Association of 18 Confirmed Susceptibility Loci for Type 2 Diabetes with Indices of Insulin Release, Proinsulin Conversion, and Insulin Sensitivity in 5327 Non-diabetic Finnish Men

Short title: Type 2 diabetes risk genes and insulin secretion

Alena Stančáková1, Teemu Kuulasmaa1, Jussi Paananen1, Anne U. Jackson2, Lori L. Bonnycastle3, Francis S. Collins3, Michael Boehnke2, Johanna Kuusisto1, Markku Laakso1

1Department of Medicine, University of Kuopio and Kuopio University Hospital, 70210 Kuopio, Finland
2Center for Statistical Genetics, Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, Michigan 48109, USA
3National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, 20892, USA

Correspondence:
Markku Laakso, MD
E mail: markku.laakso@kuh.fi

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournal.org

Submitted 27 January 2009 and accepted 27 May 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
Objective We investigated the effects of 18 confirmed type 2 diabetes risk SNPs on insulin sensitivity, insulin secretion, and conversion of proinsulin to insulin.

Research Design and Methods A total of 5327 non-diabetic men (age 58±7 years, BMI 27.0±3.8 kg/m²) from a large population-based cohort were included. Oral glucose tolerance test and genotyping of SNPs in/near PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, LOC387761, CDKN2B, IGF2BP2, CDKAL1, HNF1B, WFS1, JAZF1, CDC123, TSPAN8, THADA, ADAMTS9, NOTCH2, KCNQ1, and MTNR1B were performed. HNF1B rs757210 was excluded due to failure to achieve Hardy-Weinberg equilibrium.

Results Six SNPs (TCF7L2, SLC30A8, HHEX, CDKN2B, CDKAL1, MTNR1B) were significantly (P≤6.9x10⁻⁴) and two SNPs (KCNJ11 and IGF2BP2) were nominally (P<0.05) associated with early-phase insulin release (InsAUC_0-30/GluAUC_0-30), adjusted for age, BMI and insulin sensitivity (Matsuda ISI). Combined effects of these 8 SNPs reached -32% reduction in InsAUC_0-30/GluAUC_0-30 in carriers of ≥11 vs. ≤3 weighted risk alleles. Four SNPs (SLC30A8, HHEX, CDKAL1, TCF7L2) were significantly or nominally associated with indices of proinsulin conversion. Three SNPs (KCNJ11, HHEX, TSPAN8) were nominally associated with Matsuda ISI (adjusted for age and BMI). The effect of HHEX on Matsuda ISI became significant after additional adjustment for InsAUC_0-30/GluAUC_0-30. Nine SNPs did not show any associations with examined traits.

Conclusions Eight type 2 diabetes-related loci were significantly or nominally associated with impaired early-phase insulin release. Effects of SLC30A8, HHEX, CDKAL1, and TCF7L2 on insulin release could be partially explained by impaired proinsulin conversion. HHEX might influence both insulin release and insulin sensitivity.
Impaired insulin secretion and insulin resistance, two main pathophysiological mechanisms leading to type 2 diabetes, have a significant genetic component (1). Recent studies have confirmed a total of 20 genetic loci reproducibly associated with type 2 diabetes (2-13). Three were previously known (PPARG, KCNJ11 and TCF7L2), while 17 loci were recently discovered either by genome-wide association studies (SLC30A8, HHEX-ID, LOC387761, CDKN2A/2B, IGF2BP2, CDKAL1, FTO, JAZF1, CDC123/CAMK1D, TSPAN8/LGR5, THADA, ADAMTS9, NOTCH2, KCNQ1, MTNR1B), or candidate gene approach (WFS1 and HNF1B). The mechanisms by which these genes contribute to the development of type 2 diabetes are not fully understood.

PPARG is the only gene from the 20 confirmed loci previously associated with insulin sensitivity (14,15). Association with impaired beta-cell function has been reported for 14 loci (KCNJ11, SLC30A8, HHEX-ID, CDKN2A/2B, IGF2BP2, CDKAL1, FTO, JAZF1, CDC123/CAMK1D, TSPAN8/LGR5, THADA, ADAMTS9, NOTCH2, KCNQ1, MTNR1B) (6,12,13,16-38). Although associations of variants in HHEX (16-22), CDKAL1 (6,21-26), TCF7L2 (22,27-30), and MTNR1B (13,31,32) with impaired insulin secretion seem to be consistent across different studies, information concerning other genes is limited (12,18-25,33-38). The mechanisms by which variants in these genes affect insulin secretion are unknown. However, a few recent studies suggested that variants in TCF7L2 (22,39-42), SLC30A8 (22), CDKAL1 (22), and MTNR1B (31) might influence insulin secretion by affecting the conversion of proinsulin to insulin. Variants of FTO have been shown to confer risk for type 2 diabetes through their association with obesity (7,16) and therefore were not included in this study.

Large population-based studies can help to elucidate the underlying mechanisms by which SNPs of different risk genes predispose to type 2 diabetes. Therefore, we investigated confirmed type 2 diabetes-related loci for their associations with insulin sensitivity, insulin secretion, and conversion of proinsulin to insulin in a population-based sample of 5327 non-diabetic Finnish men.

SUBJECTS AND METHODS

Subjects. A total of 5327 non-diabetic men from the ongoing population-based cross-sectional METSIM (Metabolic Syndrome in Men) Study (10,26,43) were included in the study (age 58±7 years, BMI 27.0±3.8 kg/m²). 3594 (68%) subjects had normal glucose tolerance, 884 (17%) had isolated impaired fasting glucose, 503 (9%) had isolated impaired glucose tolerance, and 346 (6%) had both impaired fasting glucose and impaired glucose tolerance. Subjects with type 2 diabetes (N=898) were excluded from the analyses. Subjects aged from 45 to 70 years were randomly selected from the population register of Kuopio town, Eastern Finland (population of 95,000) for the METSIM study. Every participant had one-day outpatient visit to the Clinical Research Unit at the University of Kuopio. Blood samples were drawn after 12 hours of fasting followed by an OGTT. The study was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital, and carried out in accordance with the Helsinki Declaration.

Clinical measurements. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

Oral glucose tolerance test. A 2-h OGTT (75 g of glucose) was performed, with samples for plasma glucose, insulin, and proinsulin drawn at 0, 30 and 120 min.
Glucose tolerance was evaluated according to the WHO criteria (44).

**Laboratory measurements.** Plasma glucose was measured by enzymatic hexokinase photometric assay (Konelab Systems Reagents, Thermo Fischer Scientific, Vantaa, Finland), insulin by immunoassay (ADVIA Centaur Insulin IRI, no 02230141, Siemens Medical Solutions Diagnostics, Tarrytown, NY), and proinsulin by immunoassay (Human Proinsulin Ria kit Linco Research, St. Charles, MO). Proinsulin data were available for 2697 subjects.

**Genotyping.** Genotyping of 19 SNPs was performed with the TaqMan Allelic Discrimination Assay (Applied Biosystems) (PPARG rs1801282, KCNJ11 rs5219, TCF7L2 rs7903146, SLC30A8 rs13266634, HHEX rs1111875, LOC387761 rs7480010, CDKN2B rs10811661, IGF2BP2 rs4402960, CDKAL1 rs7754840, HNF1B rs757210, WFS1 rs10010131, JAZF1 rs864745, CDCA123 rs12779790, TSPAN8 rs7961581, THADA rs7578597, ADAMTS9 rs4607103, NOTCH2 rs10923931, KCNNQ1 rs2283228) and Sequenom iPLEX gold SBE (Sequenom) (MTNR1B rs10830963). TaqMan genotyping call rate was 100%, and error rate 0% among 4.5% of DNA samples genotyped in duplicate. Sequenom iPLEX call rate for MTNR1B rs10830963 was 96.8%, and error rate 0% among 4.2% of DNA samples genotyped in duplicate. All SNPs were consistent with Hardy-Weinberg equilibrium (P>0.05) except for HNF1B rs757210 (P<0.0001). This SNP was therefore omitted from all statistical analyses.

**Calculations.** The trapezoidal method was used to calculate glucose, insulin, and proinsulin area under the curve (AUC) during OGTT. Early-phase insulin release (InsAUC0-30/GluAUC0-30) was calculated as the total insulin area under the curve divided by the total glucose area under the curve during the first 30 min of an OGTT. Matsuda index of insulin sensitivity (Matsuda ISI) was calculated as reported previously (45). In our previous validation study, InsAUC0-30/GluAUC0-30 had the highest correlation (r=0.666) with the 1st phase insulin secretion in an intravenous glucose tolerance test among 11 different indices tested, and Matsuda ISI had the highest correlation with lean body mass adjusted M value from the euglycemic hyperinsulinemic clamp (r=0.776) among six different indices tested (46). Four indices of proinsulin conversion were calculated: proinsulin/insulin ratio in the fasting state (Proins0/Ins0), an index of proinsulin conversion to insulin during the first 30 min (ProinsAUC0-30/InsAUC0-30), 30 to 120 min (ProinsAUC30-120/InsAUC30-120) and 0 to 120 min (ProinsAUC0-120/InsAUC0-120) of an OGTT. All indices of proinsulin conversion were multiplied by 100. All calculations were based on glucose, insulin, and proinsulin concentrations at 0, 30, and 120 min of an OGTT. Disposition index (DI) was calculated as InsAUC0-30/GluAUC0-30 x Matsuda ISI. To estimate a combined impact of multiple type 2 diabetes risk alleles (denoted as the risk allele throughout the text) on InsAUC0-30/GluAUC0-30 we calculated a genetic risk score as a sum of weighted risk alleles (47) at SNPs significantly or nominally associated with InsAUC30/GluAUC30 in initial analyses. For each subject, the number of risk alleles (0,1,2) per SNP was weighted for their effect sizes (shown in Table 1; average effect size per allele among 8 SNPs was 1.58, which was considered as one weighted risk allele), and the sum of weighted alleles for each subject was rounded to closest integer. Subjects with ≤3 and ≥11 weighted risk alleles were pooled to obtain larger numbers.

**Statistical analysis.** Effect sizes [B (SE)] per copy of the risk allele were estimated by linear regression adjusted for age, using untransformed dependent variables, as previously described (13). P values were calculated using logarithmically transformed variables (all except for age) due
to their skewed distribution, and were adjusted for age in the primary analyses. In the secondary analyses, additional adjustment was performed as follows: effects of SNPs on InsAUC_{0-30}/GluAUC_{0-30} and ProinsAUC_{0-30}/InsAUC_{0-30} were adjusted for age, BMI and Matsuda ISI (in order to examine effects independent of obesity and insulin sensitivity), and effects of SNPs on Matsuda ISI and disposition index were adjusted for age and BMI. Effect of genetic risk score on InsAUC_{0-30}/GluAUC_{0-30} was analyzed by linear regression adjusted for age, BMI and Matsuda ISI due to significant association of genetic risk score with these covariates. Hardy-Weinberg equilibrium was tested by χ²-test. Statistical analyses were conducted with the SPSS 14 programs (SPSS, Chicago, IL). \(P<0.05\) was considered nominally significant, \(P<6.9x10^{-4}\) calculated using Bonferroni correction for multiple comparisons was considered statistically significant, given 72 independent tests for 18 SNPs and 4 outcomes measured [obesity (BMI), insulin release (InsAUC_{0-30}/GluAUC_{0-30}), insulin sensitivity (Matsuda ISI), and proinsulin conversion (ProinsAUC_{0-30}/InsAUC_{0-30})]. Power of the current sample was estimated using the Bioconductor’s GeneticsDesign package version 1.1 (http://www.bioconductor.org/packages/2.3/bioc/html/GeneticsDesign.html). We had power \(\geq 80\%\) to detect changes from 5 to 8\% per copy of the risk allele for InsAUC_{0-30}/GluAUC_{0-30}, Matsuda ISI, and DI for SNPs with minor allele frequency (MAF) larger than 30\%, and power \(\geq 80\%\) to detect a change of \(-15\%\) in ProinsAUC_{0-30}/InsAUC_{0-30} for SNPs with MAF larger than 30\%.

**RESULTS**

**Primary analyses.** Primary analyses were carried out under the additive model adjusted for age.

**Obesity.** None of the 18 SNPs was significantly associated with BMI, although for four SNPs (TCF7L2 rs7903146, CDC123 rs12779790, TSPAN8 rs7961581, and MTNR1B rs10830963) the association was nominally significant \((P=0.018, 0.006, 0.031, \text{and} 0.035)\). The effect sizes were \(<1\%\) per type 2 diabetes risk allele. In order to examine obesity-independent effects of all SNPs, we additionally adjusted their effects for BMI.

**Insulin sensitivity.** None of the 18 SNPs had significant effect on Matsuda ISI in a primary analysis. Two SNPs, HHEX rs1111875 and KCNJ11 rs5219, were nominally associated with Matsuda ISI, with effect sizes ranging from +2 to +4\% per risk allele \((P=0.010 \text{ and } 0.005)\) (Table 1). Adjustment for BMI did not have a major impact on these associations, but revealed another nominal association between TSPAN8 rs7961581 and Matsuda ISI \((P=0.008, \text{ effect size } -2\% \text{ per risk allele})\). However, both KCNJ11 rs5219 and HHEX rs1111875 were also associated with InsAUC_{0-30}/GluAUC_{0-30}. Adjustment for InsAUC_{0-30}/GluAUC_{0-30} abolished the effect of KCNJ11 rs5219 \((P=0.906)\), but strengthened the effect of HHEX rs1111875 on Matsuda ISI \((P=3.6x10^{-5})\).

**Insulin release.** Altogether, eight SNPs (in or near KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2B, IGF2BP2, CDKAL1, and MTNR1B) were nominally or significantly associated with InsAUC_{0-30}/GluAUC_{0-30}. The largest effects on InsAUC_{0-30}/GluAUC_{0-30} (from -6 to -9\% per risk allele) were observed for TCF7L2 rs7903146, HHEX rs1111875, CDKAL1 rs7754840, and MTNR1B rs10830963, and were statistically significant in both primary analyses and analyses adjusted for age, BMI and Matsuda ISI (Table 1). Effect sizes of the SNPs in/near KCNJ11, SLC30A8, CDKN2B, and IGF2BP2 were \(<-5\%\) per risk allele. Adjustment of effects of these SNPs for BMI and Matsuda ISI in addition to age attenuated the initially significant effect of KCNJ11 rs5219 \((P=0.024)\), strengthened the
Type 2 diabetes risk genes and insulin secretion

associations of \( SLC30A8 \) rs13266634 and \( CDKN2B \) rs10811661 to significant level (\( P=3.2\times10^{-4} \), and \( 1.7\times10^{-4} \)), and did not change nominal association of \( IGF2BP2 \) rs4402960 with InsAUC_{0-30}/GluAUC_{0-30} (\( P=0.004 \)) (Table 1).

Proinsulin conversion. Four SNPs (in/near \( HHEX \), \( SLC30A8 \), \( TCF7L2 \), and \( CDKAL1 \)) were associated with ProinsAUC_{0-30}/InsAUC_{0-30}, with effect sizes ranging from +3 to +6% per risk allele (Tables 1 and 2). For \( HHEX \) rs1111875 and \( SLC30A8 \) rs13266634 the effects were significant regardless of adjustments used (adjusted for age: \( P=9.7\times10^{-6} \) and \( 1.9\times10^{-5} \); adjusted for age, BMI and Matsuda ISI: \( P=6.5\times10^{-6} \) and \( 1.2\times10^{-5} \)). In contrast, adjustment for BMI and Matsuda ISI attenuated the significant effect of \( CDKAL1 \) rs7754840 to nominal level (\( P=0.002 \)), and strengthened nominal effect of \( TCF7L2 \) rs7903146 to significant level (\( P=6.0\times10^{-5} \)).

Similar results, although slightly attenuated, were obtained when alternative indices of proinsulin conversion based on proinsulin and insulin AUCs during 0-120 min or 30-120 min of an OGTT were used (ProinsAUC_{0-120}/InsAUC_{0-120} and ProinsAUC_{30-120}/InsAUC_{30-120}, Table 2). \( SLC30A8 \) rs13266634 and \( TCF7L2 \) rs7903146 were also nominally associated with fasting proinsulin/insulin ratio (Proins_{0}/Ins_{0}, Table 2). Overall, these results were consistent with associations of \( TCF7L2 \), \( SLC30A8 \), \( HHEX \) and \( CDKAL1 \) with insulin release, since the risk alleles associated with lower insulin release were also associated with higher proinsulin/insulin ratio.

Disposition index. Most of the insulin release-related SNPs (in or near \( TCF7L2 \), \( SLC30A8 \), \( HHEX \), \( CDKN2B \), \( IGF2BP2 \), \( CDKAL1 \), and \( MTNR1B \)) were also significantly or nominally associated with DI (Table 1). The largest effects ranging from -3 to -6% per risk allele were observed for \( MTNR1B \) rs10830963 (\( P=6.7\times10^{-11} \)), \( HHEX \) rs1111875 (\( P=2.5\times10^{-9} \)), \( TCF7L2 \) rs7903146 (\( P=8.3\times10^{-5} \)), \( CDKN2B \) rs10811661 (\( P=4.3\times10^{-4} \)) and \( CDKAL1 \) rs7754840 (\( P=1.6\times10^{-4} \)). Adjustment for BMI did not attenuate these associations, except for that of \( CDKN2B \) (\( P=0.001 \)).

Given the number of tests (18 tests for each variable) we would expect 0.9 \( P \) values <0.05 per variable at random. The number of associations with \( P<0.05 \) was larger than expected (9 for InsAUC_{0-30}/GluAUC_{0-30}, 4 for ProinsAUC_{0-30}/InsAUC_{0-30}, 2 for Matsuda ISI, and 7 for DI in primary analyses), suggesting that the associations we found were not likely to occur by chance. However, it should be mentioned that in spite of the large sample size we did not have sufficient power (>80%) to detect small effects (<6% per risk allele) on different traits examined for 9 of 18 SNPs investigated.

We repeated all analyses in the subgroup of subjects with normal glucose tolerance (\( N=3594 \)) (Suppl. Table 1). The effect sizes were mostly similar although associations were generally slightly weaker due to a smaller sample size. In contrast, in analyses including both non-diabetic subjects and 442 subjects with newly diagnosed type 2 diabetes the associations described above were somewhat more statistically significant with similar effect sizes, and revealed nominal associations of \( CDC123 \) rs12779790 and \( ADAMTS9 \) rs4607103 with DI (\( P=0.001 \) and 0.043, adjusted for age and BMI, effect sizes ~2% per risk allele, Suppl. Table 2).

Combining effect of risk alleles on insulin release. We combined the risk alleles at 8 SNPs significantly or nominally associated with InsAUC_{0-30}/GluAUC_{0-30} (\( KCNJ11 \), \( TCF7L2 \), \( SLC30A8 \), \( HHEX \), \( CDKN2B \), \( IGF2BP2 \), \( CDKAL1 \) and \( MTNR1B \)) to evaluate their combined effects on insulin release. InsAUC_{0-30}/GluAUC_{0-30} gradually decreased with an increasing number of risk alleles (relative effect size -4% per allele, \( P=9.3\times10^{-44} \) adjusted for age, BMI, and Matsuda ISI). Subjects with \( \geq 11 \) weighted
risk alleles (N=190) had decreased InsAUC 30/GluAUC 30 by -32% compared with subjects with \( \leq 3 \) weighted risk alleles (N=163) (Figure 1). We also performed similar analysis using non-weighted risk alleles. The difference in InsAUC 30/GluAUC 30 between subjects with \( \leq 3 \) and \( \geq 11 \) risk alleles was -37% (relative effect size -4% per risk allele, \( P = 3.8 \times 10^{-28} \)).

**DISCUSSION**

In this large population-based study we investigated the effects of confirmed type 2 diabetes risk variants on insulin secretion, insulin sensitivity, and proinsulin processing. We showed in 5327 non-diabetic Finnish men that 8 of 18 type 2 diabetes-related variants were significantly (TCF7L2, SLC30A8, HHEX, CDKN2B, CDKAL1, and MTNR1B) or nominally (KCNJ11 and IGF2BP2) associated with early-phase insulin release (InsAUC 0-30/GluAUC 0-30) after adjustment for age, BMI and Matsuda ISI. InsAUC 0-30/GluAUC 0-30 decreased gradually with increasing number of type 2 diabetes risk alleles in these SNPs, and was -32% less in subjects with \( \geq 11 \) than with \( \leq 3 \) risk alleles. Furthermore, 4 variants (TCF7L2, SLC30A8, HHEX, and CDKAL1) were also associated with proinsulin conversion (ProinsAUC 0-30/InsAUC 0-30). SNPs in/near KCNJ11, HHEX, and TSPAN8 were nominally associated with Matsuda ISI (adjusted for age and BMI).

Insulin secretion has an important genetic component, as suggested by twin studies reporting heritability estimates >50% (1), and a majority of diabetes-susceptibility genes have been shown to associate with parameters of insulin secretion (48). Our finding of 8 SNPs associated with insulin secretion, alone or in combination, provides additional evidence on the importance of the genes regulating insulin secretion as risk genes for type 2 diabetes. An observation similar to our results was reported in a study by Pascoe et al. (27), where carriers of 9 or more risk alleles in 7 genes exhibited reduced insulin secretion (assessed by the insulinogenic index) by -21.8% and reduced glucose sensitivity of beta-cells by -26.6% compared to carriers of 4 or less risk alleles. In our study, the largest effects on InsAUC 0-30/GluAUC 0-30 were observed for HHEX, MTNR1B, TCF7L2, and CDKAL1 (effect sizes ranging from -6 to -9% per risk allele). This finding is in agreement with previous studies, which have also quite consistently reported associations of these genes with impaired insulin secretion (6,13,17,30-32). Effects of SNPs in KCNJ11, SLC30A8, IGF2BP2, and CDKN2B on insulin secretion were <5% in our study. Previous studies examining these SNPs for an association with insulin secretion have been inconclusive (6,18-19,22-25,35), most probably due to insufficient power to detect modest effects of these SNPs. A few studies have reported associations of variants of WFS1 (36), TSPAN8 (33), JAZF1 (33), CDC123 (33), LOC387761 (24), and KCNQ1 (12) with insulin secretion, but our study failed to confirm such an association.

The mechanisms by which the insulin secretion-related genes influence insulin release have remained largely unknown. One of the plausible mechanisms proposed by previous studies is impaired conversion of proinsulin to insulin. In our study, four SNPs were significantly (SLC30A8 rs13266634, HHEX rs1111875, and TCF7L2 rs7903146) or nominally (CDKAL1 rs7754840) associated with the proinsulin/insulin ratio during first 30 min of an OGGT (adjusted for age, BMI, and Matsuda ISI). Variants in SLC30A8 and TCF7L2 were also nominally associated with fasting proinsulin/insulin ratio. Association of TCF7L2 rs7903146 with proinsulin levels (40,41) or proinsulin/insulin ratio (39,42) has been previously reported. Although the mechanisms behind this association are not
clear, impaired glucagon-like peptide 1 signalling seems to be involved (49). In a recent study, the association of SLC30A8 rs13266634, CDKAL1 rs7754840, and TCF7L2 rs7903146 with the proinsulin/insulin AUC ratio during OGTT was also shown (22). Our finding that HHEX variant is associated with impaired proinsulin conversion has not previously been reported. Our results suggest that SNPs in/near TCF7L2, CDKAL1, SLC30A8 and HHEX may affect insulin secretion, at least partially, through impaired proinsulin conversion. Although we had proinsulin data from almost 2700 subjects, the power of our study was limited to detect effect sizes <15% in the ProinsAUC0-30/InsAUC0-30 ratio. Therefore, even larger studies are needed to identify SNPs significantly associated with defects in proinsulin conversion.

PPARG has been the only clear insulin sensitivity-related gene among 20 diabetes-susceptibility loci confirmed by GWA studies. We observed only a small effect (-2% per risk allele) of PPARG rs1801282 (Pro12Ala) on Matsuda ISI, which was close to be nominally significant (P=0.054, adjusted for age and BMI). Similar small effects (~2% per risk allele) on Matsuda ISI were observed for variants in/near KCNJ11, HHEX, and TSPAN8 in our study, but none of them reached significant level after adjustment for age and BMI. In a recent study by Staiger et al. (34), a trend for association of TSPAN8 rs7961581 with Matsuda ISI and HOMA-IR indices of insulin sensitivity/resistance has also been reported. However, association of HHEX rs1111875 became significant after additional adjustment for InsAUC0-30/GluAUC0-30 in our study, and the risk allele was associated with higher Matsuda ISI. Although HHEX is primarily a candidate gene for impaired insulin secretion, it remains to be elucidated whether it also affects tissue-specific insulin sensitivity independently of changes in insulin secretion.

HHEX rs1111875 was associated with all traits examined in our study, and particularly its effects on InsAUC0-30/GluAUC0-30 and ProinsAUC0-30/InsAUC0-30 ratios were the most significant among all examined SNPs. Although the association of the HHEX locus with insulin secretion is well established (16-22), its association with insulin sensitivity and proinsulin conversion has not been previously reported. Further studies are needed to elucidate the molecular mechanisms of SNPs of the HHEX gene (or other genes near rs1111875) in regulating glucose homeostasis.

Our study has limitations. Only Finnish men were included in our study, and therefore we can not be sure whether our results are applicable to women and to different ethnic or racial groups. However, no evidence exists that the gender could modify the effects of diabetes susceptibility genes on glucose metabolism. We used surrogate markers of insulin secretion and insulin sensitivity derived from an OGTT, because the application of more accurate methods (intravenous glucose tolerance test, euglycemic clamp) is not feasible in a study having thousands of participants. Finally, in spite of the large sample size we did not have sufficient power (>80%) to detect small effects (<6% per allele) of examined SNPs on Matsuda ISI and InsAUC0-30/GluAUC0-30, which may explain negative findings for 9 of 18 SNPs in PPARG, LOC387761, WFS1, JAZF1, CDC123, THADA, ADAMTS9, NOTCH2, and KCNQ1.

In summary, we showed in a large cohort of Finnish men that 8 of 18 type 2 diabetes-related loci were significantly (TCF7L2, SLC30A8, HHEX, CDKN2B, CDKAL1 and MTNR1B) or nominally (KCNJ11, and IGF2BP2) associated with impaired early-phase insulin release, which decreased by -32% in carriers of ≥11 vs. ≤3 weighted type 2 diabetes risk alleles at these loci. Effects of TCF7L2, SLC30A8, HHEX,
and CDKAL1 on insulin secretion could be explained, at least in part, by impaired conversion of proinsulin to insulin. HHEX might influence both insulin release and insulin sensitivity.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Academy of Finland (Contract no. 124243), The Finnish Heart Foundation, The Finnish Diabetes Foundation, TEKES (Contract no. 1510/31/06), Commission of the European Community (LSHM-CT-2004-512013 EUGENE2, and HEALTH-F2-2007-201681) (all to M. Laakso), NIH grant DK62370 (to M. Boehnke), and The National Human Genome Research Institute Intramural project number 1 Z01 HG000024 (to F.S. Collins).
REFERENCES

1. Schousboe K, Visscher PM, Henriksen JE, Hopper JL, Sørensen TI, Kyvik KO: Twin study of genetic and environmental influences on glucose tolerance and indices of insulin sensitivity and secretion. *Diabetologia* 46:1276-1283, 2003

2. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881-885, 2007

3. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roij JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Boström K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Råstam L, Speliotes EK, Taskinen MR, Tuomilehto J, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmquist J, Laurila E, Sjögren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirm GW, Ma Q, Parikh H, Richardson D, Riche D, Purcell S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331-1336, 2007

4. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS; Wellcome Trust Case Control Consortium (WTCCC), McCarthy MI, Hattersley AT: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336-1341, 2007

5. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341-1345, 2007

6. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorradottir S, Bjarnason H, Ng MC, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So WY, Ma RC, Andersen G, Borch-Johnsen K, Jorgensen T, van Vliet-Oostapchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* 39:770-775, 2007

7. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD,
Hattersley AT, McCarthy MI: A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316:889-894, 2007

8. Gudmundsson J, Sulem P, Steinthorsdottir V, Berghthorsson JT, Thorleifsson G, Manolescu A, Rafnar T, Gudbjartsson D, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Blonldal T, Stacey SN, Helgason A, Gunnarsdottir S, Olafsdottir A, Kristinsson KT, Birgisdottir B, Ghosh S, Thorlacius S, Magnusdottir D, Stefandsdottir G, Kristjansson K, Bagger Y, Wilensky RL, Reilly MP, Morris AD, Kimber CH, Adeyemo A, Chen Y, Zhou J, So WY, Tong PC, Ng MC, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Tres A, Fuertes F, Ruiz-Echarri M, Asin L, Saez B, van Boven E, Klaver S, Swinkels DW, Aben KK, Graif T, Cashy J, Suarez BK, van Vierssen Trip O, Frigge ML, Ober C, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Palmer CN, Rotimi C, Chan JC, Pedersen O, Sigurdsson G, Benediktsson R, Jonsson E, Einarsson GV, Mayordomo JI, Catalona WJ, Kiemeney LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet* 39:977-983, 2007

9. Sandhu MS, Weedon MN, Fawcett KA, Wasson J, Debenham SL, Daly A, Lango H, Frayling TM, Neumann R, Sgerva R, Pharoah PD, Palmer CN, Kimber C, Cavendale R, Morris AD, McCarthy MI, Walker M, Hitman G, Glaser B, Permutt MA, Hattersley AT, Wareham NJ, Barroso I: Common variants in WFS1 confer risk of type 2 diabetes. *Nat Genet* 39:951-953, 2007

10. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, de Bakker PI, Abecasis GR, Almgren P, Anderson G, Ardlie K, Boström KB, Bergman RN, Bonnycastle LL, Borch-Johnsen K, Burtt NP, Chen H, Chines PS, Daly MJ, Deodhar P, Ding CJ, Doney AS, Duren W, Elliott KS, Erdos MR, Frayling TM, Freathy RM, Gianniny L, Grallert H, Grarup N, Groves CJ, Guiducci C, Hansen T, Herder C, Hitzman GA, Hughes TE, Isomaa B, Jackson AU, Jørgensen T, Kong A, Kubalanza K, Kuuvilla FG, Kuusisto J, Langenberg C, Lango H, Lauritzen T, Li Y, Lindgren CM, Lyssenko V, Marvellle AF, Meisinger C, Midithjell K, Mohlke KL, Morken MA, Morris AD, Narisu N, Nilsson P, Owen KR, Palmer CN, Payne F, Perry JR, Pettersen E, Platou C, Prokopenko I, Qi L, Qin L, Rayner NW, Rees M, Roix JJ, Sandbaek A, Shields B, Sjögren M, Steinthorsdottir V, Stringham HM, Swift AJ, Thorleifsson G, Thorsteinsdottir U, Timpson NJ, Tuomi T, Tuomilehto J, Walker M, Watanabe RM, Weedon MN, Willer CJ; Wellcome Trust Case Control Consortium, Illig T, Hveem K, Hu FB, Laakso M, Stefansson K, Pedersen O, Wareham NJ, Barroso I, Hattersley AT, Collins FS, Groop L, McCarthy MI, Boehnke M, Altshuler D: Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 40:638-645, 2008

11. Unoki H, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, Andersen G, Ng DP, Holmkvist J, Borch-Johnsen K, Jorgensen T, Sandbaek A, Lauritzen T, Hansen T, Nurbaya S, Tsunoda T, Kubo M, Babazono T, Hirose H, Hayashi M, Iwamoto Y, Kashiwagi A, Kaku K, Kawamori R, Tai ES, Pedersen O, Kamatani N, Kadowaki T, Kikkawa R, Nakamura Y, Maeda S: SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. *Nat Genet* 40:1098-1102, 2008

12. Yasuda K, Miyake K, Horikawa Y, Hara K, Osawa H, Furuta H, Hirota Y, Mori H, Jonsson A, Sato Y, Yamagata K, Hinokio Y, Wang HY, Tanahashi T, Nakamura N, Oka Y, Iwasaki N, Iwamoto Y, Yamada Y, Seino Y, Maegawa H, Kashiwagi A, Takeda J, Maeda E, Shin HD, Cho YM, Park KS, Lee HK, Ng MC, Ma RC, So WY, Chan JC, Lyssenko V, Tuomi T, Nilsson P, Groop L, Kamatani N, Sekine A, Nakamura Y, Yamamoto K, Yoshida T, Tokunaga K, Itakura
Type 2 diabetes risk genes and insulin secretion

M, Makino H, Nanjo K, Kadowaki T, Kasuga M: Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. *Nat Genet* 40:1092-1097, 2008

13. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, Potter SC, Erdos MR, Sanna S, Hottinger JJ, Wheeler E, Kaakinen M, Lyssenko V, Chen WM, Ahmadi K, Beckmann JS, Bergman RN, Bochud M, Bonnycastle LL, Buchanan TA, Cao A, Cervino A, Coin L, Collins FS, Crisponi L, de Geus EJ, Dehghan A, Deloukas P, Doney AS, Elliott P, Freimer N, Gateva V, Herder C, Hofman A, Hughes TE, Hunt S, Illig T, Inouye M, Isomaa B, Johnson T, Kong A, Krestyaninova M, Kuusisto J, Laakso M, Lim N, Lindblad U, Lindgren CM, McCann OT, Mohlke KL, Morris AD, Naitza S, Orrù M, Palmer CN, Pouta A, Randall J, Rathmann W, Saramies J, Scheet P, Scott LJ, Scuteri A, Sharp S, Sijbrands E, Smit JH, Song K, Steinthorsdottir V, Stringham HM, Tuomi T, Tuomilehto J, Uitterlinden AG, Voight BF, Waterworth D, Wichmann HE, Willemsen G, Witteman JC, Yuan X, Zhao JH, Zeggini E, Schlessinger D, Sandhu M, Boomsma DI, Uda M, Spector TD, Penninx BW, Altshuler D, Vollenweider P, Jarvelin MR, Lakatta E, Waeger G, Fox CS, Peltonen L, Groop LC, Mooser V, Cupples LA, Thorsteinsdottir U, Boehnke M, Barroso I, Van Duijn C, Dupuis J, Watanabe RM, Stefansson K, McCarthy MI, Wareham NJ, Meigs JB, Abecasis GR: Variants in MTNR1B influence fasting glucose levels. *Nat Genet* 41:77-81, 2009

14. Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K, Drivsholm T, Berglund L, Hansen T, Lithell H, Pedersen O: Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant caucasians. *Diabetologia* 44:1170-1176, 2001

15. Fritsche A, Madaus A, Tschritter O, Ozeker M, Wulle EL, Machicao F, Häring H, Stumvoll M: Polymorphism of pro12Ala in peroxisome proliferator activated receptor gamma 2 (PPARgamma2): beta cell function and insulin sensitivity. *Dtsch Med Wochenschr* 126:580-584, 2001

16. Freathy RM, Timpson NJ, Lawlor DA, Pouta A, Ben-Shlomo Y, Ruokonen A, Ebrahim S, Shields B, Zeggini E, Weedon MN, Lindgren CM, Lango H, Melzer D, Ferrucci L, Paolisso G, Neville MJ, Karpe F, Palmer CN, Morris AD, Elliott P, Jarvelin MR, Smith GD, McCarthy MI, Hattersley AT, Frayling TM: Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. *Diabetes* 57:1419-1426, 2008

17. Staiger H, Stančáková A, Zilinskaite J, Vänttinen M, Hansen T, Marini MA, Hammarstedt A, Jansson PA, Sesti G, Smith U, Pedersen O, Lilaas M, Stefan N, Fritsche A, Häring HU: A candidate type 2 diabetes polymorphism near the HHEX locus affects acute glucose-stimulated insulin release in European populations: results from the EUGENE2 study. *Diabetes* 57:514-517, 2008

18. Staiger H, Machicao F, Stefan N, Tschritter O, Thamer C, Kantartzis K, Schäfer SA, Kirchhoff K, Fritsche A, Häring HU: Polymorphisms of pro12Ala in peroxisome proliferator activated receptor gamma 2 (PPARgamma2): beta cell function and insulin sensitivity. *Dtsch Med Wochenschr* 126:580-584, 2001

19. Moore AF, Jablonski KA, McAteer JB, Saxena R, Pollin TI, Franks PW, Hanson RL, Shuldiner AR, Knowler WC, Altshuler D, Florez JC; Diabetes Prevention Program Research Group: Extension of type 2 diabetes genome-wide association scan results in the diabetes prevention program. *Diabetes* 57:2503-2510, 2008

20. Grarup N, Rose CS, Andersson EA, Andersen G, Nielsen AL, Albrechtsen A, Clausen JO, Rasmussen SS, Jørgensen T, Sandbaek A, Lauritzen T, Schmitz O, Hansen T, Pedersen O: Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2
diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes* 56:3105-3111, 2007

21. Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M; RISC Consortium; UK: Type 2 Diabetes Genetics Consortium. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. *Diabetes* 56:3101-3104, 2007

22. Kirchhoff K, Machicao F, Haupt A, Schäfer SA, Tscherritter O, Staiger H, Stefan N, Häring HU, Frischa A: Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia* 51:597-601, 2008

23. Rong R, Hanson RL, Ortiz D, Wiedrich C, Kobes S, Knowler WC, Bogardus C, Baier LJ: Association Analysis of Variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761 and CDKN2B with Type 2 Diabetes and Related Quantitative Traits in Pima Indians. *Diabetes* 58:478-488, 2008

24. Palmer ND, Goodarzi MO, Langefeld CD, Ziegler J, Norris JM, Haffner SM, Bryer-Ash M, Bergman RN, Wagenknecht LE, Taylor KD, Rotter JI, Bowden DW: Quantitative trait analysis of type 2 diabetes susceptibility loci identified from whole genome association studies in the Insulin Resistance Atherosclerosis Family Study. *Diabetes* 57:1093-1100, 2008

25. Groenewoud MJ, Dekker JM, Frischa A, Reiling E, Nijpels G, Heine RJ, Maassen JA, Machicao F, Schäfer SA, Häring HU, 't Hart LM, van Haeften TW: Variants of CDKAL1 and IGF2BP2 affect first-phase insulin secretion during hyperglycaemic clamps. *Diabetologia* 51:1659-1663, 2008

26. Stančáková A, Pihlajamäki J, Kuusisto J, Stefan N, Frischa A, Häring H, Andreozzi F, Succurro E, Sesti G, Boesgaard TW, Hansen T, Pedersen O, Jansson PA, Hammarstedt A, Smith U, Laakso M; EUGENE2 Consortium: Single-nucleotide polymorphism rs7754840 of CDKAL1 is associated with impaired insulin secretion in non-diabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. *J Clin Endocrinol Metab* 93:1924-1930, 2008

27. Pascoe L, Frayling TM, Weedon MN, Mari A, Tura A, Ferrannini E, Walker M; RISC Consortium: Beta cell glucose sensitivity is decreased by 39% in non-diabetic individuals carrying multiple diabetes-risk alleles compared with those with no risk alleles. *Diabetologia* 51:1989-1992, 2008

28. Palmer ND, Lehtinen AB, Langefeld CD, Campbell JK, Haffner SM, Norris JM, Bergman RN, Goodarzi MO, Rotter JI, Bowden DW: Association of TCF7L2 gene polymorphisms with reduced acute insulin response in Hispanic Americans. *J Clin Endocrinol Metab* 93:304-309, 2008

29. Munoz J, Lok KH, Gower BA, Fernandez JR, Hunter GR, Lara-Castro C, De Luca M, Garvey WT: Polymorphism in the transcription factor 7-like 2 (TCF7L2) gene is associated with reduced insulin secretion in non-diabetic women. *Diabetes* 55:3630-3634, 2006

30. Saxena R, Gianniny L, Burtt NP, Lyssenko V, Giuducci C, Sjögren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, Lindblad U, Daly MJ, Tuomi T, Hirschhorn JN, Ardlie KG, Groop LC, Altshuler D: Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 55:2890-2895, 2006

31. Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, Spégl P, Bugliani M, Saxena R, Fex M, Pulizzi N, Isomaa B, Tuomi T, Nilsson P, Kuusisto J, Tuomilehto J, Boehnke M,
Altshuler D, Sundler F, Eriksson JG, Jackson AU, Laakso M, Marchetti P, Watanabe RM, Mulder H, Groop L: Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nat Genet* 41:82-88, 2009
32. Staiger H, Machicao F, Schäfer SA, Kirchhoff K, Kantartzis K, Guthoff M, Silbernagel G, Stefan N, Häring HU, Fritsche A: Polymorphisms within the novel type 2 diabetes risk locus MTNR1B determine beta-cell function. *PLoS ONE* 3:e3962, 2008
33. Grarup N, Andersen G, Krarup NT, Albrechtsen A, Schmitz O, Jørgensen T, Borch-Johnsen K, Hansen T, Pedersen O: Association testing of novel type 2 diabetes risk alleles in the JAZF1, CDC123/CAMK1D, TSPAN8, THADA, ADAMTS9, and NOTCH2 loci with insulin release, insulin sensitivity, and obesity in a population-based sample of 4,516 glucose-tolerant middle-aged Danes. *Diabetes* 57:2534-2540, 2008
34. Staiger H, Machicao F, Kantartzis K, Schäfer SA, Kirchhoff K, Guthoff M, Silbernagel G, Stefan N, Fritsche A, Häring HU: Novel meta-analysis-derived type 2 diabetes risk loci do not determine prediabetic phenotypes. *PLoS ONE* 3:e3019, 2008
35. Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glümer C, Thorsteinsson B, Borch-Johnsen K, Hansen T, Pedersen O: The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52:573-577, 2003
36. Sparsø T, Andersen G, Albrechtsen A, Jørgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Wasson J, Permutt MA, Glaser B, Madsbad S, Pedersen O, Hansen T: Impact of polymorphisms in WFS1 on prediabetic phenotypes in a population-based sample of middle-aged people with normal and abnormal glucose regulation. *Diabetologia* 51:1646-1652, 2008
37. Florez JC, Jablonski KA, McAteer J, Sandhu MS, Wareham NJ, Barroso I, Franks PW, Altshuler D, Knowler WC; Diabetes Prevention Program Research Group; Testing of diabetes-associated WFS1 polymorphisms in the Diabetes Prevention Program. *Diabetologia* 51:451-457, 2008
38. Wegner L, Hussain MS, Pilgaard K, Hansen T, Pedersen O, Vaag A, Poulsen P: Impact of TCF7L2 rs7903146 on insulin secretion and action in young and elderly Danish twins. *J Clin Endocrinol Metab* 93:4013-4019, 2008
39. González-Sánchez JL, Martínez-Larrad MT, Zabena C, Pérez-Barba M, Serrano-Ríos M: Association of variants of the TCF7L2 gene with increases in the risk of type 2 diabetes and the proinsulin:insulin ratio in the Spanish population. *Diabetologia* 51:1993-1997, 2008
40. Dahlgren A, Zethelius B, Jensevik K, Syyänen AC, Berne C; ULSAM Cohort: Variants of the TCF7L2 gene are associated with beta cell dysfunction and confer an increased risk of type 2 diabetes mellitus in the ULSAM cohort of Swedish elderly men. *Diabetologia* 50:1852-1857, 2007
41. Loos RJ, Franks PW, Francis RW, Barroso I, Gribble FM, Savage DB, Ong KK, O'Rahilly S, Wareham NJ: TCF7L2 polymorphisms modulate proinsulin levels and beta-cell function in a British Europid population. *Diabetes* 56:1943-1947, 2007
42. Stolerman ES, Manning AK, McAteer JB, Fox CS, Dupuis J, Meigs JB, Florez JC: TCF7L2 variants are associated with increased proinsulin/insulin ratios but not obesity traits in the Framingham Heart Study. *Diabetologia* 52:614-620, 2009
43. Wang J, Kuusisto J, Vänttinen M, Kuulasmaa T, Lindström J, Tuomilehto J, Uusitupa M, Laakso M: Variants of the transcription 7-like 2 (TCF7L2) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired glucose tolerance. *Diabetologia* 50:1192-1200, 2007
Type 2 diabetes risk genes and insulin secretion

44. World Health Organization: Definition, Diagnosis and Classification of Diabetes: Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva, Department of Noncommunicable Disease Surveillance; 1999

45. Matsuda M, DeFronzo RA: Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22:1462-1470, 1999

46. Stančáková A, Javorský M, Kuulasmaa T, Hahner SM, Kuusisto J, Laakso M: Changes in Insulin Sensitivity and Insulin Release in Relation to Glycemia and Glucose Tolerance in 6414 Finnish Men. Diabetes 58:1212-1221, 2009

47. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, Lettre G, Lim N, Lyon HN, McCarroll SA, Papadakis K, Qi L, Randall JC, Roccavecca RM, Sanna S, Scheet P, Weedon MN, Wheeler E, Zhao JH, Jacobs LC, Prokopenko I, Soranzo N, Tanaka T, Timpson NJ, Almgren P, Bennett A, Bergman RN, Bingham SA, Bonnycastle LL, Brown M, Burtt NP, Chines P, Coin L, Collins FS, Connell JM, Cooper C, Smith GD, Dennison EM, Deodhar P, Elliott P, Erdos MR, Estrada K, Evans DM, Gianniny L, Gieger C, Gillson CJ, Guiducci C, Hackett R, Hadley D, Hall AS, Havulinna AS, Hebebrand J, Hofman A, Isomaa B, Jacobs KB, Johnson T, Jousilahti P, Jovanovic Z, Khaw KT, Kraft P, Kuokkanen M, Kuusisto J, Laitinen J, Lakatta EG, Luan J, Luben RN, Mangino M, McArdle WL, Meitinger T, Mulas A, Munroe PB, Narisu N, Ness AR, Northstone K, O’Rahilly S, Purmann C, Rees MG, Ridderstråle M, Ring SM, Rivadeneira F, Ruokonen A, Sandhu MS, Saramies J, Scott LJ, Scuteri A, Silander K, Sims MA, Song K, Stephens J, Stevens S, Stringham HM, Tung YC, Valle TT, Van Duijn CM, Vimalaswaran KS, Vollenweider P, Waeger G, Wallace C, Watanabe RM, Waterworth DM, Watkins N; Wellcome Trust Case Control Consortium, Wittman JC, Zeggini E, Zhai G, Zillikens MC, Altmseher D, Caulfield MJ, Chanock SJ, Farooqi IS, Ferrucci L, Guralnik JM, Hattersley AT, Hu FB, Jarvelin MR, Laakso M, Mooser V, Ong KK, Ouwehand WH, Salomaa V, Samani NJ, Spector TD, Tuomi T, Tuomilehto J, Uda M, Uitterlinden AG, Wareham NJ, Deloukas P, Frayling TM, Groop LC, Hayes RB, Hunter DJ, Mohlke KL, Peltonen L, Schlessinger D, Strachan DP, Wichmann HE, McCarthy MI, Boehnke M, Barroso I, Abecasis GR, Hirschhorn JN; Genetic Investigation of ANthropometric Traits Consortium: Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41:25-34, 2009

48. Perry JR, Frayling TM: New gene variants alter type 2 diabetes risk predominantly through reduced beta-cell function. Curr Opin Clin Nutr Metab Care 11:371-377, 2008

49. Schäfer SA, Tschritter O, Machicao F, Thamer C, Stefan N, Gallwitz B, Holst JJ, Dekker JM, Thart LM, Nijpels G, van Haeften TW, Häring HU, Fritsche A: Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. Diabetologia 50:2443-2450, 2007
### Table 1: Associations of 18 SNPs with early-phase insulin release (InsAUCₐ₀₋₃₀/GluAUCₐ₀₋₃₀), proinsulin conversion (ProinsAUCₐ₀₋₃₀/InsAUCₐ₀₋₃₀), insulin sensitivity (Matsuda ISI), and disposition index (DI = InsAUCₐ₀₋₃₀/GluAUCₐ₀₋₃₀ x Matsuda ISI) in non-diabetic subjects.

| SNP      | Gene         | Alleles | MAF (%) | InsAUCₐ₀₋₃₀ / GluAUCₐ₀₋₃₀ | ProinsAUCₐ₀₋₃₀ / InsAUCₐ₀₋₃₀ | Matsuda ISI | Disposition index |
|----------|--------------|---------|---------|-----------------------------|-------------------------------|-------------|-------------------|
|          |              |         |         | Effect size B (SE) | P | P* | Effect size B (SE) | P | P* | Effect size B (SE) | P | P* |
| PPARC    | C/G          | rs1801282 | 15.5    | 0.63 (0.57) | 0.316 | 0.664 | 0.14 (0.45) | 0.991 | 0.560 | -0.11 (0.11) | 0.364 | 0.054 | -0.30 (1.99) | 0.958 | 0.810 |
| KCNJ11   | G/A          | rs5219   | 47.7    | -1.14 (0.41) | 0.04 | 0.025 | 0.49 (0.32) | 0.115 | 0.531 | 0.25 (0.08) | 0.005 | 0.008 | -1.32 (1.40) | 0.362 | 0.231 |
| TCF7L2   | C/T          | rs7903146 | 17.7    | -1.78 (0.53) | 0.05 | 0.07 | 0.75 (0.42) | 0.002 | 6.0E-04 | 0.12 (0.11) | 0.228 | 0.920 | -6.51 (1.87) | 8.3E-05 | 3.4E-06 |
| SLC30A8  | C/T          | rs13266634 | 39.1    | -0.83 (0.41) | 0.013 | 3.2E-04 | 0.73 (0.33) | 1.2E-05 | 0.05 | -0.00 (0.08) | 0.871 | 0.679 | -4.19 (1.46) | 0.001 | 4.2E-04 |
| HHEX     | C/T          | rs1111875 | 46.9    | -2.73 (0.40) | 12 | 14 | 0.80 (0.32) | 9.7E-06 | 6.5E-06 | 0.17 (0.08) | 0.010 | 0.017 | -8.89 (1.42) | 2.5E-09 | 1.2E-10 |
| LOC387761| A/G          | rs7480010 | 17.5    | -0.51 (0.54) | 0.540 | 0.290 | -0.33 (0.44) | 0.829 | 0.194 | 0.17 (0.11) | 0.087 | 0.345 | 3.57 (1.91) | 0.949 | 0.189 |
| CDKN2B   | A/G          | rs10811661 | 14.5    | -1.15 (0.58) | 0.021 | 1.7E-04 | 0.31 (0.47) | 0.285 | 0.211 | -0.03 (0.12) | 0.847 | 0.413 | -6.30 (1.99) | 4.3E-04 | 0.001 |
| IGFBP2   | C/A          | rs4402960 | 32.1    | -1.34 (0.43) | 0.004 | 0.004 | 0.14 (0.34) | 0.263 | 0.368 | 0.08 (0.09) | 0.182 | 0.440 | -4.22 (1.53) | 0.038 | 0.014 |
| CDKAL1   | G/C          | rs7754840 | 37.0    | -1.68 (0.42) | 5 | 6 | 0.32 (0.34) | 3.1E-04 | 0.001 | 0.12 (0.08) | 0.181 | 0.176 | -5.25 (1.48) | 1.6E-04 | 6.4E-05 |
| WFS1     | G/A          | rs10010131 | 45.0    | -0.56 (0.41) | 0.048 | 0.397 | 0.01 (0.33) | 0.402 | 0.081 | 0.14 (0.08) | 0.055 | 0.100 | 0.23 (1.44) | 0.986 | 0.808 |
| JAZF1    | A/G          | rs864745  | 48.5    | 0.15 (0.41) | 0.551 | 0.554 | -0.25 (0.32) | 0.792 | 0.968 | -0.10 (0.08) | 0.198 | 0.067 | -1.31 (1.43) | 0.301 | 0.241 |
| CDC123   | A/G          | rs12779790 | 21.5    | -0.82 (0.49) | 0.059 | 0.062 | -0.07 (0.39) | 0.486 | 0.598 | 0.07 (0.10) | 0.369 | 0.433 | -2.36 (1.73) | 0.196 | 0.043 |
| TSPAN8   | A/G          | rs7961581 | 19.4    | 0.23 (0.51) | 0.525 | 0.891 | -0.29 (0.41) | 0.120 | 0.310 | -0.15 (0.10) | 0.343 | 0.008 | -0.75 (1.80) | 0.635 | 0.308 |
| THADA    | A/G          | rs7578597 | 5.0     | -2.09 (0.93) | 0.263 | 0.232 | -1.24 (0.73) | 0.425 | 0.267 | 0.04 (0.18) | 0.659 | 0.373 | -3.51 (3.27) | 0.355 | 0.410 |
| ADAMTS9  | G/A          | rs4607103 | 26.1    | -0.66 (0.47) | 0.335 | 0.221 | -0.04 (0.37) | 0.087 | 0.069 | -0.04 (0.09) | 0.809 | 0.587 | -2.13 (1.65) | 0.308 | 0.332 |
| NOTCH2   | C/A          | rs10923931 | 13.8    | -0.56 (0.59) | 0.228 | 0.668 | -0.95 (0.47) | 0.360 | 0.080 | 0.16 (0.12) | 0.054 | 0.060 | 1.21 (2.09) | 0.244 | 0.300 |
| KCNJ1    | A/C          | rs2283228 | 6.2     | -1.03 (0.84) | 0.161 | 0.093 | 0.31 (0.66) | 0.176 | 0.353 | 0.10 (0.17) | 0.701 | 0.284 | -3.29 (2.96) | 0.162 | 0.221 |
| MTNR1B   | C/G          | rs10830963 | 36.0    | -2.02 (0.42) | 1.4E-07 | 1.0E-13 | -0.21 (0.33) | 0.301 | 0.189 | 0.03 (0.08) | 0.577 | 0.436 | -9.65 (1.47) | 6.7E-11 | 3.8E-13 |

Effect size shown is B-coefficient (SE) per copy of the type 2 diabetes risk allele, and was calculated using untransformed variables adjusted for age by linear regression. P values were calculated using log-transformed variables (due to their skewed distribution) by linear regression. P values are adjusted for age; P* values are adjusted for age, BMI, and Matsuda ISI; P† values are adjusted for age and BMI. In the entire cohort, means±SE of examined parameters and the number of subjects with available data were as follows: InsAUC₁₀₋₃₀/GluAUC₁₀₋₃₀ 12.5±0.23 (N=2697), ProinsAUC₁₀₋₃₀/InsAUC₁₀₋₃₀ 30.4±0.29 pmol/mmol (N=5298), ProinsAUC₁₀₋₃₀/InsAUC₁₀₋₃₀ × Matsuda ISI 7.03±0.06 [mg/dl, mU/l]
Type 2 diabetes risk genes and insulin secretion

(N=5295), and DI 163.7±1.02 (N=5295). P values significant after correction for multiple testing (P<6.9x10^{-4}) are in bold. Risk alleles are underlined. Results for the additive model are presented.

Table 2: Associations of 4 SNPs with proinsulin/insulin ratio at fasting state (Proins\textsubscript{0}/Ins\textsubscript{0}), during 0 to 30 min (ProinsAUC\textsubscript{0-30}/InsAUC\textsubscript{0-30}), 30 to 120 min (ProinsAUC\textsubscript{30-120}/InsAUC\textsubscript{30-120}) and 0 to 120 min (ProinsAUC\textsubscript{0-120}/InsAUC\textsubscript{0-120}) of an OGTT in non-diabetic subjects

| Gene   | Alleles | Proins\textsubscript{0}/Ins\textsubscript{0} | ProinsAUC\textsubscript{0-30}/InsAUC\textsubscript{0-30} | ProinsAUC\textsubscript{30-120}/InsAUC\textsubscript{30-120} | ProinsAUC\textsubscript{0-120}/InsAUC\textsubscript{0-120} |
|--------|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| SNP    | MAF (%) | Effect size B (SE) P P*       | Effect size B (SE) P P*       | Effect size B (SE) P P*       | Effect size B (SE) P P*       |
| TCF7L2 | C/T     | 1.20 (1.22) 0.042 0.021       | 0.75 (0.42) 0.002 6.0E-04 0.005 1.1E-03 | 0.55 (0.44) 0.005 1.1E-03 0.004 0.001 |
| SLC30A8| C/T     | 1.59 (0.96) 0.006 0.003       | 0.73 (0.33) 1.9E-05 1.2E-05 0.64 (0.35) 1.1E-04 4.2E-05 | 0.64 (0.34) 1.1E-05 4.2E-05 0.001 6.6E-04 |
| HHEX   | C/T     | 0.74 (0.94) 0.365 0.622       | 0.80 (0.32) 9.7E-06 6.5E-06 | 0.69 (0.34) 0.002 0.002 | 0.71 (0.33) 0.001 6.6E-04 |
| CDKAL1 | G/C     | -0.39 (0.98) 0.313 0.775      | 0.32 (0.34) 3.1E-04 0.001 | 0.36 (0.35) 0.003 0.009 | 0.35 (0.35) 0.002 0.005 |

Effect size shown is B-coefficient (SE) per copy of the type 2 diabetes risk allele, and was calculated using untransformed variables adjusted for age by linear regression. P values were calculated using log-transformed variables (due to their skewed distribution) by linear regression. P values are adjusted for age, BMI and Matsuda ISI. In the entire cohort, means±SE of examined parameters and the number of subjects with available data were as follows: Proins\textsubscript{0}/Ins\textsubscript{0} 36.3±0.67 (N=2712), ProinsAUC\textsubscript{0-30}/InsAUC\textsubscript{0-30} 12.5±0.23 (N=2697), ProinsAUC\textsubscript{30-120}/InsAUC\textsubscript{30-120} 14.1±0.24 (N=2693), ProinsAUC\textsubscript{0-120}/InsAUC\textsubscript{0-120} 13.8±0.24 (N=2692). P values significant after correction for multiple testing (P<6.9x10^{-4}) are in bold. Risk alleles are underlined. Results for the additive model are presented.
Figure 1. Early-phase insulin release (InsAUC$_{0-30}$/GluAUC$_{0-30}$) according to the number of risk alleles in 8 insulin secretion-related SNPs (KCNJ11 rs5219, TCF7L2 rs7903146, SLC30A8 rs13266634, HHEX rs1111875, CDKN2B rs10811661, IGF2BP2 rs4402960, CDKAL1 rs7754840 and MTNR1B rs10830963). For each subject, the number of type 2 diabetes risk alleles (0,1,2) per SNP was weighted for their effect sizes (shown in Table 1; average effect size per risk allele among 8 SNPs was 1.58, which was considered as one weighted risk allele). Effect of the number of the risk alleles on InsAUC$_{0-30}$/GluAUC$_{0-30}$ was significant ($P=9.3 \times 10^{-44}$, adjusted for age, BMI and Matsuda ISI). Data are shown as means±SE (adjusted for age, BMI and Matsuda ISI). Bars show numbers of subjects in each category.