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ARTICLE

Systematic evaluation of validated type 2 diabetes and glycaemic trait loci for association with insulin clearance

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Abstract
Aims/hypothesis Insulin clearance is a highly heritable trait, for which few quantitative trait loci have been discovered. We sought to determine whether validated type 2 diabetes and/or glycaemic trait loci are associated with insulin clearance.
Methods Hyperinsulinaemic–euglycaemic clamps were performed in two Hispanic-American family cohorts totalling 1329 participants in 329 families. The Metabochip was used to fine-map about 50 previously identified loci for type 2 diabetes, fasting glucose, fasting insulin, 2 h glucose or HbA_{1c}. This resulted in 17,930 variants, which were tested for association with clamp-derived insulin clearance via meta-analysis of the two cohorts.
Results In the meta-analysis, 38 variants located within seven loci demonstrated association with insulin clearance ($p<0.001$). The top signals for each locus were rs10241087 (*DGKB/TMEM195* [*TMEM195* also known as *AGMO*]) ($p=4.4\times10^{-5}$); chr1:217605433 (*LYPLAL1*) ($p=3.25\times10^{-4}$); rs2380949 (*GLIS3*) ($p=3.4\times10^{-4}$); rs55903902 (*FADS1*) ($p=5.6\times10^{-4}$); rs849334 (*JAZF1*) ($p=6.4\times10^{-4}$); rs35749 (*IGF1*) ($p=6.7\times10^{-4}$); and rs9460557 (*CDKAL1*) ($p=6.8\times10^{-4}$).
Conclusions/interpretation While the majority of validated loci for type 2 diabetes and related traits do not appear to influence insulin clearance in Hispanics, several of these loci do show evidence of association with this trait. It is therefore possible that these loci could have pleiotropic effects on insulin secretion, insulin sensitivity and insulin clearance.

Keywords Association · Insulin clearance · Meta-analysis · Type 2 diabetes mellitus

Abbreviations

DIAGRAM	DIAbetes Genetics Replication And Meta-analysis
GWAF	Genome-Wide Association Analyses with Family Data
GWAS	Genome-wide association study
HTN-IR	Hypertension-Insulin Resistance
MACAD	Mexican-American Coronary Artery Disease
MAF	Minor allele frequency

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MAGIC	Meta-Analyses of Glucose and Insulin-related traits Consortium
PC	Principal component
SNP	Single nucleotide polymorphism
SSPI	Steady-state plasma insulin

Introduction

Type 2 diabetes arises when increases in insulin secretion become insufficient to overcome tissue insulin resistance. However, reduced insulin clearance (removal of insulin from the circulation) is another physiological response to insulin resistance that might delay the development of diabetes [1]. Reduced insulin clearance at baseline was found to predict incident diabetes after 5 years of follow-up independently of baseline insulin secretion [2]. Further studies are needed to characterise the role of insulin clearance in the genesis of diabetes.

We and others have established that there is a genetic basis for insulin clearance. Early evidence came from studies that found racial and/or ethnic differences in insulin clearance. For example, compared with non-Hispanic whites, African-Americans and Hispanics have lower insulin clearance [3, 4]. Furthermore, non-diabetic first-degree relatives of diabetic individuals have lower insulin clearance than individuals without a family history of diabetes [5]. We were the first to report the heritability of insulin clearance; in a study of Mexican-Americans, insulin clearance had a higher heritability (h^2=0.58) than either fasting insulin (h^2=0.38) or insulin sensitivity (h^2=0.44) [6]. We confirmed the high heritability of insulin clearance in an independent Hispanic cohort (h^2=0.73) [7]. Heritability studies in other ethnic groups are needed.

Despite this evidence for genetic regulation, few quantitative trait loci for insulin clearance have been identified. Validated genes for type 2 diabetes and related glucose and insulin traits are a logical source of candidate genes for insulin clearance. The most obvious of these is *IDE*, encoding insulin degrading enzyme, which cleaves insulin in cells that clear it. Genome-wide association studies (GWAS) of type 2 diabetes identified single nucleotide polymorphisms (SNPs) (rs1111875, rs5015480) linked with a region encompassing *IDE* and the neighbouring genes *HHEX* and *KIF11* [8–11]. Subsequently, rs1111875 was found to be associated with reduced insulin clearance [12]. Another study found an association between the *IDE* SNP rs1887922 and decreased hepatic insulin degradation [13]; this SNP is not in linkage disequilibrium with the type 2 diabetes SNPs noted above [14]. The Ala allele of the Pro12Ala variant (rs1801282) in the peroxisome proliferator activated receptor gamma (*PPARG*) gene is associated with reduced risk of type 2 diabetes mellitus [9–11, 15]. In carriers of the Ala allele, insulin clearance was found to be significantly greater, with lower NEFA levels [16].

It is clear that genes for diabetes and related traits that have been discovered and validated by GWAS have been inadequately studied as loci for insulin clearance, as there are now over 100 such loci for type 2 diabetes, fasting glucose, fasting insulin, 2 h glucose and HbA_{1c}. Our goal, therefore, was to systematically evaluate such loci for association with insulin clearance, using the Metabochip [17], which contains fine-mapping content for a large number of these loci.

Methods

Participants and phenotyping The current study was conducted in the two independent family cohorts in which we had previously documented the heritability of insulin clearance [6, 7]. The Hypertension-Insulin Resistance (HTN-IR) cohort consists of Los Angeles Hispanic-American families ascertained via a proband with essential hypertension. The recruitment and phenotyping of this cohort have been described previously [18]. After data cleaning as described below, we studied 638 participants from 148 HTN-IR families. The participants had undergone a euglycaemic clamp with steady-state insulin levels as a means of phenotyping insulin clearance.

The second cohort is the Mexican-American Coronary Artery Disease (MACAD) study [6]. After data cleaning as described below, 181 families were included in the current study, comprising 691 participants who were drawn from the offspring generation (adult offspring of probands with coronary artery disease and the spouses of those offspring) and underwent phenotyping for insulin clearance.

By design, participants undergoing detailed phenotyping in both cohorts were free of known diabetes and clinically manifest cardiovascular disease, thus avoiding secondary changes in phenotype caused by overt disease. A small percentage of participants in both cohorts were newly diagnosed with diabetes as a result of their participation in the studies. These participants were not taking any glucose-lowering medications when they were phenotyped.

Participants in the HTN-IR and MACAD cohorts underwent the hyperinsulinaemic–euglycaemic clamp procedure performed with the same techniques and assays. Insulin was measured using a kit (Human Insulin Specific RIA; Linco Research, St Charles, MO, USA) in which the cross-reactivity with proinsulin was <0.2%. During the clamp [19], a priming dose of human insulin (Novolin, Clayton, NC, USA) was followed by a constant-rate infusion of insulin (60 $mU m^{-2} min^{-1}$) for 120 min, the aim being to achieve a plasma insulin concentration of 600

pmol/l or greater. Blood was sampled every 5 min and the rate of co-infused 20% (20 g/100 ml) dextrose was adjusted to maintain plasma glucose concentrations at 5.27 to 5.55 mmol/l. Blood samples were drawn for glucose and insulin measurement at −30, −20, −10, 100, 110 and 120 min. The metabolic clearance rate of insulin ($ml m^{-2} min^{-1}$) is calculated as the insulin infusion rate divided by the final steady-state plasma insulin level (SSPI) (average of insulin levels at 100, 110 and 120 min) during the euglycaemic clamp. Because all our participants received the same insulin infusion rate (60 $mU m^{-2} min^{-1}$), the SSPI is a direct measure of insulin clearance at steady state and was the trait used in genetic analyses in this study [6].

All studies were approved by the Institutional Review Boards at the participating institutions. All participants gave informed consent before participation.

Metabochip genotyping and quality control in HTN-IR and MACAD cohorts Both cohorts were genotyped using the Metabochip, which was designed to provide high-throughput genotyping for replication and fine-mapping of GWAS results for cardiac (e.g. myocardial infarction, QT interval, blood pressure), metabolic (e.g. diabetes, fasting glucose, fasting insulin), anthropometric (e.g. BMI, WHR) and lipid traits [17].

Genotyping was performed at the Medical Genetics Institute at Cedars-Sinai Medical Center, using custom Infinium II technology, following the manufacturer's protocol (Illumina, San Diego, CA, USA) [20]. Quality control sampling led to 16 samples being removed for low genotyping rates (<98%) or low p10GC scores. Another 26 participants were removed for sex mismatch, which was determined using Genome Studio (Illumina).

Following these quality control steps, 640 HTN-IR and 693 MACAD participants with SSPI data were included in the study. The genotyping rate in these samples was 99.98% (HTN-IR) and 99.96% (MACAD). Across the two projects, 22 pairs of sample duplicates were run (representing 1% of the entire sample run as either within-plate or across-plate duplicates), yielding an average reproducibility of 99.99%.

For the quality control pipeline, 196,475 SNPs were available from Genome Studio (Illumina). Of these, 37,337 SNPs were excluded due to quality control variables that included poor cluster formation and a SNP failure rate of >2%. Further quality control excluded SNPs with: (1) a minor allele frequency (MAF) of <1% (HTN-IR: 37,238; MACAD: 35,992); (2) Hardy–Weinberg equilibrium at $p<1\times10^{-7}$ (HTN-IR: three SNPs; MACAD: seven SNPs); or (3) observed heterozygosity of >53% (HTN-IR: 1,135; MACAD: 709 SNPs). The final number of SNPs available after quality control was about 120,000.

In this study, we first used the fine-mapping content selected for the Metabochip by the DIAGRAM (DIAbetes Genetics Replication And Meta-analysis) and MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium) consortia. DIAGRAM selected content to fine-map loci for type 2 diabetes; MAGIC content fine-mapped loci for fasting glucose, fasting insulin, 2 h glucose and HbA_{1c}. The two consortia selected 50 loci for fine-mapping (Electronic supplementary material [ESM] Table 1). Of these, five loci (*ADCY5*, *G6PC2*, *GCK*, *KCNQ1* and *MTNR1B*) were selected for multiple traits; thus, the number of unique loci examined in this study was 45. Of the approximately 200,000 SNPs available on the Metabochip [17], the 45 loci fine-mapped for type 2 diabetes and related glycaemic traits encompass a total of 33,110 SNPs. Of these, 14,473 passed quality control in both cohorts and were analysed as part of the present study (the majority of SNPs failed quality control due to low MAF).

In 2012, the DIAGRAM and MAGIC consortia conducted large-scale association meta-analyses using the Metabochip, leading to the discovery of over 50 additional loci for type 2 diabetes, fasting glucose, 2 h glucose and fasting insulin [21, 22]. Of these, nine were fine-mapped on the Metabochip to investigate signals for traits including WHR, BMI, and HDL- and LDL-cholesterol (ESM Table 2). Given that two of these loci overlapped (*GRB14/COBLL1*), we analysed these eight unique fine-mapped loci in addition to the 45 fine-mapped loci described above, making for a total of 53 loci. The eight loci were fine-mapped by 7,848 SNPs, 3,457 of which passed quality control in both cohorts and were analysed. Therefore, the total number of SNPs analysed was 17,930 (14,473+3,457).

Population stratification A potential pitfall in association analysis is population stratification, in which systematic differences in ancestry are associated with phenotypes that might be associated with disease. The Hispanic-American population is significantly substructured [23]; it is therefore important to address the possible confounding effect of stratification. We computed principal components (PCs) of ancestry for unrelated founders and then projected them to all family members using SMARTPCA, which is distributed with the software package EIGENSTRAT [24]. The PC analysis was performed using 43,000 autosomal SNPs in the HTN-IR project and 43,000 SNPs in the MACAD project, with SNPs selected for minimal linkage disequilibrium ($r^2<0.2$). Two outliers (defined as >10 standard deviations) were identified in each of the two cohorts; these participants were excluded from association analyses, yielding sample sizes of 638 for the HTN-IR and 691 for the MACAD cohorts (for clinical characteristics, see Table 1). In the HTN-IR cohort, the top PC explained around 1.35% and the second PC around 0.5% of genetic variance; the remaining PCs each explained less than 0.4% of variance. We therefore adjusted for the top two PCs in the HTN-IR

Table 1 Characteristics of the participants in the two cohorts

Trait	HTN	MACAD	*p* value
Families (*n*)	148	181	n/a
Participants (*n*)	638	691	n/a
Male sex (%)	40.3	42.8	0.37
Age (years)	37.4±14.4	34.6±8.9	<0.0001
BMI (kg/m^2)	28.9±5.7	28.9±5.0	0.99
Diabetes (%)	6.9	2.5	<0.0001
Fasting insulin (pmol/l)	91.7±57.0	84.6±49.2	0.014
Fasting glucose (mmol/l)	5.39±0.50	5.12±0.63	<0.0001
SSPI (pmol/l)	829.4±187.1	810.6±233.4	0.11

Unless otherwise indicated, data are mean±SD (quantitative traits) or per cent (qualitative traits)

n/a, not applicable

association analysis. In the MACAD cohort, the top three PCs explained 1.11%, 0.55%, and 0.43% respectively, with the remaining PCs each explaining less than 0.4%. Therefore, the top three PCs were adjusted for in the MACAD association analyses.

Association analysis Association between individual candidate SNPs and insulin clearance measures was evaluated using the general estimating equations (GEE1) method implemented in the Genome-Wide Association Analyses with Family Data (GWAF) program [25]. GWAF uses functions in existing R packages to properly model the residual correlations in families in the test of genotype–phenotype association. The additive genetic model was used in both cohorts. In all analyses, $\log_{10}$-transformed SSPI was the dependent variable, with potential confounding factors, including age, sex, BMI and diabetes status being included, together with the top PCs, as covariates. We combined the results of the HTN-IR and MACAD cohorts using fixed-effects, inverse-variance weighting meta-analysis as implemented in METAL [26].

The power of this two-cohort meta-analysis to detect association is shown in ESM Table 3. With the two cohorts studied, we had 85% power to detect SNPs that explain 1.4% of SSPI variance. Even for a SNP explaining as little as 1% of variance, we had a power of 64% to detect association.

The Metabochip comprises a large number of SNPs and therefore a large number of statistical tests. Given the chip's focus on fine-mapping, and the fact that it contains a large number of variants in linkage disequilibrium, a simple correction for the total number of SNPs is not appropriate. Instead, we adjusted for the number of loci examined (53 loci) and defined the significance cut-off as $p<10^{-3}$ after Bonferroni's correction ($p=0.05/53$, i.e. approximately 1×10^{-3}). Only loci containing SNPs that met this level of significance in the meta-analysis were evaluated further.

Results

Characteristics of the cohorts are given in Table 1. Participants in the HTN-IR cohort were slightly older and had slightly higher fasting glucose and insulin values than participants in the MACAD cohort. They were comparable in BMI, sex distribution and SSPI.

In the HTN-IR and MACAD cohorts, 17,930 SNPs passed quality control measures and were used for association testing. The meta-analysis results did not show significant genomic inflation, with minimal deviation from what would have been expected by chance ($\lambda_{\text{genomic control}}=1.015$). Of the 45 original diabetes and related trait loci, six harboured 29 SNPs associated with SSPI at $p<0.001$ in the meta-analysis of both cohorts. These consisted of 19 SNPs in the *DGKB/TMEM195* locus, and of four, two, two, one and one SNPs in *IGF1*, *JAZF1*, *FADS1*, *CDKAL1* and *GLIS3*, respectively. None of these SNPs were identical to or in linkage disequilibrium with the index SNP used by DIAGRAM or MAGIC to select the loci for fine-mapping (ESM Table 4). Table 2 shows the associations of the lead SNP within each of the six loci. The beta values are very similar in each cohort, indicating that the associations are not driven by one study. The similar MAFs suggest that there were no unrecognised ethnic differences between the two cohorts.

Regional association plots for these six loci are given in Fig. 1. Regarding the signal at the *DGKB/TMEM195* locus, we found that the variant with the lowest *p* value, rs10241087 ($p=4.43\times10^{-5}$), had little supporting evidence of nearby associated variants with which it was in linkage disequilibrium (Fig. 1a). On the other hand, the variant with the second best *p* value, rs1431520 ($p=2.1\times10^{-4}$), manifested a robust locus of associated SNPs in the area between 14.4 and 14.6 Mb; this SNP was therefore selected as the index SNP for the regional plot in Fig. 1b. Relatively robust signals were also observed at *JAZF1* (Fig. 1d) and *IGF1* (Fig. 1g).

Of the loci recently associated with type 2 diabetes and related glycaemic traits by Metabochip studies in DIAGRAM and MAGIC [21, 22], eight were fine-mapped on the Metabochip. One of these, nearest to the *LYPLAL1* gene, contained nine SNPs associated with SSPI at $p<0.001$ in the meta-analysis of both cohorts. None of these SNPs were identical to or in linkage disequilibrium with the index SNP (rs4846567) used by the Genetic Investigation of ANthropometric Traits (GIANT) consortium to select the locus for fine-mapping of a WHR signal, nor were they in linkage disequilibrium with the SNP (rs4846565) associated with fasting insulin adjusted for BMI in MAGIC [22] (ESM Table 4). Table 2 displays the association of the lead SNP in this locus; its regional association plot is given in Fig. 1h.

We examined the SNPs in *IDE* and *PPPARG*, which have previously been reported to be associated with insulin clearance [12, 13, 16]. Neither the *IDE* SNPs rs1887922 ($p=0.10$) and

Table 2 Lead SNPs in seven loci associated with insulin clearance

Genetic variant					HTN-IR			MACAD			Meta-analysis
Chr	BP	SNP	Locus	Selection category	MAF	Beta	*p* value	MAF	Beta	*p* value	*p* value
6	20,902,604	rs9460557	*CDKAL1*	T2D	0.015	0.04	0.1	0.018	0.072	0.0016	6.81E-04
7	14,240,868	rs10241087	*DGKB/TMEM195*	Fasting glucose	0.19	0.025	0.0007	0.15	0.019	0.016	4.43E-05
7	28,190,367	rs849334	*JAZF1*	T2D	0.067	0.031	0.0092	0.059	0.028	0.025	6.37E-04
9	4,279,809	rs2380949	*GLIS3*	Fasting glucose	0.075	–0.024	0.03	0.098	–0.03	0.004	3.42E-04
11	61,288,567	rs55903902	*FADS1*	Fasting glucose	0.38	0.014	0.015	0.35	0.015	0.014	5.59E-04
12	101,464,313	rs35749	*IGF1*	Fasting insulin	0.073	–0.026	0.021	0.078	–0.028	0.013	6.71E-04
1[a]	217,605,433 [a]	chr1:217605433[a]	*LYPLAL1*[a]	WHR	0.068	0.022	0.058	0.067	0.037	0.0016	3.25E-04

The insulin clearance measure in these analyses was $\log_{10}$-transformed SSPI during the euglycaemic clamp

[a] The locus in the last line is a new locus from Metabochip studies [21, 22]; all others are original loci from GWAS

BP, base position (NCBI Genome Build 36.3, www.ncbi.nlm.nih.gov/genome/guide/human/release_notes.html, accessed 26 November, 2012); Chr, chromosome; T2D, type 2 diabetes

rs1111875 (p=0.40), nor the *PPARG* SNP rs1801282 (p=0.93) were associated with SSPI in the meta-analysis.

Discussion

In this study, we examined 53 fine-mapped loci for type 2 diabetes, fasting glucose, 2 h glucose, fasting insulin and HbA_{1c} for association with insulin clearance. We found that seven loci (*DGKB/TMEM195*, *IGF1*, *JAZF1*, *FADS1*, *CDKAL1*, *GLIS3*, *LYPLAL1*) harboured SNPs associated with euglycaemic clamp-derived insulin clearance in a meta-analysis of two Hispanic cohorts. An understanding of genetic regulators of insulin clearance is important because altered insulin levels characterise several common metabolic disorders, including diabetes, metabolic syndrome, polycystic ovary syndrome and non-alcoholic fatty liver disease.

We had previously conducted a microsatellite linkage scan for insulin clearance, which identified loci on chromosomes 15 and 20 [7]. In that prior study, we used all of the Metabochip content under the −1 linkage of the odds intervals of the two linkage peaks to identify positional candidate genes for insulin clearance. Six associated loci on chromosome 15 and two loci on chromosome 20 were identified, none of which were selected to fine-map signals for diabetes or related traits [7]. They were, however, selected to fine-map systolic and diastolic blood pressure, myocardial infarction/coronary artery disease and LDL-cholesterol. Therefore, in the current study we directly examined the diabetes and related glycaemic trait loci that were fine-mapped on the Metabochip.

None of the SNPs associated with insulin clearance in the current study were identical to or in linkage disequilibrium with the SNPs identified to be associated with diabetes or related quantitative traits in the original GWAS that identified these loci (ESM Table 4). This raises the possibility that different variants in these loci associate with diabetes-related traits and with insulin clearance. Alternatively, the insulin clearance-associated SNPs might represent diabetes signals in Hispanics. We could not test this hypothesis in the current study because our cohorts are largely free of diabetes. Published studies have examined the European SNPs of only two of these genes in Hispanics, with findings including: (1) mixed results regarding the association between *CDKAL1* and type 2 diabetes [27–29]; (2) positive association between *CDKAL1* variation and reduced insulin secretion [30]; and (3) negative association between *JAZF1* variation and diabetes [27].

Of the seven loci identified, five have been found to be associated with physiological measurements of insulin secretion derived from: (1) OGTTs (*FADS1* [31, 32], *DGKB* [31, 32], *GLIS3* [32], *CDKAL1* [33], *JAZF1* [34]); (2) intravenous glucose tolerance tests (*CDKAL1* [35]); and (3) hyperglycaemic clamps (*CDKAL1* [36]). *IGF1* is thought to influence insulin sensitivity, based on its association with fasting insulin and OGTT-based insulin sensitivity assessment [31, 37]. Interestingly, none of these physiological studies examined whether variants in these loci were associated with insulin clearance. Reduced insulin response to oral glucose administration has been the most common finding used to conclude that a diabetes gene acts through insulin secretion; however, hepatic insulin clearance may significantly impact on insulin levels during an OGTT, as the liver removes around 50% of insulin at first pass [38]. Regarding insulin secretion, the most compelling functional studies have been performed for *CDKAL1*, which has been found to encode a methylthiotransferase that modifies a transfer RNA for lysine; murine beta cells deficient in CDK5 regulatory subunit associated protein 1-like 1

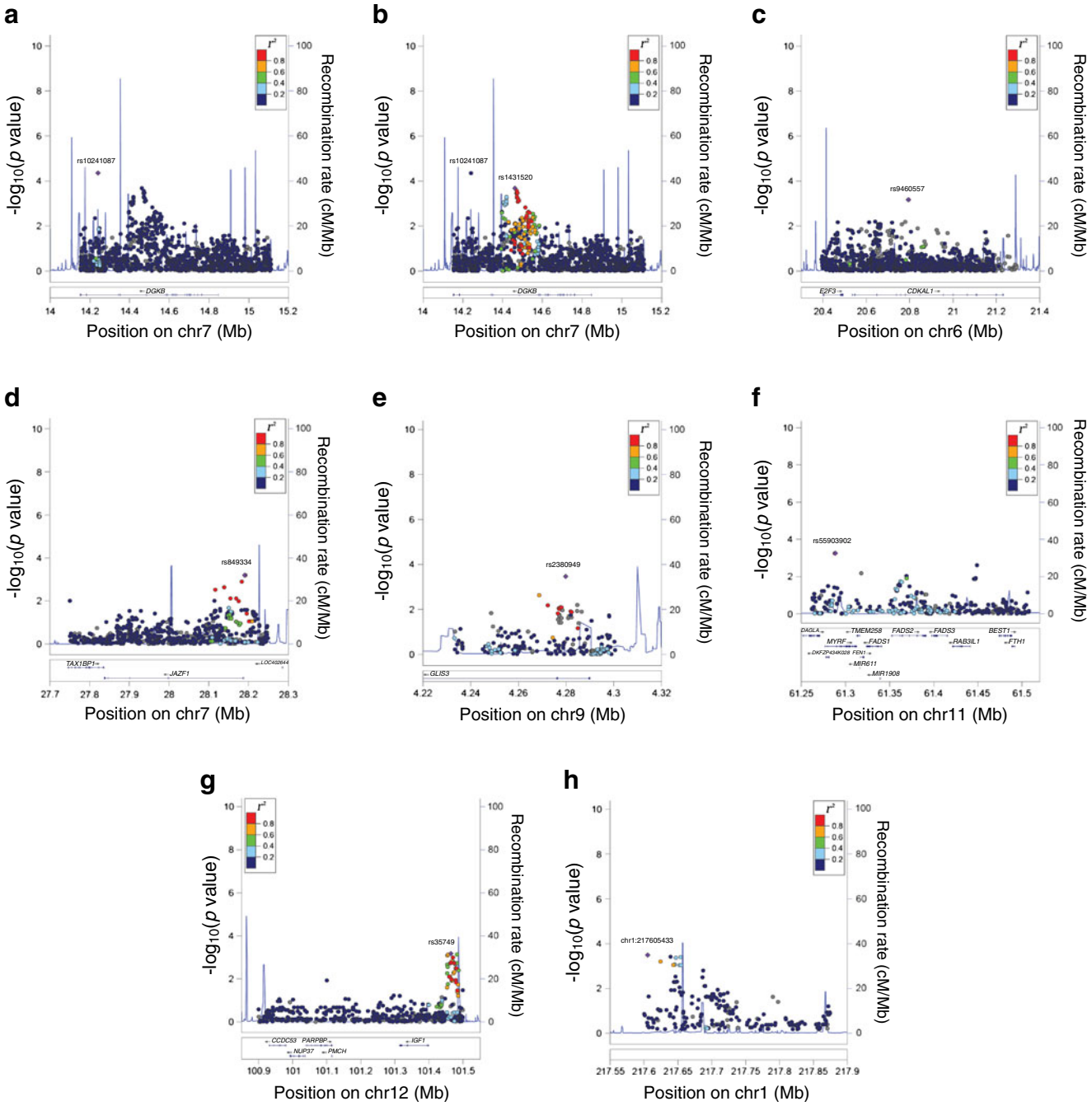

Fig. 1 Regional association plots of association with insulin clearance. The plots display genes and recombination rates in each region. SNP associations with insulin clearance are plotted as $-\log_{10}(p \text{ value})$. The SNP used as the index SNP in each plot is plotted as a purple diamond and labelled. Linkage disequilibrium of nearby SNPs with the index SNP is indicated by colors: dark blue for $r^2<0.2$; light blue for $0.2<r^2<0.4$; green for $0.4<r^2<0.6$; orange for $0.6<r^2<0.8$; and red for $r^2>0.8$. (**a**) *DGKB* (based on rs10241087), (**b**) *DGKB* (based on rs1431520), (**c**) *CDKAL1* (based on rs9460557), (**d**) *JAZF1* (based on rs849334), (**e**) *GLIS3* (based on rs2380949), (**f**) *FADS1* (based on rs55903902), (**g**) *IGF1* (based on rs35749), (**h**) *LYPLAL1* (based on chr1:217605433)

(CDKAL1) exhibited misreading of the lysine codon during proinsulin translation, resulting in an accumulation of aberrant proinsulin, endoplasmic reticulum stress, decreased insulin secretion and hyperglycaemia [39]. *GLIS3* codes for a transcription factor that plays a role in pancreatic development; it also transactivates the insulin gene in beta cells [40]. For the remaining loci, strong functional evidence that they affect pancreatic beta cell development or function has yet to be published. The primary effect of these loci may be on insulin secretion, with secondary effects on insulin clearance, as the pulsatility of insulin secretion may regulate the rate of hepatic insulin extraction [41].

Alternatively, it is possible that these loci (or a subset of them) exert pleiotropic influences on insulin secretion (insulin sensitivity for *IGF1*) and insulin clearance. For example, *GLIS3* also affects development of the liver and kidney [42], two organs that clear insulin. Pleiotropic effects of many of these loci on other traits have already been suggested by genetic association studies. For example, *GLIS3* was identified in a GWAS for type 1 diabetes [43]. Variation in *CDKAL1* has been associated with type 2 diabetes, Crohn's disease and psoriasis, the variant that affects diabetes being independent of those implicated in the other two [44].

The liver is the main organ that clears insulin. In obesity and insulin resistance, insulin clearance is reduced, possibly related to the hepatic steatosis that commonly occurs in these states. Three loci, *DGKB*, *FADS1* and *LYPLAL1*, modulate fatty acid and lipid metabolism, and may contribute to hepatic insulin resistance. Insulin action and insulin clearance are intimately linked in the liver, as the first event for both is binding of insulin to the insulin receptor, with receptor binding triggering internalisation of the insulin-insulin receptor complex by endocytosis [38]. *DGKB* encodes a diacylglycerol kinase, which regulates cellular levels of diacylglycerol by converting it to phosphatidic acid. Diacylglycerol accumulation in liver has been found to impair insulin signalling by activating protein kinase C epsilon [45]. Diacylglycerol kinase activity may also regulate the amount of diacylglycerol available for conversion to triacylglycerol by diacylglycerol acyltransferases. *FADS1* codes for delta 5 fatty acid desaturase, which adds double bonds to fatty acids, playing a central role in the generation of highly unsaturated long-chain fatty acids. Depressed delta 5 saturation has been associated with obesity and insulin resistance in multiple studies [46, 47]. Interestingly, the *FADS1* locus was identified in a recent GWAS for liver enzyme concentrations, which are often abnormal in hepatic steatosis [48]. We note that our insulin clearance signal at the *FADS1* locus encompasses several other genes, including two other related factors, *FADS2* and *FADS3* (Fig. 1f), along with a number of unrelated genes (e.g. *DAGLA*, which encodes a DAG lipase), any of which could ultimately contribute to the insulin clearance signal. *LYPLAL1* codes for a lipase whose function is not well understood. Several variants in linkage disequilibrium near *LYPLAL1* have been associated with WHR, fasting insulin, triacylglycerol and adiponectin levels; independent variants have also been associated with non-alcoholic fatty liver disease and visceral fat [22, 49–53]. An association between this WHR locus and insulin clearance is consistent with the finding that waist circumference was correlated with insulin clearance independently of BMI and insulin sensitivity [54]. The observations above raise the possibility that *DGKB*, *FADS1* and *LYPLAL1* affect insulin clearance via their effects on hepatic lipid metabolism, steatosis and/or insulin responsiveness.

IGF1 codes for insulin-like growth factor 1, which mediates the action of growth hormone. IGF-1 and growth hormone excess (acromegaly, gigantism) are known to induce insulin resistance, diabetes and other features of the metabolic syndrome. Little is known about whether IGF-1 influences insulin clearance. A small study in which six adults with severe growth hormone deficiency were treated with low-dose growth hormone found no change in insulin clearance as determined by the clamp procedure [55]. Receptors for IGF-1 and insulin are known to form hybrids [56]. We speculate that IGF-1 could affect insulin clearance by binding to hybrid receptors or by its cross-reactivity with insulin receptors, which might influence the availability of receptors for insulin internalisation.

We used HaploReg (www.broadinstitute.org/mammals/haploreg/haploreg.php, accessed 26 November 2012) to conduct a preliminary assessment of the potential functional impact of the lead SNPs in Table 2 (as well as of rs1431520 from the *DGKB* locus). This revealed strong conservation of the sequence encompassing four SNPs (rs1431520, *DGKB*; rs2380949, *GLIS3*; rs849334, *JAZF1*; rs9460557, *CDKAL1*). Each of these SNPs was found to alter the binding sites for several transcription factors. In particular, the *CDKAL1* SNP alters binding of forkhead family transcription factors; the *DGKB* SNP alters binding of homeobox family transcription factors.

We did not replicate the previously noted associations between both *PPARG* and the *HHEX/IDE* locus and insulin clearance [12, 13, 16]. One possible explanation is that those previous studies were conducted in German European individuals, while ours were conducted in Hispanic-Americans; differences in the genetic architecture of insulin clearance and/or linkage disequilibrium patterns between the ethnic groups may thus explain the non-replication. Furthermore, the German studies mainly [16] or exclusively [12, 13] used OGTT-derived measures of insulin clearance, which primarily reflect hepatic insulin clearance [16]. On the other hand, we used the euglycaemic clamp, which quantifies whole-body (hepatic and peripheral) insulin clearance. This difference in phenotyping may also have contributed to the non-replication.

One limitation of our study concerns the accuracy of SSPI as a measurement of the metabolic clearance rate of insulin. Because we did not measure C-peptide levels during the euglycaemic clamps to document suppression of endogenous insulin secretion, it is possible that our estimates of insulin clearance may underestimate the true values. However, because the proportion of SSPI represented by residual insulin secretion is expected to be small during hyperinsulinaemic infusion, we are confident that this had a minimal effect on our association results.

In conclusion, this study identified additional quantitative trait loci for insulin clearance by focusing on fine-mapping of 53 loci for diabetes and related traits. Approximately 20 additional loci for type 2 diabetes and related quantitative traits were identified by GWAS published after the design of the Metabochip. Furthermore, Metabochip studies by the DIAGRAM and MAGIC consortia have recently validated over 50 additional loci for these traits [21, 22], only eight of which we were able to study in detail, given the available fine-mapping content. Additional work will be needed in future to determine whether variants in the remaining newer loci are also associated with insulin clearance. Finally, our studies in genetic determinants of insulin clearance were conducted exclusively in Hispanics; studies in other ethnic groups are now needed to shed light on this trait, which is a major contributor to circulating insulin levels [54].

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