Genome-Wide Association Identifies Nine Common Variants Associated With Fasting Proinsulin Levels and Provides New Insights Into the Pathophysiology of Type 2 Diabetes

Rona J. Strawbridge,¹ Joséé Dupuis,²,³ Inga Prokopenko,⁴,⁵ Adam Barker,⁶ Emma Ahlvqvist,⁷ Denis Rybin,⁸ John R. Petrie,⁹ Mary E. Travers,⁴ Nabila Bouatia-Naji,¹⁰,¹¹ Antigone S. Dimas,¹²,¹³ Alexandra Nica,¹⁴,¹²,¹³ Eleanor Wheeler,¹⁴ Han Chen,² Benjamin F. Voight,¹⁵,¹⁶ Jalal Taneera,⁷ Stavroula Kanoni,¹³,¹⁷ John F. Peden,⁵,¹⁸ Fabiola Turrini,⁷,¹⁹ Stefan Gustafsson,²⁰ Carina Zabena,²¹,²² Peter Almgren,⁷ David J.P. Barker,²¹ Daniel Barnes,⁶ Elaine M. Dennison,²⁴ Johan G. Eriksson,²⁵,²⁶,²⁷,²⁸ Per Eriksson,²¹ Elodie Eury,¹⁰,¹¹ Lasse Folkersten,²⁹ Caroline S. Fox,³,³⁰ Timothy M. Frayling,³¹ Anuj Goel,³¹,³² Harvest F. Gu,³² Momoko Horikoshi,⁴,³³ Bo Isomaa,²⁷,³³ Anna L. Gloyn,⁴ John Öhrvik,¹ Clive Osmond,²⁴ Laura Pascoe,⁴ Felicity Payne,¹⁴ Avan A. Sayer,²⁴ Bengt Sennblad,¹ Angela Silveira,¹ Alena Stancáková,³⁷ Kathy Stittups,¹³ Amy J. Swift,³⁹ Ann-Christine Sylvännien,⁴¹ Tiinamaja Tuomi,²⁷,⁴² Ferdinand M. van ’t Hooft,³¹ Mark Walker,⁴³ Michael N. Weedon,⁴¹ Weijia Xie,³¹ Björn Zethelius,⁴⁴ the DIAGRAM Consortium,* the GIANT Consortium,* the MuTHER Consortium,* Tiinamaija Tuomi,²⁷,⁴² Ferdinand M. van ’t Hooft,³¹ Mark Walker,⁴³ Michael N. Weedon,⁴¹ Weijia Xie,³¹ Björn Zethelius,⁴⁴ the DIAGRAM Consortium,* the GIANT Consortium,* the MuTHER Consortium,* the CARDiOGRAM Consortium,, the CARDIOGRAM Consortium,* the GIANT Consortium,* the mUther Consortium,* and the CARDIOGRAM Consortium.*

From the ¹Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden; the ²Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts; the ³National Health, Lung, and Blood Institute’s Framingham Heart Study, Framingham, Massachusetts; the ⁴Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford, U.K.; the ⁵Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, U.K.; the ⁶MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, U.K.; the ⁷Department of Clinical Sciences, Diabetes and Endocrinology, University Hospital and Malmö University, Malmö, Sweden; the ⁸Boston University Data Coordinating Center, Boston, Massachusetts; the ⁹BHF Cardiovascular Research Centre, University of Glasgow, Glasgow, U.K.; the ¹⁰Université Lille-Nord de France, Lille, France; the ¹¹CNRS UMR 8189, Institut Pasteur de Lille, Lille, France; the ¹²Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland; the ¹³Wellcome Trust Genome Campus, Hinxton, U.K.; the ¹⁴Metabolic Disease Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge, U.K.; the ¹⁵Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts; the ¹⁶Center for Human Genetic Research and Diabetes Research Center (Diabetes Unit), Massachusetts General Hospital, Boston, Massachusetts; the ¹⁷Department of Dietetics-Nutrition, Harokopio University, Athens, Greece; the ¹⁸Department of Cardiovascular Medicine, University of Oxford, Oxford, U.K.; the ¹⁹Department of Medicine, University of Verona, Verona, Italy; the ²⁰Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; the ²¹OBER of Diabetes and Endocrinology Medicina Asociadas (CIBERDEM), Madrid, Spain; the ²²Fundación Investigación Biomédica del Hospital Clínico San Carlos, Madrid, Spain; the ²³Heart Research Center, Oregon Health and Science University, Portland, Oregon; the ²⁴MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton, U.K.; the ²⁵National Institute for Health and Welfare, Helsinki, Finland; the ²⁶Helsinki University Central Hospital, Unit of General Practice, Helsinki, Finland; the ²⁷Folkhalsan Research Centre, Helsinki, Finland; the ²⁸Department of General Practice and Primary Health Care, University of Helsinki, Helsinki, Finland; the ²⁹Experimental Cardiovascular Research Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden; the ³⁰Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts; the ³¹Institute of Biomedical and Clinical Sciences, Peninsula Medical School, Torbay Hospital, and Research Program of Molecular Medicine, University of Helsinki, Helsinki, Finland; the ³²Center for Statistical Genetics, Department of Bio-statistics, School of Public Health, University of Michigan, Ann Arbor, Michigan; the ³³Hospital for Children and Adolescents, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland; the ³⁴INSERM UMR 859, Lille, France; the ³⁵Hospital for Children and Adolescents, Helsinki University Central Hospital, Uppsala, Sweden; the ³⁶Malmö Municipal Health Care Center and Hospital, Jäsko, Finland; the ³⁷Center for Statistical Genetics, Department of Bio-statistics, School of Public Health, University of Michigan, Ann Arbor, Michigan; the ³⁸Center for Non-Communicable Diseases Pakistan, Karachi.
OBJECTIVE—Proinsulin is a precursor of mature insulin and C-peptide. Higher circulating proinsulin levels are associated with impaired β-cell function, raised glucose levels, insulin resistance, and type 2 diabetes (T2D). Studies of the insulin processing pathway could provide new insights about T2D pathophysiology.

RESEARCH DESIGN AND METHODS—We have conducted a meta-analysis of genome-wide association tests of associations of proinsulin with T2D, impaired β-cell function, and type 2 diabetes (T2D). Studies of the insulin processing pathway could provide new insights about T2D pathophysiology.

RESULTS—Nine SNPs at eight loci were associated with proinsulin levels (P < 5 × 10⁻⁸). Two loci (LARP6 and SGSM2) have not been previously related to metabolic traits, one (MADD) has been associated with fasting glucose, one (PCSK1) has been implicated in obesity, and four (TCF7L2, SLC30A8, VPS13C/C2CD4A/B, and ARAP1, formerly CENTD2) increase T2D risk. The proinsulin-raising allele of ARAP1 was associated with a lower fasting glucose (P = 1.7 × 10⁻⁴), improved β-cell function (P = 1.1 × 10⁻⁷), and lower risk of T2D (odds ratio 0.88; P = 7.8 × 10⁻⁵). Notably, PCSK1 encodes the protein proconvertase 1/3, the first enzyme in the insulin processing pathway. A genotype score composed of the nine proinsulin-raising alleles was not associated with coronary disease in two large case-control datasets.

CONCLUSIONS—We have identified nine genetic variants associated with fasting proinsulin. Our findings illuminate the biology underlying glucose homeostasis and T2D development in humans and argue against a direct role of proinsulin in coronary artery disease pathogenesis. Diabetes 60:2624–2634, 2011

Genome-wide association studies (GWAS) have uncovered dozens of common genetic variants associated with risk for type 2 diabetes (T2D; reviewed in [1]). Known associated variants in these loci account for only a small proportion of the heritable component of T2D (1), suggesting that additional loci await discovery. The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) was created under the premise that genome-wide analysis of continuous diabetes-related traits could not only identify loci regulating variation in these glycemic traits, but also yield additional T2D susceptibility loci and insights into the underlying physiology of these loci (2–5). In addition, the genetic study of T2D endophenotypes may help clarify the pathophysiologic heterogeneity of this disease by elucidating the respective roles of β-cell function, insulin secretion, processing and sensitivity, and glucose metabolism (6).

Discovery of novel genetic determinants of insulin secretion and action has primarily focused on insulin levels (3,4,7,8). Proinsulin is the molecular precursor for insulin and has relatively low insulin-like activity, and its enzymatic conversion into mature insulin and C-peptide is a critical step in insulin production and secretion (Supplementary Fig. 1). Although hyperinsulinemia typically denotes insulin resistance, high proinsulin in relation to circulating levels of mature insulin can indicate β-cell stress as a result of insulin resistance, impaired β-cell function, and/or insulin processing and secretion abnormalities (9) (Supplementary Fig. 2). There is good evidence that higher proinsulin predicts future T2D (10) and coronary artery disease (CAD) (11–13), even after taking fasting glucose levels into account. Interestingly, some loci previously associated with fasting glucose levels (MADD) or risk of T2D (TCF7L2, SLC30A8, CDKAL1) are also associated with higher circulating proinsulin (6,14–17). Therefore, genome-wide analysis of proinsulin levels could reveal additional novel loci increasing susceptibility for T2D and perhaps CAD.

Thus, to identify novel loci influencing proinsulin processing and secretion and potentially increasing susceptibility for T2D, we performed a meta-analysis of ~2.5 million directly genotyped or imputed autosomal single nucleotide polymorphisms (SNPs) from four GWAS of fasting proinsulin levels (adjusted for concomitant fasting insulin) including 10,701 nondiabetic adult men and women of European descent. Follow-up of 23 lead SNPs from the most significant association signals in up to 16,578 additional individuals of European ancestry detected nine genome-wide significant associations with proinsulin levels, including two novel signals in or near LARP6 and SGSM2, and the known glycemic loci ARAP1, MADD (two independent signals), TCF7L2, VPS13C/C2CD4A/B, SLC30A8, and PCSK1. Here we describe these genetic associations, perform fine-mapping to identify potential causal variants, assess gene expression in human tissues, and define their impact on other glycemic quantitative traits and risk of both T2D and CAD.

RESEARCH DESIGN AND METHODS

Cohort/study description. Four cohorts contributed to the discovery meta-analysis through the contribution of phenotypic and GWAS data. These included the Framingham Heart Study (n = 5,750), Precocious Coronary Artery Disease (PROCARDIS) (n = 3,259), the Fenland study (n = 1,372), and the Diabetes Genetics Initiative (DGI) (n = 311), for a total of 10,701 participants.
Eleven cohorts contributed to the follow-up efforts; these included Metabolic Syndrome in Men (METSIM) \((n = 5,122)\), Botnia Prevalence, Prediction and Prevention of diabetes (Botnia-PPP) \((n = 2,280)\), Helsinki Birth Cohort Study (HBCS) \((n = 1,640)\), the Ely study \((n = 1,568)\), the Hertfordshire study \((n = 1,016)\), Uppsala Longitudinal Study of Adult Men (ULSAM) \((n = 939)\), Relationship between Insulin Sensitivity and Cardiovascular disease (RISC) \((n = 914)\), Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) \((n = 912)\), Segovia \((n = 911)\), the Greek Health Randomized Aging Study (GHRAS) \((n = 668)\), and Stockholm Diabetes Prevention Program (SDPP) \((n = 399)\), for a total of 16,378 participants (with maximal sample for any one SNP of 15,898). We excluded individuals with known diabetes, on antidiabetic treatment, or with fasting glucose \(\geq 7 \text{ mmol/L}\) \((n = 668)\). We chose the latter because the adjusted trait and the ratio was 0.95, and the quantile-quantile GWAS plots were comparable.

We used a linear regression model with natural log transformed fasting proinsulin as the dependent variable and genotypes as predictors, with adjustment for natural-log transformed fasting insulin values, sex, age, geographical covariates (if applicable), and age squared (Framingham only) to evaluate the association under an additive genetic model. Association analysis was performed by individual studies using SNPTEST \((19)\), STATA \((20)\), PLINK \((21)\), or LMEKIN \((R\) kinship package) software \((22)\). Genome-wide association inflation coefficients were estimated for each discovery cohort using the genomic control \((GC\) method \((23)\) and applied subsequently to each individual SNP association test statistics to correct for cryptic relatedness. The \(\lambda \) GC value for the final meta-analysis of proinsulin adjusted for fasting insulin was 1.01. The inverse-variance-fixed effects meta-analysis method was used to evaluate the pooled regression estimates for additively coded SNPs using METAL \((24)\). Sex interaction effects were evaluated with a function in the GWAMA software \((25)\).

**Follow-up SNP selection and analysis.** We carried forward to stage 2 the most significant SNP from each of 21 independent loci that showed association with proinsulin in stage 1 analyses at \(P < 1 \times 10^{-5}\). Additionally, two SNPs near the \(P < 1 \times 10^{-5}\) threshold \((\text{ASAP2 and a gene desert region})\) were carried forward as a result of biological plausibility \((\text{ASAP2 is involved in vesicular transport})\) and/or consistency of direction of effect in all discovery stage 1 studies \((\text{both loci})\). We genotyped these 23 variants in 11 additional stage 2 studies totaling 16,378 nondiabetic participants of European ancestry \((\text{Supplementary Table 1};\) genotyping assays and conditions are available upon request). We meta-analyzed stage 1 and stage 2 results using inverse-variance weighted fixed effects meta-analysis methods, including up to 27,070 participants.

Additional analyses and expression and expression quantitative trait loci \((\text{eQTL})\) studies are described in the Supplementary Data.

**Proinsulin and insulin measurements.** Proinsulin \((\text{pmol/L})\) was measured from fasting whole blood, plasma, or serum or a combination of these using enzyme-linked immunosorbent or immunometric assays. Fasting insulin \((\text{pmol/L})\) was measured using either enzyme-linked immunosorbent, immuno-fluorescent, or radioimmunometric assays \((\text{Supplementary Table 1})\). **Genotyping.** Genome-wide commercial arrays \((\text{Affymetrix 500K, MIPS 50K, and Illumina Human1M/610K})\) were used by the four discovery cohorts as described in the Supplementary Data. **Statistical analyses.** We aimed to identify genetic variants associated with high proinsulin levels relative to an individual’s fasting insulin levels. This can be done by examining proinsulin-to-insulin ratios or by statistically adjusting proinsulin for fasting insulin. We chose the latter because the adjusted trait has comparable predictive value \((18)\) and displayed better statistical performance in pilot studies and adequate heritability in the Framingham Heart Study, one of the larger cohorts examined here \((h^2 = 0.36 \text{ vs. } 0.34 \text{ for the proinsulin-to-insulin ratio})\). In Framingham, correlation between the adjusted trait and the ratio was 0.95, and the quantile-quantile GWAS plots were comparable.
Heterogeneity and sex-stratified analyses. We noted some degree of heterogeneity in our joint meta-analysis (Table 1). Part of the heterogeneity arose from the MDRM1 sample, which contributed only 1% of the variance explained by the joint meta-analysis. Although the overall significance for the effect allele was 10.5% in men (P = 2.0 \times 10^{-10}) and 11.5% in women (P = 1.5 \times 10^{-9}), the allele was not selected for further testing based on a suggestive significance threshold (26). The combined results, when adjusting for both BMI and fasting glucose, revealed the same loci associated with fasting proinsulin levels at genome-wide levels of statistical significance (Table 1).
of the METSIM cohort improved the heterogeneity score and produced nominal significance for the association in men ($P = 0.02$), the effect size remained threefold stronger in women than in men ($\beta$-coefficient $0.0427 \text{ vs. } 0.0165$, respectively).

To provide further reassurance regarding any residual heterogeneity, we repeated our meta-analyses based on $P$ values (rather than $\beta$-coefficients) and meta-analyzed the resulting $z$ scores. Our findings were essentially unchanged, suggesting that heterogeneity in the $\beta$-estimates across cohorts has not produced spurious results.

**Exploration of proinsulin processing mechanisms.**

Proinsulin is initially cleaved to $32,33$-split proinsulin and further to insulin and C-peptide before secretion (Supplementary Fig. 1); we were therefore interested in the effects of the nine top SNPs on these traits. The proinsulin-raising alleles of each SNP were consistently associated with higher $32,33$-split proinsulin levels, with effect sizes following the rank order of proinsulin effect sizes. Nearly all associations reached nominal conventional levels of statistical significance in this smaller dataset of $4,103-6,343$ individuals with measures of $32,33$-split proinsulin levels ($\text{all } P < 1.5 \times 10^{-3}$, with the exception of the conditional signal at $MADD$). The insulinogenic index ($27$), which measures dynamic insulin secretion during the first $30$ min after an oral glucose load and was available in $14,956$ subjects, showed nominal associations for four loci. Of these, the proinsulin-raising alleles were associated with a lower insulinogenic index at $VPS13C/C2CD4A/B$, $TCF7L2$, and $SLC30A8$ and higher at $ARAP1$ (Table 2).

We detected no nominal associations with fasting C-peptide ($P > 0.05$). Given the differences in hepatic clearance of insulin and C-peptide, we also performed sensitivity analyses to account for any possible impact this may have had on our results. We adjusted proinsulin levels for fasting C-peptide rather than fasting insulin in two cohorts (Ely and Botnia-PPP); comparison of $\beta$-estimates showed that the majority of loci had very similar effect sizes and the same rank order was preserved, arguing against noticeable discrepancies between the two adjustment schemes.

**Association with other glycemic traits.**

To clarify potential mechanisms, the top nine signals ($ARAP1$, two at $MADD$, $PCSK1$, $TCF7L2$, $VPS13C/C2CD4A/B$, $SLC30A8$, $LARP6$, and $SGSM2$) were also examined in relation to other glucometabolic traits (fasting and $2$-h postload glucose and insulin, homeostasis model assessment estimates of $\beta$-cell function [$HOMA$-$B$] and insulin resistance [$HOMA$-$IR$] [$28$], glycated hemoglobin [$A1C$], $T2D$, and BMI [Table 3]). We investigated results available from MAGIC meta-analyses of GWAS of glycemic traits ($3$) and ob-ob meta-analyses of GWAS of glycemic traits (5) and ob-ob and produced nominal significance for $2$-h postload insulin or insulin sensitivity as estimated by the Matsuda index ($31$) (Table 3).

Associations with $T2D$ were confirmed for four known $T2D$ loci ($SLC30A8$, $ARAP1$, $VPS13C/C2CD4A/B$, and $TCF7L2$; Table 3). Counterintuitively, the proinsulin-raising allele of $ARAP1$ (formerly known as $CENTD2$ and reported as such in DIAGRAM+) ($29$) was associated with a lower fasting glucose ($0.019 \text{ mg/dL per } A$ allele; $P = 1.7 \times 10^{-5}$), lower $A1C$ ($0.023\%$; $P = 0.02$), and a lower risk of $T2D$ (odds ratio $[OR]$ $0.88$; $P = 7.8 \times 10^{-6}$; Table 3).

The two novel loci ($LARP6$ and $SGSM2$) did not show significant associations with $T2D$ ($OR [95\% CI]$: $1.01$ [$0.95–1.07$] and $1.01$ [$0.96–1.08$], respectively), indicating that if they increase $T2D$ risk they do so to an extent confined within the bounds of narrow $95\%$ CI.

**Fine-mapping, copy number variants, and tissue expression.**

We used MACH ($32$) or IMPUTE ($19$) applied to the $1000$ Genomes CEU reference panel (www.1000genomes.org) to carry out imputation of $\sim$8 million autosomal SNPs with minor allele frequency $>1\%$. Analysis of $1000$ Genomes-imputed data in the four discovery cohorts indicates that although there are low-frequency ($1–5\%$) genetic variants that influence levels of circulating proinsulin, these are found in the same loci that contain common proinsulin-influencing variants, and none of them yield substantially stronger signals than the index SNP at each locus (Supplementary Fig. 5).

Using current databases of copy number variants ($33$) and the SNAP software (http://www.broadinstitute.org/mpg/snap/index.php; CEU, HapMap release 22), we checked whether any of the proinsulin-associated SNPs were within $500 \text{ kb}$ and in linkage disequilibrium (LD) with any of the SNPs known to tag copy number variants in the human genome. No copy number variant tag SNPs were found within $500 \text{ kb}$ of our lead SNPs.

To guide identification of the gene responsible for each association signal, we also examined the gene expression profile of selected genes in each associated region across a range of human tissues, including islets and fluorescence-activated cell (FAC)-sorted $\beta$-cells (Fig. 3A–F and Supplementary Fig. 6). We defined $1$-Mb intervals around the lead SNP at each locus and prioritized biologically plausible genes as gleaned from the literature (see Box in Supplementary Data). We were able to demonstrate $\beta$-cell expression of most genes examined (Fig. 3F). However, at the $LARP6$ locus, $CT62$ is expressed exclusively in testis, likely excluding it as a relevant gene in this context. At the $ARAP1$ locus, $STARD10$ is expressed more strongly in pancreatic and islet tissue than any other tissue type; similarly, at the $VPS13C$ locus both $C2CD4A$ and $C2CD4B$ demonstrate higher expression in pancreas and islets than all other tissue types.

We also studied the expression of the transcript for the gene closest to the index SNP at each of the nine replicated loci in human islets isolated from $55$ nondiabetic and $9$ diabetic individuals. Of the nine loci, $PCSK1$ ($P = 0.02$) and $MADD$ ($P = 0.07$) demonstrated $35–45\%$ lower expression in subjects with $T2D$ compared with control subjects.

**Functional explanation.**

We evaluated whether any of the associated SNPs was in strong LD with a potentially causal variant. We used SNPer (34) to classify all SNPs in strong LD with the lead SNP ($r^2 > 0.8$) within a $1$-Mb region. We found that $PCSK1$ rs6235 codes for a non-synonymous variant (S690T), which is in perfect LD with rs6234, another missense variant (Q665E); both were
FIG. 2. Regional plots of eight genomic regions containing novel genome-wide significant associations. For each region, 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). In each panel, the stage 1 discovery SNP taken forward to stage 2 follow-up is represented by a purple diamond (with global meta-analysis $P$ value), with its stage 1 discovery $P$ value denoted by a red diamond with bolded borders. Estimated recombination rates (taken from HapMap) are plotted to reflect the local LD structure around the associated SNPs and their correlated proxies (according to a white to red scale from $r^2 = 0$ to 1, based on pairwise $r^2$ values from HapMap CEU). Gene annotations were taken from the University of California Santa Cruz genome browser. A: ARAP1 region; B: MADD region; C: PCSK1 region; D: TCF7L2 region; E: VPS13C/C2CD4A/B region; F: SLC30A8 region; G: LARP6 region; H: SGSM2 region.
predicted to be non-damaging by Polyphen (35) and SIFT (36). At SLC30A8, the proinsulin-associated SNP rs11558471 is a perfect proxy for the known T2D-associated SNP rs13266634, encoding R325W. The T allele (encoding tryptophan) is predicted to be benign by PolyPhen, but damaging by SIFT. We found no other strong ($r^2 > 0.8$) correlations in HapMap CEU with potentially functional SNPs within 1 Mb of the lead signals.

We also tested whether any of the proinsulin-associated SNPs might influence proximal (cis) expression of human transcripts, in tissues available to us that had been paired to genetic data. We found a significant association ($P = 0.01$, permutation threshold) of rs1549318 with expression levels of LARP6 in adipose tissue. SNP rs1549318 is located ~37 kb from LARP6, and the proinsulin-raising T allele is associated with lower levels of expression. Analysis of an eQTL database from human liver indicated that the proinsulin-raising A allele of the lead SNP at the SGSM2 locus (rs179456) was associated with increased liver expression of TRPS1 ($P = 0.004$).

**CAD.** We constructed unweighted and weighted genotype scores composed of the nine genome-wide significant proinsulin-raising alleles, with weights defined by the $\beta$-coefficients from our replication meta-analysis, and tested the association of these scores with CAD in the Coronary Artery Disease Genome-wide Replication And Meta-Analysis (CARDIoGRAM) (37) ($n = 22,000$ CAD case subjects and 60,000 control subjects) and CAD (38) ($n = 15,420$ CAD case subjects and 15,062 control subjects) datasets. Neither weighted nor unweighted genotype scores reached nominal significance in either dataset ($P = 0.47$ and 0.81 for unweighted and weighted scores in CARDIoGRAM, respectively; $P = 0.60$ and 0.43 for unweighted and weighted scores in CAD, respectively).

**DISCUSSION**

We report the first meta-analysis of genome-wide association datasets for circulating fasting proinsulin. We adjusted proinsulin for fasting insulin levels, aiming to capture an increase in proinsulin relative to the nonspecific activation of the insulin processing pathway induced by generalized insulin resistance (Supplementary Fig. 2). Loci that simply influence insulin resistance are typically sought by a GWAS for fasting insulin or more sophisticated measures of insulin sensitivity (3,4,6). Thus, we hoped to identify loci that indicate the inability of the $\beta$-cell to process proinsulin adequately in response to metabolic demands.

We have identified nine signals at eight loci associated with higher proinsulin levels (see Box in Supplementary Data). Two of these loci (LARP6 and SGSM2) have not been previously related to metabolic traits. A 10th signal emerged after sex-stratified analyses; an explanation for the female-specific genome-wide significant association at DDX31 requires fine-mapping to identify the causal gene. Although the function of the DDX31 gene product is unknown, other members of the DEAD-box protein family have been implicated in sex-specific processes such as spermatogenesis (39). We have also replicated at the genome-wide level previously reported nominal associations of MADD, TCF7L2, VPS13C/C2CD4A/B, SLC30A8, and PCSK1 with proinsulin (6,14-17,40). The knowledge that TCF7L2, VPS13C/C2CD4A/B, and ARAPI are established T2D loci provides reassurance that a quest for genetic determinants of proinsulin can serve to identify disease-associated signals. Interestingly, the proinsulin-raising
coefficients are adjusted for age, sex, and study-specific covariates (if applicable). OR is for T2D only as a dichotomous trait.

Table 3: Association of proinsulin loci with other glycemic traits

<table>
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<th>SNP</th>
<th>Nearest gene</th>
<th>Fasting glucose (β value)</th>
<th>HOMA-β</th>
<th>Matsuda index (β value)</th>
<th>2-h glucose (β value)</th>
<th>2-h insulin (β value)</th>
<th>HbA1c (β value)</th>
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</table>

**TABLE 3**

**Association of proinsulin loci with other glycemic traits**

The association of proinsulin loci with other glycemic traits is shown in Table 3. The table lists the nearest gene for each SNP, along with the association coefficients for fasting glucose, HOMA-β, and Matsuda index, as well as coefficients for 2-h glucose and 2-h insulin. The data includes results for T2D and control subjects, with and without the T2D-associated SNP. Additional details are provided in the text.)

**Figures**

Figures are not included in the text, but they are mentioned in the context of the study's findings. Additional details and references are provided for further understanding.

**References**


2. Additional details and references are provided for further understanding.

**Conclusions**

The study provides evidence for the association of proinsulin loci with other glycemic traits, including fasting glucose, HOMA-β, and Matsuda index. These findings have implications for understanding the genetics and biology of T2D and related conditions. Further research is needed to elucidate the mechanisms underlying these associations.
member of the NR1 subfamily of nuclear hormone receptors) are expressed in FAC-sorted \( \beta \)-cells, the relative expression of the first two is much higher in islets than in other human tissues, again suggesting that these two genes, encoding nuclear factors that are upregulated in response to inflammation, may be particularly relevant to endocrine pancreatic function.

The genome-wide association of a missense variant in \( PCSK1 \) with fasting proinsulin also serves as a positive control. \( PCSK1 \) encodes the protein prohormone convertase...
L3 (PC1), which is the first enzyme in the proinsulin processing pathway, where it cleaves proinsulin to 32,33-split proinsulin (Supplementary Fig. 1). A related enzyme, PC2, acts on 32,33-split proinsulin in the second processing step. People deficient in PC1 become obese at an early age and exhibit pituitary hypofunction because of the lack of several mature peptide hormones (45), whereas PC2-null mice demonstrate increased levels of 32,33-split proinsulin (46). The rs6235 SNP reported here results in the substitution of a serine residue for threonine at position 690 of the molecule; the minor allele (Thr) is associated with higher proinsulin levels. A nominal association of the same allele with higher BMI is only nominal here, but confirms a previous report (47). This specific amino acid change has been shown not to affect enzyme catalysis or maturation of the protein in vitro (47), but the COOH terminus of the protein (where S69OT is located, adjacent to a conserved proline residue) is known to direct the correct subcellular targeting of the protein as well as stabilizing and partially inhibiting PC1. Although one might expect lower levels of the reaction product (32,33-split proinsulin) in carriers of the risk allele, the potential diversion of the substrate down its alternate path (giving rise to 65,66-split proinsulin, whose assay typically has 60% cross-reactivity with 32,33-split proinsulin) requires further study. Alternatively, if changes in the activity of PC1 also affect that of PC2 (for instance, by competing for inhibitory peptides) one might see reductions in the catalytic function of both enzymes and accumulation of both proinsulin and 32,33-split proinsulin.

Because of the reported relationship between proinsulin levels and coronary events (11–13), the identification of genetic determinants of proinsulin levels might help shed light on whether hyperproinsulinemia is a mediator of CAD or a byproduct of a shared etiologic mechanism. If hyperproinsulinemia is causally associated with an increased risk of CAD, one might expect that SNPs that specifically and selectively raise proinsulin levels should increase the risk of CAD given an adequately powered study. We have not observed such an effect for a genotype score constructed with the genome-wide significant proinsulin association signals. Assuming conservative approximations of the reported effect sizes of proinsulin on CAD (OR ~1.5 per 1-SD increase in proinsulin) (12,13), and of the nine SNPs reported here on circulating proinsulin (5%), a CAD cohort like CARDIoGRAM has 99% power to detect an effect of proinsulin SNPs on CAD. The absence of statistical significance argues against a direct etiologic role of proinsulin on CAD.

In summary, we have identified nine loci that associate with fasting proinsulin levels. Several of these loci increase risk of T2D; interestingly, both proinsulin-raising and lowering alleles can lead to T2D through decreases in insulin secretion, indicating defects distal or proximal to the first enzymatic step in proinsulin conversion, respectively. Other genetic determinants of proinsulin levels do not necessarily lead to higher T2D risk, suggesting that it is not a mere elevation in proinsulin, but rather the specific impairment in proinsulin processing and the reaction of the β-cell to this defect that determine whether ultimately β-cell insufficiency will cause pathological hyperglycemia. The direct elevation of fasting proinsulin out of proportion to fasting insulin does not seem to increase risk of CAD.

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Please see the Supplementary Data.

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