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ORIGINAL ARTICLE

Common variants associated with changes in levels of circulating free fatty acids after administration of glucose–insulin–potassium (GIK) therapy in the IMMEDIATE trial

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Glucose–insulin–potassium (GIK) therapy may promote a shift from oxygen-wasteful free fatty acid (FFA) metabolism to glycolysis, potentially reducing myocardial damage during ischemia. Genetic variation associated with FFA response to GIK was investigated in an IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care) substudy ($n = 117$). In patients with confirmed acute coronary syndromes, associations between 132 634 variants and 12-h circulating FFA response were assessed. Between initial and 6-h measurements, three *LINGO2* variants were associated with increased levels of total FFA (*P*-value for 2 degree of freedom test, $P_{2df} \leqslant 5.51 \times 10^{-7}$). Lead *LINGO2* single-nucleotide polymorphism, rs12003487, was nominally associated with reduced 30-day ejection fraction ($P_{2df} = 0.03$). Several *LINGO2* signals were linked to alterations in epigenetic profile and gene expression levels. Between 6 and 12 h, rs7017336 nearest to *IMPA1/FABP12* showed an association with decreased saturated FFAs ($P_{2df} = 5.47 \times 10^{-7}$). Nearest to *DUSP26,* rs7464104 was associated with a decrease in unsaturated FFAs ($P_{2df} = 5.51 \times 10^{-7}$). Genetic variation may modify FFA response to GIK, potentially conferring less beneficial outcomes.

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INTRODUCTION

Under normal aerobic conditions myocardial metabolism predominantly utilizes free fatty acids (FFAs), with these substrates generating between 60 and 90 percent of all energy produced by the heart. The remaining 10–40 percent of myocardial energy is generated by carbohydrate metabolism, and in particular glycolysis.[1,2] In these conditions, FFA metabolism is advantageous, as despite requiring greater oxygen consumption it produces more energy per unit of substrate when compared with glycolysis. In the presence of ischemia, there is a shift toward glucose metabolism; however, FFA continues to be the main energy source. In addition to increasing oxygen requirements the high rate of FFA oxidation in the ischemic heart has detrimental effects such as the inhibition of pyruvate dehydrogenase, which leads to the suppression of glucose metabolism.[3,4] This results in increased lactate and proton concentration in ischemic cells compromising contractile function and reducing cardiac efficiency.[4–6] Previous studies have suggested that FFAs also exert negative effects on the vessel wall by triggering endothelial apoptosis and impairing endothelium-dependent vasodilation.[7,8]

Administration of glucose, insulin and potassium (GIK) is proposed as a therapeutic approach for acute myocardial infarction (MI), partly for promoting a shift from FFA to glucose metabolism. GIK decreases the levels of oxygen-wasting FFA in the post-MI setting by suppressing FFA release from adipose tissue stores, while at the same time maintaining elevated levels of glucose, which can then replace FFA in cardiac metabolism. Animal studies have demonstrated that if initiated at the onset of ischemia, GIK can increase and sustain production of glycolytic ATP, leading to a slower onset of irreversible damage, improved contractile function and greater recovery at reperfusion.[9–12] Supporting these findings, the IMMEDIATE (Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care) Trial, which was designed to deliver metabolic support to the ischemic myocardium immediately upon the patient's presentation with an acute coronary syndrome,[13] reported that out-of-hospital administration of GIK was associated with lower rates of cardiac arrest and in-hospital mortality, and a smaller infarct size at 30 days, even though no reduction in progression to MI was observed.[14] That study additionally identified a rapid suppression of circulating FFAs in response to GIK, suggesting a shift away from FFA metabolism to glycolysis (personal communication).

Genetic factors may also be important modifiers of response to GIK therapy, potentially influencing the production and utilization of FFAs in myocardial energy metabolism. In previous population-based studies, genetic variation in fatty acid desaturase (*FADS*) genes, ELOVL Fatty Acid Elongase 2 (*ELOVL2)*, Glucokinase (Hexokinase 4) Regulator (*GCKR)*, ALG14, UDP-N-acetylglucosaminyltransferase subunit *(ALG14)*, Lysophosphatidylglycerol Acyltransferase 1 *(LPGAT1)*, Hypoxia Inducible Factor 1, Alpha Subunit Inhibitor (*HIF1AN)* and

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others have been linked to a diverse range of functions including FA metabolism, protein glycosylation and phospholipid modeling. Many of these genes are involved in glucose- and oxygen-sensing pathways, n-3 metabolism and have been associated with circulating levels of saturated and unsaturated FAs.[15,16] In a sub-study of the IMMEDIATE Trial, we have previously identified genetic variation associated with the glucose and potassium response to GIK.[17,18] Extending these findings, we have investigated whether genetic factors were associated with variation in the circulating FFA response to GIK, both as total FFA and its sub-fractions, saturated and unsaturated fatty acids.

MATERIALS AND METHODS

Study population

The inclusion and exclusion criteria for both the IMMEDIATE Trial ($N = 871$) and the IMMEDIATE Genetic Ancillary Study ($N = 321$) have been previously described.[13,17] The aim of the Genetic Ancillary Study was to identify genetic variation that modified the response to GIK therapy initiated early in the course of acute coronary syndrome and administered for 12 h. A subset of 117 Genetic Ancillary Study participants was concurrently enrolled in the Biological Mechanisms Cohort, and underwent additional analyses including the measurement of FFAs and cardiovascular outcomes. All subjects enrolled into the Biological Mechanism Cohort had a confirmed diagnosis of acute coronary syndrome and received at least 8 h of the study drug. Approval for this study was received from the Icahn School of Medicine at Mount Sinai's Institutional Review Board, as well as from the Institutional Review Board at each participating study site.

FFA measurement

Blood samples for FFA analysis were collected into vacutainers containing EDTA and pre-loaded with tetrahydrolipstatin (an inhibitor of lipoprotein lipase, 39 μg ml^{-1} blood[19]). Plasma total and individual FFA concentrations including chain length and saturation, were measured using thin layer chromatography on silica gel G followed by gas chromatography (GC2010, Shimadzu Corp, Columbia, MD, USA) using a 100 m SP2560 capillary column. Internal standards were included to correct for analytical losses, and oxidative losses were corrected for with external standards.

Levels of FFA were obtained initially, and at ~6 and 12 h post infusion initiation, or where appropriate at the point the study drug was stopped prematurely. Due to blood samples being obtained upon arrival at the emergency department, FFA levels were not necessarily drawn in the non-fasting state. Measurements were carried out at the Tufts Clinical and Translational Science Institute core laboratory.

Genetic analysis

Genomic DNA was extracted from either whole blood or saliva using previously established methods.[17] Genotyping was carried out on all patient samples using both the Illumina Cardio-Metabochip[20] and HumanExome Beadchip.[21] The single-nucleotide polymorphism (SNP) clustering and quality control protocol implemented in this study have been described in detail previously.[17] Briefly, quality control resulted in the exclusion of SNPs that were monomorphic, deviated from Hardy–Weinberg equilibrium ($P < 0.0001$), or had low call rates ($< 95\%$). After the completion of quality control, the merged genetic data set contained 164 111 SNPs from Cardio-Metabochip and 90 138 SNPs from HumanExome Beadchip. Variants with a minor allele frequency of < 0.05 were excluded to give a final genetic data set of 132 634 common variants with a mean genotyping success rate of 99.85%. Samples were filtered for individual call rates $< 95\%$, discordance between self-reported sex and X-chromosome dosage and relatedness. The Genetic Ancillary Study cohort reported in this current study was comprised of Biological Mechanism cohort's participants only and thus included 117 individuals.

Statistical analyses

FFA phenotypes. This current study investigated the association of the primary study outcome, total FFA and secondary outcomes, saturated and unsaturated FAs, with 132 634 gene variants in patients receiving a 12-h infusion of GIK or placebo. Due to the non-linear FFA response to GIK therapy, changes in metabolite levels were assessed as initial to 6-h change (6-h measurements—initial measurements), and 6- to 12-h change (12-h measurements—6-h measurements). Saturated FA was calculated as the sum of fractions 16:0 and 18:0, which constitute the majority of saturated FAs present in the circulation, and similarly unsaturated FFA concentration was calculated from the sum of 18:1n9 and 18:2n6 fractions. Total FFA exhibited a skewed distribution and was log transformed prior to statistical analyses. Therefore, the 6-h log-changes for total FFA are interpreted as ratios. Saturated FFA and unsaturated FFA were each converted to a percent of total FFA concentration.

Cardiovascular outcomes. Cardiovascular outcomes were documented for Biological Mechanism Cohort participants. In-hospital progression to MI was adjudicated by an independent cardiology review committee.

At 30 days post admission, infarct size was determined in those that progressed to MI. Infarct size was measured by 99mTc-sestamibi single photon emission computed tomographic perfusion imaging with the central reading and infarct size determination conducted at the trial's imaging core laboratory at Tufts Medical Center.[13,14]

Association analysis of common variants. Differences in clinical characteristics between the treatment arms were compared using comparison of means and χ^2-tests where appropriate. The relationship between FAs and clinical outcomes were assessed using Pearson's correlations. In order to detect joint significance for the main SNP effect and the SNP × intervention interaction in the same linear regression model, a two degree of freedom (2 df) test was carried out as previously described.[17] As the patients included in the IMMEDIATE Trial were ethnically diverse, in order to control for population admixture we computed principal components using EIGENSTRAT[22] to include as covariates in linear regression analyses. All statistical models reported in this study also adjusted for the potential confounding factors: age, sex, treatment arm, diabetes status and the first two PCs. The analyses investigating initial to 6-h change in FFA levels additionally adjusted for the time from infusion initiation until the first blood draw.

Even though statistical analyses were carried out on 132 634 common variants (minor allele frequency $\geqslant 0.05$), it was determined that only 87 538 were uncorrelated after accounting for linkage disequilibrium (LD) using the Li and Ji approach.[23] Therefore, after adjusting for multiple comparisons, statistical significance for the primary outcomes was deemed to have been reached if $P \leqslant 5.71 \times 10^{-7}$ (0.05/87 538). For the secondary outcomes, the multiple testing-adjusted P-value was set at $P \leqslant 1.90 \times 10^{-7}$ (0.05/87 538/3). The 2 df P-value was used to assess significance regardless of whether the association was predominantly driven by the main effect of genotype and/or the genotype × treatment interaction.

Annotation of the top association signals. We searched the SNP and CNV Annotation Database (http://www.scandb.org/newinterface/about.html)[24] for expression quantitative trait loci (eQTLs) in HapMap lymphoblastoid cell line samples, derived from individuals of European and African ancestry, for associations with our top FFA signals. We repeated this search using HaploReg, a tool for exploring annotations of the noncoding genome at variants on haplotype blocks, including candidate regulatory SNPs at disease-associated loci (http://www.broadinstitute.org/mammals/haploreg/haploreg.php). We then tested the list of eQTL-related genes for the enrichment in certain biological processes and pathways using Enrichr (http://amp.pharm.mssm.edu/Enrichr/).[25]

RESULTS

Patient characteristics

Baseline patient information for the IMMEDIATE Biological Mechanisms cohort is shown in Table 1. The Biological Mechanisms Cohort was generally representative of the entire Genetic Ancillary Study Cohort reported in our previous analyses.[17,18] However, a greater proportion of participants concurrently enrolled in the Biological Mechanism cohort were of White ethnicity when compared with those that did not provide consent to be involved in this sub-study (94.8% vs 83.6%). Despite no difference in the initial total FFA levels between the GIK and placebo groups, the proportion of saturated and unsaturated FAs differed significantly between the study arms with higher saturated and lower unsaturated FA concentrations observed in the GIK group. This trend remained at 6 and 12-h measurements,

Table 1. Patient characteristics of the IMMEDIATE trial Biological Mechanism Cohort

	N	*GIK*	N	*Placebo*	P-*value*
Demographics					
Age	52	63.8 ± 13.3	64	63.8 ± 13.1	0.995
Gender, *N* = male, (%)	52	39 (75.0%)	64	45 (70.3%)	0.574
Race, *N* (%)	52		64		0.640
White	50	96.2%	60	93.8%	
Black	2	3.8%	2	3.1%	
Asian	0	0%	1	1.6%	
Other	0	0%	1	1.6%	
Hispanic, *N* (%)	3	5.8%	7	10.9%	0.324
Clinical characteristics					
Duration of treatment (hours)	52	12.0 ± 1.9	64	12.0 ± 0.3	0.958
Time from treatment initiation until first blood draw (hours)	50	2.2 ± 1.2	59	2.9 ± 1.6	0.013
Plasma total FFA, (Umol l^{-1})					
Initial total FFA	50	661.4 ± 835.2	59	740.4 ± 337.4	0.507
6-h total FFA	49	269.2 ± 211.7	55	469.7 ± 214.4	6.0×10^{-6}
12-h total FFA	51	248.7 ± 128.3	61	451.6 ± 234.5	2.2×10^{-7}
Plasma saturated FFA (16:0+18:0 as % of total FFA)					
Initial saturated FFA	50	36.9 ± 6.9	59	31.7 ± 5.1	1.9×10^{-5}
6-h saturated FFA	49	44.2 ± 6.4	55	35.2 ± 5.7	2.06×10^{-11}
12-h saturated FFA	51	45.0 ± 7.9	61	35.1 ± 5.7	8.30×10^{-12}
Plasma unsaturated FFA (18:1n9+18:2n6 as % of total FFA)					
Initial unsaturated FFA	50	47.8 ± 8.1	59	54.3 ± 6.3	8.0×10^{-6}
6-h unsaturated FFA	49	37.9 ± 7.4	55	49.3 ± 7.1	1.18×10^{-12}
12-h unsaturated FFA	51	37.4 ± 8.6	61	49.8 ± 7.1	2.29×10^{-13}
Cardiovascular outcomes					
Progression to MI, n = Yes	52	86.5% (45)	64	92.2% (59)	0.320
30-day adjusted infarct size (% of left ventricle)	36	11.4 ± 15.9	48	17.5 ± 17.5	0.100
30-day ejection fraction (%)	38	63.1 ± 11.3	48	59.8 ± 9.9	0.153

Abbreviations: FFA, free fatty acid; GIK, glucose, insulin and potassium; IMMEDIATE, *I*mmediate *M*yocardial *M*etabolic *E*nhancement *D*uring *I*nitial *A*ssessment and *T*reatment in *E*mergency care; MI, myocardial infraction.

even though the total FFA levels were significantly lower in the GIK group at both time points (Table 1). As expected, a significant difference in the initial to 6-h FFA response was observed, with those receiving GIK exhibiting a greater increase in saturated FAs (mean GIK = 7.1%, mean placebo = 3.3%; $P = 0.01$) in combination with a larger decrease in unsaturated FAs (mean GIK = − 9.6%, mean placebo = − 4.6%; $P = 1.1 \times 10^{-4}$). Despite no significant treatment difference with cardiovascular outcomes including progression to MI ($P = 0.32$), 30-day adjusted infarct size ($P = 0.10$) or 30-day ejection fraction ($P = 0.15$) in this subgroup of patients, weak correlations were observed between the initial to 6-h changes in total FFA and proportion of unsaturated FA and infarct size in individuals with confirmed MI ($r = 0.33$, $P = 0.06$ and $r = 0.36$, $P = 0.04$, respectively; Supplementary Table 1). Also, after the adjustment for treatment regimen, there was a borderline association between the FFA response (initial to 6-h and 6- to 12-h) and progression to MI ($P = 0.06$ and 0.05, respectively; Supplementary Table 2).

Associations between common variants and total FFA

The quantile–quantile (Q–Q) plots demonstrate that the chip-wide *P*-value distribution is consistent with no systematic biases in data analysis (Supplementary Figure S1). An association between three *LINGO2* intronic variants, all in LD (r^2 range 0.83–1.0), and the initial to 6-h change in total FFA was observed ($P_{2df} \leqslant 5.51 \times 10^{-7}$). Carrying the 'A' allele of the lead SNP, rs12003487 (minor allele frequency = 0.38), was associated with a 66% increase in total circulating FFA in GIK-treated participants, whereas in the placebo group this same allele was associated with a 21% decrease ($P_{2df} = 4.64 \times 10^{-7}$; Table 2, Figure 1). An additional 38 SNPs in this locus, with varying degrees of LD with the 3 lead SNPs (r^2 range 0.009–1.0), showed suggestive associations with the initial to 6-h changes in total FFA ($P = 6.34 \times 10^{-7}$ to $P = 0.001$, Figure 2). No associations with 6- to 12-h change in total FFA was observed. Additional top common variant hits that did not reach study-wise significance are shown in Supplementary Tables 3 and 4.

Association between common variants and FFA fractions

The strongest signal to be associated with a greater decrease in the proportion of saturated FFAs (over total) in GIK-treated patients between 6 and 12 h was rs7017336, located nearest to the *IMPA1* and *FABP12* genes ($P_{2df} = 5.47 \times 10^{-7}$; Table 2). The SNP nearest to *DUSP26*, rs7464104 showed the most significant association with a decrease in unsaturated FFA levels in the GIK group and an increase in unsaturated FFA in placebo-treated individuals between the 6- and 12-h measurements ($P_{2df} = 5.51 \times 10^{-7}$; Table 2). There were no significant associations between common genetic variation and initial to 6-h change in saturated and unsaturated FFAs. The top variants associated with saturated and unsaturated FFA traits are listed in Supplementary Tables S5–S8.

Associations with cardiovascular outcomes

A nominal association was observed between the lead *LINGO2* SNP, rs12003487, and 30-day ejection fraction ($P_{2df} = 0.03$). Each copy of the 'A' allele interacted with GIK to confer an 8.8% lower ejection fraction, compared with a 1.7% lower ejection fraction in

Table 2. Chip-wide significant associations for changes in fatty acid levels

			P-value (FC GIK, FC placebo)		P-value (β GIK, β placebo)		P-value (β GIK, β placebo)	
SNP	*Gene/nearest gene*	*MAF*	*Initial to 6-h total FFA*	*6- to 12-h total FFA*	*Initial to 6-h saturated FFA*	*6- to 12-h saturated FFA*	*Initial to 6-h unsaturated FFA*	*6- to 12-h unsaturated FFA*
rs12003487	*LINGO2*	0.38	**4.64×10^{-7}**[a] (1.66, 0.79)	0.93 (1.03, 1.03)	**0.02** (−3.36, 1.21)	0.77 (−1.02, −0.15)	**1.9×10^{-4}** (5.64, −1.61)	0.79 (1.13, 0.39)
rs7017336	*IMPA1/FABP12*	0.09	**0.02** (1.82, 1.23)	0.22 (1.45, 0.37)	0.73 (−1.20, −1.53)	**5.47×10^{-7}**[a] (−11.08, −4.86)	0.41 (2.63, 2.64)	**1.06×10^{-6}** (13.75, 4.99)
rs7464104	*DUSP26*	0.05	0.66 (0.91, 1.00)	**7.78×10^{-4}** (0.78, 1.30)	0.54 (−1.21, 0.42)	**4.85×10^{-6}** (3.97, −3.17)	0.84 (0.66, −0.38)	**5.51×10^{-7}**[a] (−5.11, 4.29)

Abbreviations: FC, fold change; FFA, free fatty acid; GIK, glucose, insulin and potassium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism. [a]Chip-wide statistical significance, $P<5.71\times10^{-7}$. FC and β represent the difference in levels per allele copy. At least nominal associations ($P<0.05$) are highlighted in bold.

the placebo group. No further associations were observed for FFA-associated variants and cardiovascular outcomes.

Annotation of the top association signals

Fifteen variants within *LINGO2*, all in strong LD with each other (pairwise $r^2>0.82$; $D'>0.93$) and associated with changes in FFA during the first 6-h of infusion at $P_{2df}<10^{-5}$, were found to alter gene expression of 17 genes: *UCK1*, *JMJD4*, *TST*, *SHARPIN*, *LIPE*, *GATA3*, *RILP*, *M6PRBP1*, *APRT*, *REC8*, *EFNA2*, *LDOC1L*, *FIBP*, *CORO1A*, *KATNB1*, *KIAA0284* and *LOC606724* ($P\leqslant0.0001$) in lymphoblastoid cell lines. Supplementary Table S9 depicts SNP-expressed gene pairs with the associated *P*-values. Importantly, several SNPs were eQTLs to more than one gene and the same genes were affected by different SNPs, which is consistent with the high LD between the SNPs. All of the eQTL SNPs appeared to be *trans* acting with none appearing to be an eQTL for *LINGO2* itself or were reported in other tissues.

In addition, according to ENCODE,[26] nine of these genes (*JMJD4*, *FIBP*, *UCK1*, *SHARPIN*, *TST*, *LDOC1L*, *KATNB1*, *RILP* and *APRT*) demonstrated an enrichment in histone modification in the cardiac mesoderm cells at histone H3 on lysine residue 36, *H3K36me3*, subjected to methylation of histone tails ($P=8.47\times10^{-7}$; adjusted $P=2.83\times10^{-4}$). Moreover, according to WikiPathways (2015; http://www.wikipathways.org), an enrichment of genes involved in the adiposity pathway was observed among the *LRRK2* eQTL-related genes in both *Homo sapiens* and *Mus musculus* ($P=3.10\times10^{-3}$; adjusted $P=0.025$). No other association signals were determined to be eQTLs.

DISCUSSION

In this current study we identified a study-wide significant association between multiple variants within the *LINGO2* gene and the initial to 6-h response of total FFA. Specifically, in carriers of the lead *LINGO2* SNP, rs12003487 (minor allele frequency = 0.38), the administration of GIK resulted in a 66% increase in circulating plasma total FFA. However, in the placebo group a decrease of 21% per copy of the minor allele was observed. This is despite the fact that within the first 6 h of infusion, the overall FFA levels were more substantially reduced in the GIK group compared with placebo (Table 1). These observations are consistent with previous studies in humans and animal models that have reported that GIK decreases circulating concentrations of FFA.[27–30] Moreover, extensive experimental and clinical data have shown the adverse effects of elevated FFA levels on the ischemic and infarcted myocardium.[31] Importantly, in our study, carriers of rs12003487 who were randomized to the GIK arm and whose FFA levels increased in the first 6-h of infusion, also had a poorer 30-day ejection fraction. Existing literature does not support the direct link between FFA levels and ejection fraction,[32,33] but rather suggests the relationship between high FFA and propensity to cardiac arrest.[34] Although due to the small sample size and low event rate, the link between rs12003487 and cardiac arrest could not be assessed in the present study, future studies with outcome data will need to validate this association.

LINGO2, or leucine rich repeat and Ig domain containing 2 protein, belongs to the LINGO family of genes that encode transmembrane proteins and are thought to have an important role in the transportation of signals across the membrane.[35] *LINGO*2 has been associated with essential tremor in Parkinson's disease in multiple studies,[36,37] and a small deletion at position 9p21.1, which includes the *LINGO2* gene, has also been implicated in syndromic childhood obesity.[38] Further highlighting a potential metabolic effect, genetic variation within *LINGO2* has been associated with an increased body mass index in a genome-wide association study of 249 796 individuals,[39] and in that same study a positive suggestive association was identified with fasting

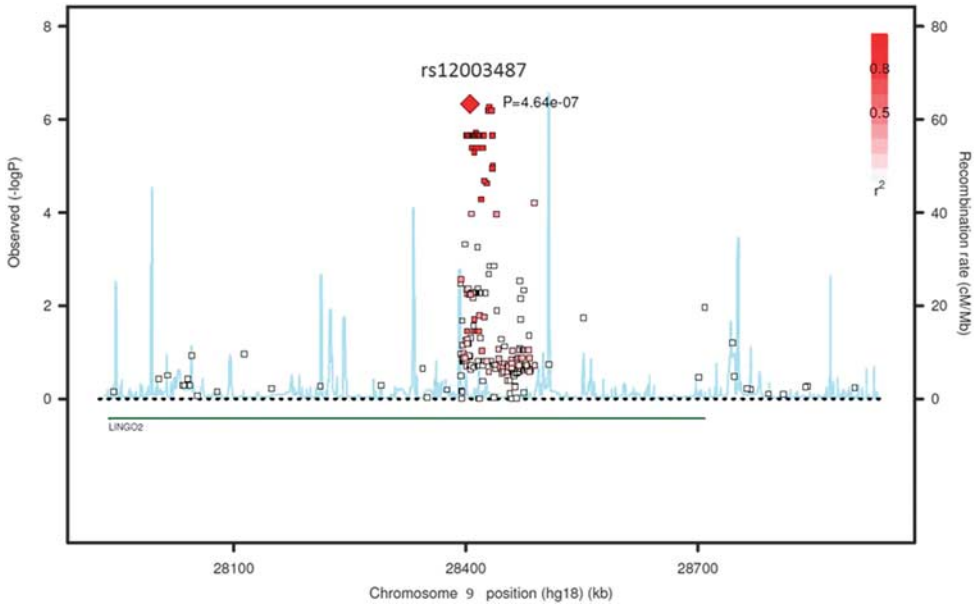

Figure 1. Regional plot of the *LINGO2* gene showing associations with initial to 6-h change in total FFA. The *x* axis shows the chromosome and physical distance (kb), the left *y* axis shows the negative base ten logarithm of the *P*-value and the right *y* axis shows recombination activity (cM/Mb) as a blue line. The degree of linkage disequilibrium of single-nucleotide polymorphisms (SNPs) with the lead SNP is indicated by a scale of intensity of red color as shown in the legend. Positions, recombination rates and gene annotations are according to the NCBI's build 36 (hg18).

insulin levels in a follow-up analysis in the MAGIC consortium.[40] This body mass index-associated variant is in weak-to-moderate LD ($r^2 = 0.3$) with rs12003487, the lead *LINGO2* variant identified in our current study.

Although further studies are warranted to determine the mechanisms for *LINGO2*'s involvement in GIK response, there are other potential explanations to the obtained results. We discovered that 14 out of the 34 top *LINGO2* signals ($P < 10^{-5}$) were eQTLs linked to differences in the expression of 17 genes. Among these genes is *LIPE* that encodes hormone-sensitive lipase. In adipose tissue and heart, hormone-sensitive lipase primarily hydrolyzes stored triglycerides to FFAs. Previous studies have also suggested a metabolic switch from lipid to carbohydrate metabolism in skeletal muscle in hormone-sensitive lipase knockout mice.[41,42] In addition, a recent paper has shown that carriers of a novel 19-bp frameshift deletion in exon 9 of *LIPE* had dyslipidemia, systemic insulin resistance and diabetes.[43] It is plausible that variation in *LINGO2* causes an increase in *LIPE* expression, resulting in an impaired metabolic switch from β-oxidation to glycolysis in cardiomyocytes. Even in the presence of glucose and insulin due to GIK, this may eventually lead to impaired contractile function and poorer cardiac efficiency. Furthermore, a number of genes whose expression is modified by the *LINGO2* variation (eQTLs) demonstrated enrichment in histone modification in the cardiac mesoderm cells at histone *H3K36* implicated in diverse processes, including alternative splicing, dosage compensation and transcriptional repression, as well as DNA repair and recombination, or were linked to the adiposity pathway. Notably, all of the *LINGO2* variants identified in our study are intronic, and none appear to be an eQTL for *LINGO2* itself. As none of these SNPs are known to have a functional impact on *LINGO2*, and there is no known biology that would suggest *LINGO2* itself acts as a transcriptional regulator, it is also possible that the associated SNPs are instead acting on regulatory elements contained within the *LINGO2* gene.

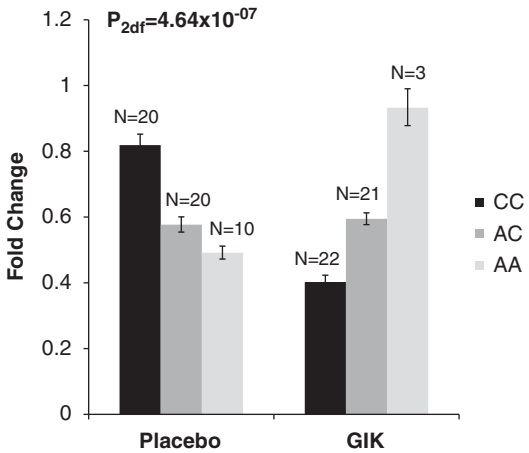

Figure 2. Association between the lead *LINGO2* SNP, rs12003487, and initial to 6-h change in total free fatty acid. GIK, glucose, insulin and potassium.

Saturated FAs (sum of 16:0 and 18:0) as percentages of total FFA increased with infusion, whereas unsaturated FAs (sum of 18:1n9 and 18:2n6) decreased in both GIK and placebo groups. The changes in both fractions were more pronounced in the GIK group. We identified an association between rs7017336 nearest to *FABP12* and *IMPA1* with saturated FFAs between 6-h and 12-h. Fatty acid-binding proteins (FABP's) have an important function in lipid trafficking, binding and transferring hydrophobic ligands including saturated and unsaturated long-chain fatty acids (C12–C20), eicosanoids, bile slats and peroxisome proliferators.[44] Currently, 10 *FABP* genes have been characterized in humans including the most recently identified and less well-studied *FABP12*.[45] *FABP12* has been mapped to the same chromosomal region as several other FABPs; which have been associated with a diverse range of cardiovascular and metabolic

phenotypes including adiposity and obesity, insulin resistance, type 2 diabetes, end-stage kidney disease, hypertension, cardiac dysfunction, atherosclerosis and the metabolic syndrome.[46–51] Genetic variation within several of the *FABP* genes has also been associated in multiple studies with these same traits.[52–55] Whether *FABP12* exhibits similar potent cardiovascular and metabolic properties remains unknown and warrants further investigation.

Also within this locus, *IMPA1* encodes the myo-inositol monophosphatase 1 gene located on chromosome 8q21. Inositol monophosphatases, including IMPA1, have been implicated in the pathogenesis of bipolar disorder, potentially via an upregulation of mitochondrial-related genes that has suggested a role for mitochondrial dysfunction in the pathophysiology of this disorder.[56] The chronic reduction of plasma FFA has also been shown to improve mitochondrial function and insulin sensitivity in obese and type 2 diabetic individuals.[57]

Nearest to *DUSP26*, rs7464104 interacted with GIK to confer a decrease in unsaturated FFAs in the final 6-h of drug infusion. *DUSP26* (dual specificity phosphatase 26) belongs to a family of dual specificity phosphatases that exhibit crucial functions in cell signaling.[58–61] *DUSP26* has multiple functions in mitogen-activated protein kinase (MAPK) signaling, which is involved in a variety of biological processes relevant to cardiovascular biology including cell growth, differentiation and apoptotic cell death.[62] MAPKs have an important role in the cardiac remodeling process,[63–65] and have been implicated in myocardial ischemia, fibrosis, hypertrophy and cardiac dysfunction.[66–68] Interestingly, elevated FFAs have also been linked to abnormal activation of MAPKs. [69,70]

There is little data available on the differential role that circulating saturated and unsaturated FA fractions may have in the context of ischemia.[71] Further studies are needed to better understand whether the distribution of FA fractions impacts cardiovascular outcomes in this setting.

Strengths of this study include the randomized nature of patient assignment in the IMMEDIATE Trial. The serial measurements obtained during infusion also allowed for the comparison of changes in FA levels, and provided information about the dynamics of the response. This study's limitations include the lack of fasting pre-treatment measurements, since the study drug was initiated by emergency medical services and the first blood draws were carried out during subsequent admission at the emergency department. Our sample size limited the statistical power to detect modest effects. Our study design does not allow for the causality of the observed associations to be determined. Although we were able to link the top *LINGO2* signals to the expression of the *LIPE* gene, which is directly involved in FA metabolism, we cannot rule out the existence of functional variant/s in one of the neighboring genes that was tagged by the *LINGO2* markers. Finally, it was not possible to replicate our findings in independent cohorts as the IMMEDIATE Trial is the first GIK clinical study that enabled DNA collection.

In summary, our findings suggest that variation in the *LINGO2* locus may modify short-term total FFA response to GIK infusion, potentially through altering the expression of the *LIPE* gene involved in FA metabolism. This may subsequently affect cardiac efficiency. Further studies are warranted to better understand the biological mechanisms of the observed associations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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