-nucleotide polymorphisms (SNPs) were clustered into genotypes using Illumina BeadStudio software. SNPs were removed if they were monomorphic, had call rates <95% or deviated from Hardy-Weinberg equilibrium (HWE, $P < 0.0001$). There were 196 725 SNPs in the MetaboChip data set. However, of these, 27 814 monomorphic SNPs were removed and 529 SNPs that failed HWE and had a low call rate were additionally excluded. There were 247 870 SNPs in the Exome Chip data set, of which 157 172 were monomorphic and 22 were eliminated owing to failure of HWE and low call rate. For a total of 4264 SNPs presented on both the chips, the SNP data with the lower call rate were removed. This resulted in the exclusion of a further 3771 SNPs from MetaboChip and 493 from Exome Chip. After the completion of quality control and exclusion of SNPs with a minor allele frequency (MAF) < 5%, the merged genetic data set contained 132 634 SNPs with a mean genotyping success rate of 99.85%. The final data set included previously analyzed glucose- and insulin-associated SNPs,¹¹ to obtain a global representation of the genetic variation most contributing to the

GIK response. Of the common variants analyzed, 12 322 (9.3%) were non-synonymous, 1855 (1.4%) synonymous, 55 680 (42.0%) intronic, 57 451 (43.3%) intergenic, 357 (0.27%) were located in the 5'-untranslated region, 1972 (1.5%) in the 3'-untranslated region and 54 (0.04%) were splice variants. Samples were filtered for individual call rates < 95% and relatedness. Duplicate concordance was assessed for three samples assayed on MetaboChip and five samples assayed on Exome Chip. Genotype concordance for each duplicate was 98.3%. After excluding two individuals that were recruited twice, and samples with a low call rate, the Genetic Ancillary Study cohort comprised 318 individuals.

Statistical analysis

Phenotype derivation—We have investigated the associations between gene variants and changes in non-fasting plasma glucose and potassium during 12-h GIK infusion. Metabolite response to treatment exhibited nonlinear trends over 12 h; therefore, the change in each trait was determined between initial and 6-h measurements (calculated as 6-h level–initial level) and between 6- and 12-h measurements (calculated as 12-h level–6-h level). Glucose data exhibited skewed distributions and were log-transformed. As a result, their 6-h log-changes are interpreted as ratios. The 6-h changes for potassium can be interpreted as absolute differences between the two measurements. Infarct size was estimated as the percentage of hypoperfused myocardium. Only patients with confirmed MI were included in this analysis. Eighteen subjects had an infarct size of 0. It was possible to have an adjudicated MI based on acute care details despite the scan (30 days later) identifying no discernible infarct. An infarct size of 59% was assigned to three individuals who died in hospital (calculated as the largest observed infarct plus 1).

Statistical analysis—A two degree of freedom test (2 d.f.) was carried out to detect joint significance for the main effect of the SNP and the SNP × intervention interaction in the same linear regression model.¹⁵ As our patient population was ethnically diverse, we computed principal components to be used as covariates in regression analyses to control for population admixture.¹¹ All models adjusted for age, sex, treatment arm, diabetes status and the first two principal components. Associations with initial to 6-h change were additionally adjusted for the time from infusion initiation to first blood draw. Top signals linked to glucose and potassium response and having MAF ≥ 10% were then tested for their association with infarct size. A MAF threshold of 10% was implemented for the infarct size analyses owing to the smaller sample size of this variable, with data only available for the individuals recruited into the biological mechanism cohort. To control for the floor effect, which resulted from the fact that multiple patients with MI had an infarct size of zero, the Tobit model was used. Statistical analyses were carried out using the *R* software package (R Foundation for Statistical Computing, Vienna, Austria).

Of 132 634 common variants, we determined the number of uncorrelated markers to be 87 538, after accounting for linkage disequilibrium using the approach by Li and Ji.¹⁶ Therefore, after adjustment for multiple hypothesis testing, a *P*-value threshold for statistical significance was set at 5.71×10^{-7} . The 2.d.f *P*-value was used to determine significance regardless of whether the effect was driven by the genotype and/or the genotype × treatment interaction.

RESULTS

Out-of-hospital and in-hospital patient characteristics of the IMMEDIATE Genetic Ancillary Study are shown in Tables 1 and 2, respectively. There were no significant differences in basic demographics including age, gender and medical history of diabetes, MI or heart failure between the randomization groups ($P > 0.05$). Although no significant difference was observed in the parent trial, in this sub-study, the same trend that patients treated with GIK were less likely to progress to MI than those given placebo reached statistical significance ($P = 0.04$, GIK = 61.1%, placebo = 71.9%). The possible explanation is that fewer patients without ACS were included in the Genetic Ancillary Study following the improved inclusion criteria applied later on in the trial, creating a more appropriate cohort of patients who would benefit the most from the therapy. In patients who progressed to MI, a trend towards a smaller infarct was observed in those treated with GIK ($P = 0.10$). There was no significant difference with treatment in the composite outcome of 1-year death or heart failure or cardiac arrest.¹⁷

Chip-wide significant associations with initial to 6-h response to GIK infusion

Analyses identified four variants that significantly modified the initial to 6-h glucose response to GIK ($P_{2df} = 4.75 \times 10^{-7}$) (Table 3).

Initial to 6-h glucose change—Carriers of the rs7229849 ‘G’ allele (MAF = 0.14), located nearest the *ADCYAP1* (adenylate cyclase-activating polypeptide 1) gene, exhibited a greater increase in glucose between the initial and 6-h measurements with GIK, but not with placebo ($P_{2df} = 1.01 \times 10^{-7}$, placebo fold change = 0.91, GIK fold change = 1.51 per ‘G’ allele). Within the *NDRG4* (N-myc downstream-regulated gene family member 4)) gene, the ‘A’ allele of rs13333449 was associated with a larger increase in glucose in GIK-treated participants only ($P_{2df} = 5.46 \times 10^{-7}$, placebo fold change = 0.96, GIK fold change = 1.39). Nominal associations were observed between both rs7229849 and rs13333449 and additional metabolite traits ($P < 0.05$). The ‘A’ allele of non-synonymous *SETX* rs1185193 (MAF = 0.17) was associated with a 1.5-fold greater increase per copy in glucose between the initial measurement and 6 h in GIK-treated subjects but not the placebo group ($P_{2df} = 4.19 \times 10^{-7}$). Conversely, the *XP04* (exportin 4) rs2585897 ‘A’ allele was associated with a 0.68-fold decrease in glucose in the first 6h of infusion in GIK patients only ($P_{2df} = 4.75 \times 10^{-7}$). Additional suggestive associations with initial to 6-h changes in glucose ($P_{2df} < 5.0 \times 10^{-5}$) are shown in Supplementary Tables S1.

Initial to 6-h potassium change—No statistically significant associations were observed for the initial to 6-h potassium response. The variants exhibiting the strongest signals were located within the *SOX5*, *SETBP1*, *PCNT* and *RUNX1* genes ($P_{2df} = 1.95 \times 10^{-5}$, Supplementary Table 2).

Chip-wide significant associations with 6–12-h response to GIK infusion

Between 6 and 12h, there were 11 variants that modified the response of glucose and 1 modified the response of potassium with GIK therapy ($P_{2df} = 4.26 \times 10^{-7}$; Table 3).

6–12-h glucose change—The minor alleles of non-synonymous *TASIR3* rs76755863 (MAF = 0.06; $P_{2df} = 4.94 \times 10^{-9}$, placebo fold change = 0.84, GIK fold change = 0.48 per ‘A’ allele), non-synonymous *DNAH5* rs2277046 (MAF = 0.08; $P_{2df} = 2.73 \times 10^{-8}$, placebo fold change = 0.99, GIK fold change = 0.41 per ‘G’ allele), intronic *PTPRG* rs1317639 (MAF = 0.23; $P_{2df} = 1.69 \times 10^{-7}$, placebo fold change = 0.97, GIK fold change = 0.71 per ‘G’ allele), intronic *MAGII* rs10510950 (MAF = 0.07; $P_{2df} = 3.81 \times 10^{-7}$, placebo fold change = 1.07, GIK fold change = 0.54 per ‘C’ allele), intronic *PTCSC3* rs1169151 (MAF = 0.46; $P_{2df} = 4.62 \times 10^{-7}$, placebo fold change = 0.95, GIK fold change = 0.73 per ‘G’ allele) and intronic *ABCB11* rs34453330 (MAF = 0.05; $P_{2df} = 5.65 \times 10^{-7}$; placebo fold change = 1.01, GIK fold change = 0.52) were associated with a larger decrease in glucose in GIK-treated individuals, as were minor alleles of rs4870000 near *AKAP12* (MAF = 0.08; $P_{2df} = 8.85 \times 10^{-8}$, placebo fold change = 1.02, GIK fold change = 0.55 per ‘A’ allele), rs12197990 near *LCA5* (MAF = 0.17; $P_{2df} = 1.99 \times 10^{-7}$, placebo fold change = 1.01, GIK fold change = 0.65 per ‘G’ allele) and rs17445665 near *ARFGEF2* (MAF = 0.25; $P_{2df} = 4.03 \times 10^{-7}$, placebo fold change = 0.95, GIK fold change = 0.68 per ‘A’ allele). Conversely, the ‘A’ allele of the *STRADA* variant, rs2137143, (MAF = 0.06) was associated with a greater increase in glucose between 6 and 12 h with GIK but not with placebo ($P_{2df} = 3.70 \times 10^{-9}$, placebo fold change = 1.02, GIK fold change = 2.28 per ‘A’ allele). A variant, rs12641551, near *ACSL1*, (MAF = 0.38) exhibited a similar association, conferring a larger change in glucose between 6 and 12 h in the GIK-treated subjects only ($P_{2df} = 3.38 \times 10^{-8}$, placebo fold change = 0.99, GIK fold change = 1.38 per ‘G’ allele).

Seven of these variants showed additional nominal associations with other metabolite traits ($P < 0.05$; Table 3).

6–12-h potassium change—Near *AHCYL1* (adenosylhomocysteinase-like 1) and *CSF1* (colony stimulating factor 1), the ‘A’ allele of rs17025286 (MAF = 0.06) was associated with a larger decrease in potassium between 6 and 12 h in the GIK group compared with placebo ($P_{2df} = 4.12 \times 10^{-8}$, β placebo = $-0.08 \text{ mmol l}^{-1}$, β GIK = $-0.82 \text{ mmol l}^{-1}$ per ‘A’ allele). Additional suggestive associations with 6–12-h changes in metabolites ($P_{2df} < 5.0 \times 10^{-5}$) are shown in Supplementary Tables S3 and S4.

Associations between metabolite-associated variants and 30-day adjusted infarct size

Of the 16 variants significantly associated with glucose and potassium response to 12-h infusion of GIK, two exhibited an additional nominal association with 30-day adjusted infarct size ($P_{2df} < 0.05$). The most significant of these, rs12641551 ($P_{2df} = 0.016$), which exhibited a greater increase in glucose in the last 6 h of infusion, interacted with treatment to confer a bigger infarct in the GIK group (β , GIK = 11.11% of LV per ‘G’ allele) and a smaller infarct in the placebo-treated subjects (β , placebo = -5.82% of LV per ‘G’ allele; Figure 1). The ‘A’ allele of *XPO4* rs2585897, which conferred a greater decrease in glucose in the first 6 h of GIK infusion, was associated with a smaller infarct in both the placebo (β , placebo = -10.48% of LV per ‘A’ allele) and GIK arms (β , GIK = -8.52% of LV per ‘A’ allele, $P_{2df} = 0.049$; Figure 2).

There were no associations between these variants and either progression to MI and/or the combined outcome of 1-year death and/or heart failure and/or cardiac arrest (data not shown). The lack of association with the later outcome may be confounded by the fact that at least half of all cardiac arrests occurred before patients could survive to have samples collected, and primarily occurred in placebo participants.

DISCUSSION

Despite extensive experimental data supporting the role of GIK in cardioprotection during ischemia, GIK remains an investigational therapy currently not recommended for routine use in the treatment of patients with an ACS because clinical trials administering GIK to patients with ACS have produced inconsistent results.¹⁸ The IMMEDIATE trial provided GIK support within the first hours of ACS and addressed the hypothesis that this treatment can be beneficial only if administered early enough to salvage the ischemic myocardium.^{6,12} Variable blood metabolite levels that we found associated with specific polymorphisms suggests that differences in genes involved in processes including cell growth and differentiation, kinase activity, cellular transport and homeostasis may also affect the GIK response. The study design is unable to directly assess the functional role of genetic variants in tissue metabolite levels in response to GIK, but our data provide the first demonstration that genetic factors can influence the GIK response in blood. We speculate that some of the genetic factors described here may have altered blood metabolite levels by changing their uptake into tissues such as the heart.

We identified a genetic variant near *ACSL1* that was significantly associated with a greater increase in glucose during GIK infusion. This variant was also associated with larger infarct size in individuals who progressed to MI compared with those treated with placebo. *ACSL1* is an isozyme of the long-chain fatty acid coenzyme A ligase family that converts free long-chain fatty acids into fatty acyl-CoA esters, and thereby has a key role in lipid biosynthesis and fatty acid degradation. Analysis of expression patterns in animal models have indicated that *ACSL1* contributes to fatty acid metabolism in the heart and the development of cardiac hypertrophy.¹⁹ Variants in or near *ACSL1* have been associated with type 2 diabetes,²⁰ metabolic syndrome,²¹ cardiorespiratory fitness²² and success of resuscitation from sudden cardiac arrest.²³ Although the functional consequences of rs12641551 are not known, the present study suggests that genetic variation may affect fatty acid degradation and cellular energy stores leading to the oxidative stress and apoptosis inherent in ischemia. Alternatively, glucose uptake by tissues including the heart could be negatively impacted upon.

Our study also demonstrated that an intronic variant in *XPO4* was associated with a greater decrease in glucose levels during the first 6 h of GIK infusion, as well as a much smaller infarct in individuals with MI in both treatment arms. *XPO4* mediates nuclear export of eIF5A (eukaryotic translation initiation factor 5A),²⁴ which is the only protein to contain the amino acid hypusine. A higher content of the eIF5A protein has been found in differentiated skeletal muscle cells. After treatment with a hypusination inhibitor, which reversibly abolished the differentiation process, an increase in glucose consumption and lactate production, as well as a decrease in glucose and palmitic acid, has been observed.²⁵ A

unique hypusine modification of eIF5A has also been shown to promote islet beta cell inflammation and dysfunction in mice, suggesting it as a potential therapeutic target for preserving islet function under inflammatory conditions.²⁶ Furthermore, eIF5A may be a potential target of rosiglitazone, one of the thiazolidinedione oral anti-diabetic drugs.²⁷ The decrease in glucose levels that was observed during the initial hours of GIK infusion in carriers of the *XPO4* variant may indicate better glucose uptake by the heart, ultimately contributing to a smaller infarct size in those that progressed to MI.

Differences in glucose, but not outcomes, were observed for SNPs within *TAS1R3*, *DNAH5*, *PTPRG*, *MAG11*, *PTCSC3*, *STRADA*, *ABCB11* and *NDRG4*, and near *AKAP12*, *ARFGF2* and *ADCYAP1* ($P = 5.71 \times 10^{-7}$). Specifically, a SNP near *ADCYAP1* was associated with glucose response to GIK. *ADCYAP1* is a pancreatic neuropeptide that stimulates insulin secretion in a glucose-dependent manner and has an important role in islet actions and mechanisms. Variation in human *ADCYAP1* has been linked to type 2 diabetes.²⁸ Intronic variants in two neighboring genes, *PTPRG* and *MAG11*, were also associated with glucose response. Variation in *PTPRG* has been associated with type 2 diabetes, heart rate and the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle, or QT interval.^{29,30} Noticeably, both of these genes are located near the type 2 diabetes candidate gene *ADAMTS9* that is believed to be involved in liver and adipocyte insulin resistance.³¹ Genetic variation within the genomic region of *ABCB11*, which encodes the ATP-binding cassette subfamily B (MDR/TAP) member 11, has been associated with fasting glucose^{32,33} and HbA1c³⁴ levels in multiple studies. The *NDRG4* gene is known to be expressed in the central nervous system and the heart of zebrafish, and is involved in the regulation of cardiomyocyte growth and proliferation.³⁵ Variants in the *NDRG4* genomic region have also been associated with QT interval duration.^{36,37} Finally, two of the associated genes, *PTCSC3* and *AKAP12*, are within expression quantitative trait loci linked to glucose levels, insulin sensitivity, fat distribution and/or left ventricular contractility, further supporting the role of these genomic regions in the GIK response (see Supplementary Box).

Potassium is an important component of GIK, and without it the effects of glucose and insulin would be to cause hypokalemia, thereby contributing to arrhythmias.¹⁸ We detected a variant in the *CSF1/AHCYL1* locus that was associated with a steep decrease in plasma potassium levels during the final 6 h of GIK infusion. *CSF1* is a cytokine that controls the production, differentiation and function of macrophages. Murine macrophage-specific CSF1 has been shown to stimulate glucose uptake as a necessary early response for cell survival and subsequent DNA synthesis,³⁸ inhibits the voltage-activated inward-rectifying potassium conductance, and appears to be one of the earliest cellular responses to intracellular mediators.³⁹ *AHCYL1* encodes a protein involved in the conversion of homocysteine isoforms. Homocysteine is an important risk factor for the development of human heart disease, including atrial fibrillation and heart failure. Elevated homocysteine levels have been shown to cause electrophysiological disturbances of potassium currents in human atrial myocytes.⁴⁰ Although both *CSF1* and *AHCYL1* are plausible candidates to impact potassium metabolism in myocytes, additional studies are required to elucidate the role of rs17025286 in GIK response.

Strengths of this study include the randomized assignment of GIK in individuals at high risk of ACS, which reduces the effect of confounding factors that can be a major challenge for association studies based on cross-sectional and observational data. Repeated measurements during infusion also allowed for the comparison of changes in biomarker levels between the intervention group and placebo, providing information about the dynamics of the response.

We also acknowledge several limitations of our study, including the lack of baseline biomarker measurements. As the study drug was initiated by emergency medical services and the first blood measurements were carried out during emergency department admission, no pre-treatment measurements are available. Importantly, in spite of the large glucose load in the GIK infusion, endogenous insulin secretion as indicated by unchanged C-peptide levels (data not shown) did not vary significantly over the 12-h infusion, suggesting that the relative levels of glucose and insulin during GIK infusions were balanced in regard to the impact on blood glucose levels. However, metabolite levels were non-fasting, precluding us from a formal estimation of insulin resistance, as assessed by the homeostasis model assessment index, HOMA2-IR. Our sample size limited the statistical power to detect modest effects; however, 44% of the pharmacogenetic studies reported in the genome-wide association studies catalog⁴¹ as of March 2014 also have a sample size of < 400. Single-photon emission computed tomographic imaging, which has been used by several clinical trials to quantify infarct size,⁴² was performed only on a subset of IMMEDIATE Trial participants. The IMMEDIATE Trial is the first GIK clinical study that enabled DNA analysis, making replication efforts in independent cohorts infeasible.

In summary, our findings suggest that genetic variation may modify response to 12-h GIK infusion, and contribute to metabolic function and cardiovascular outcomes in ACS. The loci linked to GIK response in the current study may help inform future studies for prioritizing candidates for functional studies and replication.

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Figure 1. Association between rs12641551 near *ACSL1* and (a) 6–12-h change in glucose and (b) 30-day adjusted infarct size. GIK, glucose, insulin and potassium.

Figure 2. Association between *XPO4* rs2585897 and (a) initial to 6-h change in glucose and (b) 30-day adjusted infarct size. GIK, glucose, insulin and potassium.

Table 1

Demographics, pre-hospital clinical characteristics and medical history by treatment group for the IMMEDIATE Genetic Ancillary Study

	<i>n</i>	GIK	<i>n</i>	Placebo	<i>P</i> -value ^a
<i>Demographics/Clinical characteristics</i>					
Age (years)	157	63.8 ± 12.5	160	63.0 ± 13.6	0.60
Gender, <i>n</i> = male (%)	157	117(74.5)	160	120(75.0)	0.92
Ethnicity, <i>n</i> (%)	157		160		0.07
White		132(84.1)		146(91.3)	
Black		17(10.8)		7(4.4)	
Asian		4(2.5)		1(0.6)	
Other		4(2.5)		6(3.8)	
Hispanic, <i>n</i> (%)	157	15(9.5)	159	25(15.7)	0.01
BMI (kgm ⁻²)	150	28.6 ± 5.5	152	28.8 ± 6.6	0.77
Time for symptom onset until infusion initiation (hours)	128	2.7 ± 3.9	141	2.3 ± 2.7	0.33
<i>Medical history, n (%)</i>					
Previous myocardial infarction (Yes)	157	55(35.0)	160	57(35.6)	0.91
History of diabetes (yes)	157	49(31.2)	160	38(23.8)	0.14
Previous heart failure (yes)	157	27(17.2)	160	24(15.0)	0.38

Abbreviations: BMI, body mass index; GIK, glucose, insulin and potassium; IMMEDIATE, immediate myocardial metabolic enhancement during initial assessment and treatment in emergency care.

^a Statistical significance $P < 0.05$

Data are presented as mean ± s.d. and percentages.

In-hospital clinical characteristics and metabolite measurements by treatment group for the IMMEDIATE Genetic Ancillary Study

Table 2

	<i>n</i>	GIK	<i>n</i>	Placebo	<i>P</i> -value ^d
<i>Clinical characteristics</i>					
Duration of treatment (hours)	157	8.4 ± 4.8	132	9.1 ± 4.5	0.17
Time from treatment initiation until first blood draw—standard of care (hours)	156	0.64 ± 0.76	159	0.55 ± 0.61	0.28
Time from treatment initiation until first blood draw—biological mechanisms cohort (hours)	50	2.21 ± 1.20	55	2.63 ± 1.27	0.09
<i>Non-fasting plasma glucose levels (mg dL⁻¹)^b</i>					
Initial blood glucose	156	188.7(177.7–202.4)	159	154.5(145.5–165.7)	3.10 × 10 ⁻⁵
6-h blood glucose	92	188.6(167.3–214.9)	114	148.4(138.4–159.2)	3.70 × 10 ⁻⁴
12-h blood glucose	137	146.9(131.6–162.4)	142	142.6(134.3–151.4)	0.64
<i>Non-fasting plasma potassium levels (mmol L⁻¹)</i>					
Initial potassium	155	3.99 ± 0.64	159	3.93 ± 0.55	0.37
6-h potassium	91	4.35 ± 0.52	112	4.08 ± 0.46	9.30 × 10 ⁻⁵
12-h potassium	146	4.43 ± 0.58	152	3.97 ± 0.46	3.95 × 10 ⁻¹³
<i>Cardiovascular outcomes</i>					
Progression to myocardial infarction, <i>n</i> = yes	157	96(61.1%)	160	115(71.9%)	0.04
30-day adjusted infarct size (% of left ventricle)	36	11.4 ± 15.9	48	17.5 ± 17.5	0.10
1-year death/heart failure/cardiac arrest	156	22(14.1%)	159	31(19.5%)	0.23

Abbreviations: GIK, glucose, insulin and potassium; IMMEDIATE, immediate myocardial metabolic enhancement during initial assessment and treatment in emergency care.

^aStatistical significance *P* < 0.05.^bData were log-transformed before analysis.

Data are presented as geometric means and 95% confidence intervals. Ratios are presented for initial to 6-h and for 6–12-h changes for logged data. Data are presented as unadjusted means ± s.d. or percentages unless otherwise stated.

Table 3

Chip-wide significant associations for glucose and potassium traits, and additional nominal associations

SNP	Position	Chr	Gene/nearest gene (s)	Function	A1	MAF	P-value (FC placebo, FC GIK)		P-value (β placebo, β GIK)	
							Initial to 6-h glucose	6–12-h glucose	Initial to 6-h potassium	6–12-h potassium
rs76755863	1266738	1	<i>TASIR3</i>	Non-synonymous	A	0.06	0.001 (0.93, 1.62)	4.94 $\times 10^{-8}$ ^a (0.84, 0.48)	0.082 (0.11, -0.37)	0.075 (-0.20, 0.23)
rs17025286	110503433	1	<i>CSF1</i> (distance = 29817); <i>AHCYL1</i> (distance = 23954)	Intergenic	A	0.06	0.132 (1.15, 1.18)	0.529 (0.92, 0.92)	0.138 (-0.008, 0.37)	4.12 $\times 10^{-08a}$ (-0.08, -0.82)
rs34453330	169781530	2	<i>ABCB11</i>	Intron	A	0.05	0.012 (1.12, 1.43)	5.65 $\times 10^{-07a}$ (1.01, 0.52)	0.492 (-0.03, -0.21)	0.800 (0.05, 0.09)
rs1317639	61569213	3	<i>PTPRG</i>	Intron	G	0.23	0.001 (1.01, 1.28)	1.69 $\times 10^{-07a}$ (0.97, 0.71)	0.661 (0.07, -0.04)	0.250 (-0.11, -0.05)
rs10510950	65972434	3	<i>MAGI1</i>	Intron	C	0.07	0.112 (0.88, 1.23)	3.81 $\times 10^{-07a}$ (1.07, 0.54)	0.690 (-0.01, -0.14)	0.625 (0.05, -0.12)
rs2277046	13717471	5	<i>DNAH5</i>	Non-synonymous	G	0.08	0.082 (1.17, 1.33)	2.73 $\times 10^{-08a}$ (0.99, 0.41)	0.524 (0.03, -0.24)	0.244 (0.21, -0.003)
rs12641551	185767941	4	<i>ACSL1</i> (distance = 19969)	Intergenic	G	0.38	0.844 (0.97, 0.99)	3.38 $\times 10^{-08a}$ (0.99, 1.38)	0.937 (-0.01, 0.02)	0.454 (0.01, 0.08)
rs12197990	80140783	6	<i>LCA5</i> (distance = 53925)	Intergenic	G	0.17	0.132 (1.06, 1.17)	1.99 $\times 10^{-07a}$ (1.01, 0.65)	0.948 (-0.03, -0.01)	0.234 (0.01, -0.15)
rs4870000	151536785	6	<i>AKAP12</i> (distance = 24349)	Intergenic	A	0.08	0.386 (1.09, 1.14)	8.85 $\times 10^{-08a}$ (1.02, 0.55)	0.596 (0.13, -0.08)	0.485 (-0.15, 0.01)
rs1185193	135203409	9	<i>SETX</i>	Non-synonymous	A	0.17	4.19 $\times 10^{-07a}$ (0.98, 1.50)	0.086 (1.09, 0.87)	0.691 (0.06, 0.07)	0.378 (0.06, 0.11)
rs2585897	21398979	13	<i>XPO4</i>	Intron	A	0.21	4.75 $\times 10^{-07a}$ (0.98, 0.68)	0.101 (1.04, 1.17)	0.944 (0.01, 0.03)	0.091 (0.05, 0.17)
rs1169151	36640601	14	<i>PTCSC3</i>	Intron	G	0.46	0.429 (1.02, 1.08)	4.26 $\times 10^{-07a}$ (0.95, 0.73)	0.059 (-0.12, -0.14)	0.151 (0.11, -0.02)
rs13333449	58502210	16	<i>NDRG4</i>	Intron	A	0.33	5.46 $\times 10^{-07a}$ (0.96, 1.39)	0.005 (1.02, 0.81)	0.778 (-0.03, -0.05)	0.301 (0.10, -0.02)
rs2137143	61805401	17	<i>STRADA</i>	Intron	A	0.06	0.216 (0.91, 0.80)	3.70 $\times 10^{-08a}$ (1.02, 2.28)	0.753 (-0.11, 0.05)	0.321 (-0.04, 0.23)
rs7229849	994946	18	<i>ADCYAP1</i> (distance = 82773)	Intergenic	G	0.14	1.01 $\times 10^{-07}$ (0.91, 1.51)	0.038 (0.99, 0.81)	0.135 (-0.21, 0.05)	0.050 (0.02, -0.22)
rs17445665	477510491	20	<i>ARFGGEF2</i> (distance = 27784)	Intergenic	A	0.25	0.004 (1.01, 1.28)	4.03 $\times 10^{-07a}$ (0.95, 0.68)	0.736 (-0.04, -0.06)	0.914 (-0.02, -0.03)

Abbreviations: FC, fold change; GIK, glucose, insulin and potassium; MAF, minor allele frequency.

^a Chip-wide statistical significance, $P < 5.71 \times 10^{-7}$.

A1 represents minor allele. FC and β represent the difference in levels per allele copy. At least nominal associations ($P < 0.05$) are highlighted in bold.