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Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge

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79Full membership list of the GIANT consortium is provided in the Supplementary Note.

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AUTHOR CONTRIBUTIONS


COMPELING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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Abstract

Glucose levels 2 h after an oral glucose challenge are a clinical measure of glucose tolerance used in the diagnosis of type 2 diabetes. We report a meta-analysis of nine genome-wide association studies (n = 15,234 nondiabetic individuals) and a follow-up of 29 independent loci (n = 6,958–30,620). We identify variants at the GIPR locus associated with 2-h glucose level (rs10423928, $\beta$ (s.e.m.) = 0.09 (0.01) mmol/l per A allele, $P = 2.0 \times 10^{-15}$). The GIPR A-allele carriers also showed decreased insulin secretion ($n = 22,492$; insulinogetic index, $P = 1.0 \times 10^{-17}$; ratio of insulin to glucose area under the curve, $P = 1.3 \times 10^{-16}$) and diminished incretin effect ($n = 804$; $P = 4.3 \times 10^{-8}$). We also identified variants at ADCY5 (rs2877716, $P = 4.2 \times 10^{-16}$), VPS13C (rs17271305, $P = 4.1 \times 10^{-8}$), GCKR (rs1260326, $P = 7.1 \times 10^{-11}$) and TCF7L2 (rs7903146, $P = 4.2 \times 10^{-10}$) associated with 2-h glucose. Of the three newly implicated loci (GIPR, ADCY5 and VPS13C), only ADCY5 was found to be associated with type 2 diabetes in collaborating studies ($n = 35,869$ cases, $89,798$ controls, OR = 1.12, 95% CI 1.09–1.15, $P = 4.8 \times 10^{-18}$).

Type 2 diabetes (T2D) is defined as a state of chronic hyperglycemia defined as elevated glucose levels measured either when fasting or 2 h after glucose challenge (2-h glucose).
during an oral glucose tolerance test (OGTT). GWAS have contributed to the identification of many established T2D-associated loci. More recently, collaborative efforts of the Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC) and other investigators have led to the discovery of genetic variation associated with fasting glucose levels in nondiabetic individuals, with MTNR1B additionally conferring risk of T2D. Not all loci associated with fasting glucose showed association with T2D, suggesting that GWAS of quantitative traits related to diabetes can also identify physiological loci that provide mechanistic insights into normal trait variation. An accompanying study by MAGIC has identified 16 loci associated with fasting glucose or fasting insulin in a GWAS-based meta-analysis; 9 of these loci are newly identified, and 5 also show evidence for association with T2D.

Although there are common mechanisms, such as insulin secretion, that regulate fasting and stimulated glucose levels, there are distinct mechanisms regulating glucose levels after an oral glucose challenge. For example, oral glucose intake engenders the incretin effect, in which intestinal cells release insulin secretagogues, mainly glucagon-like peptide 1 (GLP1) and gastric inhibitory polypeptide (GIP), leading to a higher insulin response compared to that from a matched intravenous glucose stimulation. Additionally, numerous epidemiological studies have shown that OGTT 2-h glucose levels predict cardiovascular disease morbidity and mortality, even in the nondiabetic range of hyperglycemia.

Two-hour glucose level is a heritable quantitative trait (heritability ($h^2 = 0.40$)) that has been associated with diabetes, and assessing the genetic contribution to variability in 2-h glucose provides an opportunity to identify genetic variation underlying this trait in nondiabetic individuals and to test the secondary hypothesis that these loci may also contribute to T2D susceptibility. Here we performed a meta-analysis of several 2-h glucose GWAS to expand our understanding of post–oral glucose challenge physiology in nondiabetic individuals.

A meta-analysis combining 9 discovery GWAS ($n = 15,234$) and replication stages with up to 29 SNPs in 17 studies comprising up to 30,620 individuals of European descent revealed 5 loci associated with 2-h glucose at genome-wide significance ($P = 5 \times 10^{-8}$; see Online Methods, Table 1, Fig. 1, Supplementary Fig. 1 and Supplementary Tables 1 and 2). Three loci were newly associated with 2-h glucose in an analysis adjusted for age, sex, BMI and study-specific covariates: GIPR (gastric inhibitory polypeptide receptor, rs10423928, $\beta$ (s.e.m.) = 0.09 (0.01) mmol/l per A allele, $P = 2.0 \times 10^{-15}$), VPS13C (vacuolar protein sorting 13 homolog C, rs17271305, $\beta$ (s.e.m.) = 0.06 (0.01) mmol/l per G allele, $P = 4.1 \times 10^{-8}$) and ADCY5 (adenylate cyclase, 5 rs2877716, $\beta$ (s.e.m.) = 0.09 (0.01) mmol/l per C allele, $P = 4.2 \times 10^{-16}$). The ADCY5 locus was also identified by an accompanying study reporting meta-analysis in MAGIC for fasting glucose levels ($r^2 = 0.82$ to the most significant fasting glucose SNP rs11708067). The remaining loci identified here included the previously published fasting glucose–associated gene GCKR (glucokinase (hexokinase 4) regulator, missense SNP rs1260326, $P = 7.1 \times 10^{-11}$) and the established T2D-associated gene TCF7L2 (transcription factor 7-like 2, rs12243326 with $r^2 = 0.79$ to most significant T2D SNP rs7903146, $P = 4.2 \times 10^{-10}$).

To determine whether these associations reflected differences in fasting glucose levels or whether they primarily influenced the incremental response to a glucose challenge, we repeated our association analysis including fasting glucose as a covariate (Table 1 and Supplementary Table 2). Adjusting for fasting glucose resulted in increased effect size for the GCKR, GIPR and VPS13C loci and supported their specific role in post-challenge glucose regulation. In contrast, adjusting for fasting glucose slightly decreased the effect for
the ADCY5 and TCF7L2 loci, which suggested that the risk alleles in both genes increase glucose levels both in the fasting and post-challenge state.

In meta-analyses available from MAGIC, fasting glycemic traits variants at the GIPR, VPS13C and ADCY5 loci were not associated with fasting insulin or insulin resistance as measured by homeostasis model assessment, which may reflect the inadequacy of the crude measures used here or may reflect a lack of power to detect small effects (Supplementary Table 3). Associations of risk alleles in GCKR and TCF7L2 with fasting glycemic traits have been reported previously. In a large Swedish meta-analysis ($n = 27,628$), the GIPR rs10423928 2-h glucose–raising allele was significantly associated with lower BMI ($P_{\text{meta}} = 7.5 \times 10^{-5}$, V.L. and L.G., unpublished data).

GIP is one of the two incretin hormones that stimulate insulin response after an oral glucose challenge. It has been shown that the incretin effect is impaired in individuals with T2D; specifically, in individuals with T2D, stimulated GIP secretion appears normal and their insulinotropic response to GIP is reduced. GIPR is therefore a biologically plausible candidate for mediating insulin secretion after oral glucose challenge. We tested associations of GIPR variants with indices of oral glucose–stimulated insulin secretion in up to 13 studies with samples measured at multiple times during the OGTT (Table 2 and Supplementary Table 4). The rs10423928 A allele associated with increased 2-h glucose was also associated with lower insulinoenic index ($\beta$ (s.e.m.) = $-0.08$ (0.01) $\mu$U/mmol, $P = 1.0 \times 10^{-17}$), which represents a reduction in the early phase of insulin secretion. The rs10423928 A allele was also associated with a lower ratio of insulin to glucose area under the curve (AUC$_{\text{ins/gluc}}$, $\beta$ (s.e.m.) = $-0.05$ (0.01) pmol/mmol, $P = 1.3 \times 10^{-16}$), which is an integrated measure of insulin response over the 2-h OGTT. Furthermore, the rs10423928 A allele was associated with lower 2-h insulin level (adjusted for 2-h glucose, $\beta$ (s.e.m.) = $-0.04$ (0.01) pmol/l, $P = 2.0 \times 10^{-13}$).

Because GIP is involved in the insulin response specific to an oral glucose challenge, GIPR variation was not expected to influence the insulin response to an intravenous glucose load. We tested the insulin response in 1,509 nondiabetic participants from four studies who underwent an intravenous glucose tolerance test (IVGTT). No association was observed with measures of acute insulin response (AIR) from the IVGTT ($P = 0.12$; Supplementary Table 5), even though the study had >97% power to detect an effect explaining 1% trait variance ($\alpha = 0.05$). We also derived an estimate of the incretin effect by comparing the insulin response to oral versus intravenous glucose administered to the same 804 nondiabetic individuals from the Botnia, Denmark and EUGENE2-Kuopio studies. Individuals carrying the A risk allele of rs10423928 in GIPR showed a significantly lower incretin effect ($\beta$ (s.e.m.) = $-0.012$ (0.004), $P = 4.3 \times 10^{-4}$; Fig. 2 and Supplementary Table 5). Our results are consistent with animal studies, in which mice with targeted deletion of Gipr showed mild glucose intolerance and reduced insulin secretion in response to an oral glucose challenge but showed normal fasting glucose and normal insulin secretion in response to an intraperitoneal glucose challenge.

The variant in GIPR most significantly associated with 2-h glucose (rs10423928) is an intronic SNP with no known function based on FastSNP (see URL section). Notably, rs10423928 is in strong linkage disequilibrium ($r^2 = 0.93$) with a missense mutation (at rs1800437, resulting in the substitution E354Q). Some groups have explored the E354Q substitution as a candidate for association with T2D. One study showed that people homozygous for the Gln354-encoding allele of this gene had lower fasting and post oral-load C-peptide levels, suggesting a role for GIPR in insulin secretion; this is in line with our observations. In small T2D case-control studies, no association has been observed at GIPR. We performed a meta-analysis of 16 T2D association studies ($n = 19,091$).
We assessed the mRNA expression patterns of GIPR and the nearest upstream (EML2) and downstream (SNRPD2) genes in a human tissue panel (Fig. 3). All three genes were expressed in the pancreas, but only GIPR had strong specific mRNA expression in the sorted pancreatic beta cells, supporting the implication of GIPR in insulin secretion. No significant difference in GIPR, EML2 or SNRPD2 mRNA expression in pancreatic islets was seen based on the rs10423928 genotype (for GIPR P = 0.76, n = 19; Supplementary Note).

As adenylate cyclases have been implicated in the cAMP pathway of GLP-1 and GIP-induced insulin release by beta cells24,25, we also tested for association of the most significant ADCY5 variant with measures of insulin response and risk of T2D. The 2-h glucose-raising C allele of rs2877716 was associated with lower 2-h insulin (P = 1.4 × 10^{-6}) but was not associated with AUC_{ins/gluc} (P = 0.16) or with the insulinogenic index (P = 0.23; Table 2 and Supplementary Table 4). The lack of association with the two latter indices suggests that ADCY5 is unlikely to be directly involved in insulin secretion in response to an oral glucose challenge and may not operate in the same pathway as GIPR. In support of our observations, the mRNA expression pattern of ADCY5 reported in the recent MAGIC study on fasting glucose traits6 shows that ADCY5 is most highly expressed in heart and brain tissues, with weaker expression in the pancreas, islets and sorted beta cells. Finally, we found that the rs2877716 C allele was also associated with increased risk of T2D (OR = 1.12, 95% CI 1.09–1.15, P = 4.8 × 10^{-18}) in a separate meta-analysis of 25 association studies (total n = 35,869 cases, 89,798 controls; Table 3 and Supplementary Table 6) and was associated with increased risk of developing future T2D in 16,061 individuals from the Malmo Preventive Project (OR = 1.19, 95% CI 1.10–1.29, P = 3.13 × 10^{-5}; see Supplementary Note). Taken together, our results do not support a role for ADCY5 in early insulin secretion in response to an oral glucose load, but it remains to be determined how it (or another causal gene at the locus) contributes to risk for T2D.

We tested association of the VPS13C variant with insulin secretion indices because of its novelty and unknown function (Table 2 and Supplementary Table 4). The risk allele G of rs17271305 associated with higher 2-h glucose was also associated with lower 2-h insulin (P = 7.5 × 10^{-11}). rs17271305 showed no association with AUC_{ins/gluc} (P = 0.86) but was nominally associated with insulogenic index (P = 0.01). The VPS13C variant was not associated with T2D (OR = 0.97, 95% CI 0.94–1.00, P = 0.08) (Table 3 and Supplementary Table 6), suggesting that it may contribute to normal variation in 2-h glucose but not susceptibility to T2D. Investigation of the mRNA expression profiles of VPS13C revealed the presence of transcripts in several organs including brain, adipose tissue, liver, pancreas, and, most strongly, in sorted beta cells (Fig. 3). Analysis of the neighboring gene FAM148A indicated a pancreatic tissue-specific mRNA expression profile, mainly in beta cells (Fig. 3); however, its expression was not altered by VPS13C genotype in pancreatic islets (P = 0.9, n = 19; Supplementary Note).

VPS13C spans 208 kb on chromosome 15 and encodes a protein homolog of the yeast vacuolar protein sorting 13. This family of proteins is involved in trafficking of membrane proteins between the trans-Golgi network and the prevacuolar compartment26. rs17271305, identified by the 2-h glucose meta-analysis, is 101 kb from the FAM148B association signal (rs11071657) identified by the MAGIC fasting glucose meta-analysis6, but could represent...
an independent signal, as rs17271305 is weakly correlated with rs11071657 ($r^2 = 0.28$ in HapMap CEU, $P_{2-h \text{ glucose}} = 0.002$). Detailed fine-mapping and functional analyses will be needed to definitively establish the causal gene and variant(s) at this locus.

In conclusion, we report a GWAS for glucose levels 2 h after an oral glucose challenge, and we have investigated the role of newly discovered 2-h glucose variants in influencing normal physiology and potentially influencing risk of T2D. We identified five loci associated with 2-h glucose, in GIPR, VPS13C, ADCY5, GCKR and TCF7L2. As the physiological roles of GCKR and TCF7L2 variants have been examined in detail previously\textsuperscript{17,27}, we focused on the three newly identified associated loci. ADCY5 variants are associated with fasting\textsuperscript{6} and 2-h glucose levels and with an increased risk of T2D, highlighting the fact that investigation of diabetes-related quantitative traits can lead to identification of additional T2D-associated loci. VPS13C variants may contribute to normal variation in 2-h glucose, but their effect on T2D pathogenesis is unclear.

Our association results suggest a role for GIPR in the incretin effect and in early pathophysiologic pathways that could lead to impaired glucose tolerance and T2D in humans. Previously, it was hypothesized that patients with T2D might express a smaller amount of GIPR or defective GIPR\textsuperscript{28}. Meier et al. observed that individuals with T2D and a subgroup of the first-degree relatives of these individuals had a blunted insulin response to GIP, supporting the hypothesis that a defect of the GIPR could be part of the T2D pathophysiology\textsuperscript{29}. Future studies should examine how GIPR variants may modify response to treatments targeting the enteroinsular axis.

### Methods

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

### Acknowledgments

The authors would like to thank the many colleagues who contributed to collection and phenotypic characterization of the clinical samples, as well as genotyping and analysis of the GWA data. We gratefully acknowledge those who agreed to participate in these studies. A full list of acknowledgments and funding support for each study is described in the Supplementary Note.

### References


Figure 1.
Regional plots of five genome-wide significant associations for 2 hour glucose based on 2 hour glucose discovery analysis adjusted for age, sex, BMI and study-specific covariates. (a–e) For each of the GCKR (a), ADCY5 (b), TCF7L2 (c), VPS13C (d) and GIPR (e) regions, directly genotyped and imputed SNPs are plotted with their meta-analysis $P$ values (as $-\log_{10}$ values) as a function of genomic position (NCBI Build 36; hg 18). In each panel, the SNP taken forward for replication (large red diamond) and joint discovery and replication $P$ value (blue diamond) are shown. Estimated recombination rates (HapMap) are plotted to reflect the local linkage disequilibrium structure around the associated SNPs and their correlated proxies ($0 < r^2 < 1$, represented on a white to red scale, based on pairwise $r^2$ values from HapMap CEU). Gene annotations were taken from the UCSC genome browser.
Figure 2.
Percent incretin effect in the Botnia, Denmark and EUGENE2-Kuopio studies of nondiabetic individuals \((n = 804)\) by \(GIPR\) rs10423928 genotype. Mean and s.d. for each study are displayed by genotype (see Supplementary Table 5 for details). Incretin effect was adjusted for age, sex and BMI and study-specific covariates.
Figure 3.
mRNA expression in human tissues of the genes located in the \textit{GIPR} (a) and \textit{VPS13C} (b) regions. Expression data is relative expression levels measured by quantitative RT-PCR. All samples were run in triplicate and normalized to the GAPDH relative expression level. AU, arbitrary units.
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Table 1

Genome-wide significant loci for 2-h glucose during an OGTT from 26 studies in nondiabetic individuals

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<td>7.05 × 10⁻¹¹</td>
<td>0.10 (0.01)</td>
<td>9.23 × 10⁻²¹</td>
<td>2.26 × 10⁻²¹</td>
<td></td>
</tr>
<tr>
<td>rs287716</td>
<td>3</td>
<td>124577141</td>
<td>ADCY5</td>
<td>C/T</td>
<td>0.77</td>
<td>0.10 (0.02)</td>
<td>6.26 × 10⁻⁶</td>
<td>0.09 (0.01)</td>
<td>1.21 × 10⁻¹¹</td>
<td>0.09 (0.01)</td>
<td>4.19 × 10⁻¹⁶</td>
<td>0.07 (0.01)</td>
<td>1.68 × 10⁻¹¹</td>
<td>7.98 × 10⁻¹²</td>
<td></td>
</tr>
<tr>
<td>rs1224326</td>
<td>10</td>
<td>114778805</td>
<td>TCF7L2</td>
<td>C/T</td>
<td>0.21</td>
<td>0.13 (0.02)</td>
<td>1.20 × 10⁻⁹</td>
<td>0.05 (0.02)</td>
<td>1.27 × 10⁻³</td>
<td>0.08 (0.01)</td>
<td>4.23 × 10⁻¹⁰</td>
<td>0.07 (0.01)</td>
<td>9.99 × 10⁻⁹</td>
<td>1.17 × 10⁻¹⁰</td>
<td></td>
</tr>
<tr>
<td>rs17271305</td>
<td>15</td>
<td>60120272</td>
<td>VPS13C</td>
<td>G/A</td>
<td>0.42</td>
<td>0.09 (0.02)</td>
<td>1.04 × 10⁻⁶</td>
<td>0.05 (0.02)</td>
<td>1.58 × 10⁻³</td>
<td>0.06 (0.01)</td>
<td>4.11 × 10⁻⁸</td>
<td>0.07 (0.01)</td>
<td>4.33 × 10⁻¹¹</td>
<td>8.41 × 10⁻¹¹</td>
<td></td>
</tr>
<tr>
<td>rs10423928</td>
<td>19</td>
<td>50874144</td>
<td>GIPR</td>
<td>A/T</td>
<td>0.18</td>
<td>0.15 (0.03)</td>
<td>3.33 × 10⁻⁶</td>
<td>0.09 (0.01)</td>
<td>2.30 × 10⁻¹¹</td>
<td>0.09 (0.01)</td>
<td>1.98 × 10⁻¹⁵</td>
<td>0.11 (0.01)</td>
<td>2.56 × 10⁻²⁰</td>
<td>5.94 × 10⁻¹⁸</td>
<td></td>
</tr>
</tbody>
</table>

Results from fixed effects, inverse variance meta-analysis of 9 GWA (ARIC, BLSA, CHS stage1&2, CoLaus, DGI, Fenland, FHS, FUSION, Sorbs) and 17 follow-up studies (Amish, BotniaPPP, CHS stage3, DIAGEN, ELY, FrenchFamilyMembers, FrenchHaguenau, FrenchObeseAdults, FUSION stage2, HERFORD, Inter99, METSIM, NHANES, RISC, Roche, ULSAM, Whitehall II) with adjustment for age, sex and BMI. Position based on hg18, NCBI build36. Combined discovery and replication P values for 2-h glucose adjusted for age and sex (no BMI), and further adjusted for fasting glucose are also presented. Replication meta-analysis results and joint discovery and replication meta-analysis results include proxy SNPs with r² > 0.8 in HapMap CEU.

Allele frequencies based on HapMap phase II CEU sample. FG adj, adjusted for fasting glucose in addition to age, sex, BMI and study-specific covariates (center).
Table 2

Effect of ADCY5, VPS13C and GIPR variants on indices of insulin response during an OGTT

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Nearest gene</th>
<th>Effect allele</th>
<th>n</th>
<th>Effect (s.e.m.) µU/mmol (BMI-adj)</th>
<th>P value (BMI-adj)</th>
<th>P value</th>
<th>Effect (s.e.m.) pmol/mmol (BMI-adj)</th>
<th>P value (BMI-adj)</th>
<th>P value</th>
<th>Effect (s.e.m.) pmol/l (BMI-adj)</th>
<th>P value (BMI-adj)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2877716</td>
<td>3</td>
<td>ADCY5</td>
<td>C</td>
<td>19,461</td>
<td>−0.011 (0.009)</td>
<td>0.23</td>
<td>0.22</td>
<td>−0.010 (0.007)</td>
<td>0.16</td>
<td>0.18</td>
<td>30,987</td>
<td>1.43 × 10^{-6}</td>
<td>3.09 × 10^{-6}</td>
</tr>
<tr>
<td>rs17271305</td>
<td>15</td>
<td>VPS13C</td>
<td>G</td>
<td>13,911</td>
<td>0.024 (0.010)</td>
<td>0.01</td>
<td>0.02</td>
<td>−0.001 (0.007)</td>
<td>0.86</td>
<td>0.76</td>
<td>23,842</td>
<td>7.45 × 10^{-11}</td>
<td>2.58 × 10^{-10}</td>
</tr>
<tr>
<td>rs10423928</td>
<td>19</td>
<td>GIPR</td>
<td>A</td>
<td>22,529</td>
<td>−0.076 (0.009)</td>
<td>1.00 × 10^{-17}</td>
<td>2.44 × 10^{-20}</td>
<td>−0.051 (0.007)</td>
<td>9.50 × 10^{-17}</td>
<td>3.39 × 10^{-20}</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AUC_{ins/gluc}, area under the curve for insulin divided by area under the curve for glucose.
Table 3

Meta-analysis of T2D association studies for SNPs at previously unknown 2-h glucose–associated loci

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Nearest gene</th>
<th>Effect allele</th>
<th>n studies</th>
<th>n cases</th>
<th>n controls</th>
<th>T2D fixed effects</th>
<th>T2D random effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2877716</td>
<td>3</td>
<td>ADCY5</td>
<td>c</td>
<td>25</td>
<td>35,869</td>
<td>89,798</td>
<td>1.12 (1.09–1.15)</td>
<td>1.12 (1.08–1.16)</td>
</tr>
<tr>
<td>rs17271305</td>
<td>15</td>
<td>VPS13C</td>
<td>g</td>
<td>13</td>
<td>15,180</td>
<td>32,556</td>
<td>0.97 (0.94–1.00)</td>
<td>0.99 (0.94–1.04)</td>
</tr>
<tr>
<td>rs10423928</td>
<td>19</td>
<td>GIPR</td>
<td>a</td>
<td>16</td>
<td>19,091</td>
<td>38,508</td>
<td>1.07 (1.03–1.12)</td>
<td>1.07 (1.02–1.12)</td>
</tr>
</tbody>
</table>

Proxies rs11708067 with $r^2 = 0.82$ in HM CEU to rs2877716 used in eight studies; rs1717195 with $r^2 = 0.95$ in HM CEU used in two studies. Proxy rs12913951 with $r^2 = 0.71$ in HM CEU to rs17271305 used in two studies.

Proxy rs116728660 with $r^2 = 0.95$ in HM CEU to rs10423928 used in three studies.