Association of 18 Confirmed Susceptibility Loci for Type 2 Diabetes With Indices of Insulin Release, Proinsulin Conversion, and Insulin Sensitivity in 5,327 Nondiabetic Finnish Men

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OBJECTIVE—We investigated the effects of 18 confirmed type 2 diabetes risk single nucleotide polymorphisms (SNPs) on insulin sensitivity, insulin secretion, and conversion of proinsulin to insulin.

RESEARCH DESIGN AND METHODS—A total of 5,327 nondiabetic men (age 58 ± 7 years, BMI 27.0 ± 3.8 kg/m^2) from a large population-based cohort were included. Oral glucose tolerance tests and genotyping of SNPs in or 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 because of failure to achieve Hardy-Weinberg equilibrium.

RESULTS—Six SNPs (*TCF7L2*, *SLC30A8*, *HHEX*, *CDKN2B*, *CDKAL1*, and *MTNR1B*) were significantly ($P < 6.9 \times 10^{-4}$) 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 eight 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*, and *TCF7L2*) were significantly or nominally associated with indexes of proinsulin conversion. Three SNPs (*KCNJ11*, *HHEX*, and *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. ***Diabetes*** **58:2129–2136, 2009**

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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 20 genetic loci reproducibly associated with type 2 diabetes (2–13). Three were previously known (*PPARG*, *KCNJ11*, and *TCF7L2*), whereas 17 loci were recently discovered either by genome-wide association studies (*SLC30A8*, *HHEX-IDE*, *LOC387761*, *CDKN2A/2B*, *IGF2BP2*, *CDKAL1*, *FTO*, *JAZF1*, *CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, *NOTCH2*, *KCNQ1*, and *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 β-cell function has been reported for 14 loci (*KCNJ11*, *SLC30A8*, *HHEX-IDE*, *CDKN2A/2B*, *IGF2BP2*, *CDKAL1*, *TCF7L2*, *WFS1*, *HNF1B*, *JAZF1*, *CDC123/CAMK1D*, *TSPAN8/LGR5*, *KCNQ1*, and *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 (12,18–25,27,33–38). 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 single nucleotide polymorphisms (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 5,327 nondiabetic Finnish men.

RESEARCH DESIGN AND METHODS

A total of 5,327 nondiabetic 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/m^2). Of these, 3,594 (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, Eastern Finland (population of 95,000) for the METSIM study. Every participant had a 1-day outpatient visit to the Clinical Research Unit at the University of Kuopio. Blood samples were drawn after 12 h of fasting followed by an oral glucose tolerance test (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. BMI was calculated as weight (killograms) divided by height (meters) squared.

OGTT. 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 World Health Organization 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 2,697 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, *CDC123* rs12779790, *TSPAN8* rs7961581, *THADA* rs7578597, *ADAMTS9* rs4607103, *NOTCH2* rs10923931, *KCNQ1* 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 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 ($InsAUC_{0-30}/GluAUC_{0-30}$) 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, $InsAUC_{0-30}/GluAUC_{0-30}$ had the highest correlation ($r = 0.666$) with the first-phase insulin secretion in an intravenous glucose tolerance test among 11 different indexes 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 indexes tested (46). Four indexes of proinsulin conversion were calculated: proinsulin/insulin ratio in the fasting state ($Proins_0/Ins_0$), an index of proinsulin conversion to insulin during the first 30 min ($ProinsAUC_{0-30}/InsAUC_{0-30}$), 30–120 min ($ProinsAUC_{30-120}/InsAUC_{30-120}$), and 0–120 min ($ProinsAUC_{0-120}/InsAUC_{0-120}$) of an OGTT. All indexes 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 was calculated as $InsAUC_{0-30}/GluAUC_{0-30} \times$ Matsuda ISI. To estimate a combined impact of multiple type 2 diabetes risk alleles (denoted as the risk allele throughout the text) on $InsAUC_{0-30}/GluAUC_{0-30}$ we calculated a genetic risk score as a sum of weighted risk alleles (47) at SNPs significantly or nominally associated with $InsAUC_{0-30}/GluAUC_{0-30}$ 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 eight 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) because of 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 (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 because of significant association of genetic risk score with these covariates. Hardy-Weinberg equilibrium was tested by χ^2 test. Statistical analyses were conducted with the SPSS 14 programs (SPSS, Chicago, IL). $P < 0.05$ was considered nominally significant, and $P < 6.9 \times 10^{-4}$ calculated using Bonferroni correction for multiple comparisons was considered statistically significant, given 72 independent tests for 18 SNPs and four 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 ≥80% to detect changes from 5 to 8% per copy of the risk allele for $InsAUC_{0-30}/GluAUC_{0-30}$, Matsuda ISI, and disposition index for SNPs with minor allele frequency >30%, and power ≥80% to detect a change of ~15% in $ProinsAUC_{0-30}/InsAUC_{0-30}$ for SNPs with minor allele frequency 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 4 SNPs (*TCF7L2* rs7903146, *CDC123* rs12779790, *TSPAN8* rs7961581, and *MTNR1B* rs10830963) the association was nominally significant ($P = 0.018$, 0.006, 0.031, and 0.035). The effect sizes were <−1% per type 2 diabetes risk allele. 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 ($P = 0.010$ 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 ($P = 0.008$, effect size −2% 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 ($P = 3.6 \times 10^{-5}$).

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 or 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 associations of *SLC30A8* rs13266634 and *CDKN2B* rs10811661 to significant level ($P = 3.2 \times 10^{-4}$ and 1.7×10^{-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 or 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: $P = 9.7 \times 10^{-6}$ and 1.9×10^{-5}; adjusted for age, BMI, and Matsuda ISI: $P = 6.5 \times 10^{-6}$ and 1.2×10^{-5}). In contrast, adjustment for BMI and Matsuda ISI attenuated the significant effect of *CDKAL1* rs7754840 to nominal level ($P =$

TABLE 1
Associations of 18 SNPs with early-phase insulin release ($InsAUC_{0-30}/GluAUC_{0-30}$), proinsulin conversion ($ProinsAUC_{0-30}/InsAUC_{0-30}$), insulin sensitivity (Matsuda ISI), and disposition index (disposition index = $InsAUC_{0-30}/GluAUC_{0-30} \times$ Matsuda ISI) in nondiabetic subjects

	Alleles	$InsAUC_{0-30}$ / $GluAUC_{0-30}$			$ProinsAUC_{0-30}$ / $InsAUC_{0-30}$			Matsuda ISI			Disposition index		
Gene SNP	MAF (%)	Effect size B (SE)	*P* values	**P* values	Effect size B (SE)	*P* values	**P* values	Effect size B (SE)	*P* values	†*P* values	Effect size B (SE)	*P* values	†*P* values
PPARG rs1801282	C/G 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 rs5219	G/A 47.7	−1.14 (0.41)	**3.8E-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 rs7903146	C/T 17.7	−1.78 (0.53)	**3.9E-05**	**9.8E-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 rs13266634	C/T 39.1	−0.83 (0.41)	0.013	**3.2E-04**	0.73 (0.33)	**1.9E-05**	**1.2E-05**	−0.00 (0.08)	0.871	0.679	−4.19 (1.46)	0.001	**4.2E-04**
HHEX rs1111875	C/T 46.9	−2.73 (0.40)	**3.2E-12**	**1.4E-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 rs7480010	A/G 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)	0.094	0.189
CDKN2B rs10811661	A/G 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
IGF2BP2 rs4402960	C/A 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 rs7754840	G/C 37.0	−1.68 (0.42)	**3.4E-05**	**2.2E-06**	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 rs10010131	G/A 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 rs864745	A/G 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 rs12779790	A/G 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 rs7961581	A/G 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 rs7578597	A/G 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 rs4607103	G/A 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 rs10923931	C/A 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
KCNQ1 rs2283228	A/C 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 rs10830963	C/G 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 (because of 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: $InsAUC_{0-30}/GluAUC_{0-30}$ 30.4 ± 0.29 pmol/mmol (n = 5,298), $ProinsAUC_{0-30}/InsAUC_{0-30}$ 12.5 ± 0.23 (n = 2,697), Matsuda ISI 7.03 ± 0.06 (mg/dl, mU/l; n = 5,295), and disposition index 163.7 ± 1.02 (n = 5,295). *P* values significant after correction for multiple testing ($P < 6.9 \times 10^{-4}$) are in bold. Risk alleles are underlined. Results for the additive model are presented.

TABLE 2
Associations of four SNPs with proinsulin/insulin ratio at fasting state ($Proins_0/Ins_0$), during 0–30 min ($ProinsAUC_{0-30}/InsAUC_{0-30}$), 30–120 min ($ProinsAUC_{30-120}/InsAUC_{30-120}$), and 0–120 min ($ProinsAUC_{0-120}/InsAUC_{0-120}$) of an OGTT in nondiabetic subjects

Gene SNP	Alleles		$Proins_0/Ins_0$			$ProinsAUC_{0-30}/InsAUC_{0-30}$			$ProinsAUC_{30-120}/InsAUC_{30-120}$			$ProinsAUC_{0-120}/InsAUC_{0-120}$		
	MAF (%)		Effect size B (SE)	*P* values	**P* values	Effect size B (SE)	*P* values	**P* values	Effect size B (SE)	*P* values	**P* values	Effect size B (SE)	P values	**P* values
TCF7L2 rs7903146	C/T 17.7		1.20 (1.22)	0.042	0.021	0.75 (0.42)	0.002	**6.0E-04**	0.55 (0.44)	0.005	**1.1E-03**	0.57 (0.43)	0.004	0.001
SLC30A8 rs13266634	C/T 39.1		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)	**8.2E-05**	**2.8E-05**
HHEX rs1111875	C/T 46.9		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 rs7754840	G/C 37.0		−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 (because of their skewed distribution) by linear regression. *P* values are adjusted for age; **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: $Proins_0/Ins_0$ 36.3 ± 0.67 (n = 2,712), $ProinsAUC_{0-30}/InsAUC_{0-30}$ 12.5 ± 0.23 (n = 2,697), $ProinsAUC_{30-120}/InsAUC_{30-120}$ 14.1 ± 0.24 (n = 2,693), $ProinsAUC_{0-120}/InsAUC_{0-120}$ 13.8 ± 0.24 (n = 2,692). *P* values significant after correction for multiple testing ($P < 6.9 \times 10^{-4}$) are in bold. Risk alleles are underlined. Results for the additive model are presented.

0.002), and strengthened nominal effect of *TCF7L2* rs7903146 to significant level ($P = 6.0 \times 10^{-4}$). Similar results, although slightly attenuated, were obtained when alternative indexes 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, because the risk alleles associated with lower insulin release were 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 disposition index (Table 1). The largest effects ranging from −3 to −6% per risk allele were observed for *MTNR1B* rs10830963 ($P = 6.7 \times 10^{-11}$), *HHEX* rs1111875 ($P = 2.5 \times 10^{-9}$), *TCF7L2* rs7903146 ($P = 8.3 \times 10^{-5}$), *CDKN2B* rs10811661 ($P = 4.3 \times 10^{-4}$), and *CDKAL1* rs7754840 ($P = 1.6 \times 10^{-4}$). 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 (nine for $InsAUC_{0-30}/GluAUC_{0-30}$, four for $ProinsAUC_{0-30}/InsAUC_{0-30}$, two for Matsuda ISI, and seven for disposition index in primary analyses), suggesting that the associations we found were not likely to occur by chance. However, it should be mentioned that despite 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 = 3,594) (supplemental Table 1, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0117/DC1). The effect sizes were mostly similar, although associations were generally slightly weaker because of a smaller sample size. In contrast, in analyses including both nondiabetic 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 disposition index (P = 0.001 and 0.043, adjusted for age and BMI, effect sizes ~−2% per risk allele, supplemental Table 2).

Combined effect of risk alleles on insulin release. We combined the risk alleles at eight 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, $P = 9.3 \times 10^{-44}$ adjusted for age, BMI, and Matsuda ISI). Subjects with ≥11 weighted risk alleles (n = 190) had decreased $InsAUC_{0-30}/GluAUC_{0-30}$ by −32% compared with subjects with ≤3 weighted risk alleles (n = 163) (Fig. 1). We also performed similar analysis using nonweighted risk alleles. The difference in $InsAUC_{0-30}/GluAUC_{0-30}$ between subjects with ≤3 and ≥11 risk alleles was −37% (relative effect size −4% per risk allele, $P = 3.8 \times 10^{-28}$).

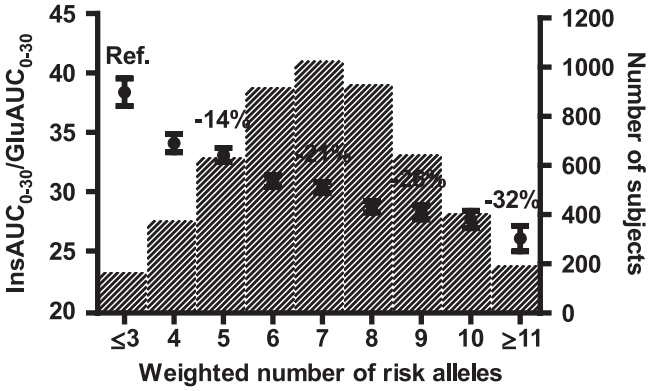

FIG. 1. Early-phase insulin release ($InsAUC_{0-30}/GluAUC_{0-30}$) according to the number of risk alleles in eight insulin secretion–related SNPs (*KCNJ11* rs5219, *TCF7L2* rs7903146, *SLC30A8* rs13266634, *HHEX* rs1111875, *CDKN2B* rs10811661, *IGF2BP2* rs4402960, *CDKAL1* rs7754840, and *MTNR1B* 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 eight 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.

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 5,327 nondiabetic 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 ≥11 than with ≤3 risk alleles. Furthermore, four variants (*TCF7L2*, *SLC30A8*, *HHEX*, and *CDKAL1*) were also associated with proinsulin conversion ($ProinsAUC_{0-30}/InsAUC_{0-30}$). SNPs in or 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 eight 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 nine or more risk alleles in seven genes exhibited reduced insulin secretion (assessed by the insulinogenic index) by −21.8% and reduced glucose sensitivity of β-cells by −26.6% compared with carriers of four 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 because of 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 the first 30 min of an OGTT (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 signaling seems to be involved (49). In a recent study (22), the association of *SLC30A8* rs13266634, *CDKAL1* rs7754840, and *TCF7L2* rs7903146 with the proinsulin/insulin AUC ratio during OGTT was also shown. Our finding that *HHEX* variant is associated with impaired proinsulin conversion has not previously been reported. Our results suggest that SNPs in or near *TCF7L2*, *CDKAL1*, *SLC30A8*, and *HHEX* may affect insulin secretion, at least partially, through impaired proinsulin conversion. Although we had proinsulin data from almost 2,700 subjects, the power of our study was limited to detect effect sizes <15% in the $ProinsAUC_{0-30}/InsAUC_{0-30}$ 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 genome-wide association 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 or 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 homeostasis model assessment of insulin resistance indexes of insulin sensitivity or resistance has also been reported. However, association of *HHEX* rs1111875 became significant after additional adjustment for $InsAUC_{0-30}/GluAUC_{0-30}$ 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 $InsAUC_{0-30}/GluAUC_{0-30}$ and $ProinsAUC_{0-30}/InsAUC_{0-30}$ 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 cannot be sure whether our results are applicable to women and to different ethnic or racial groups. However, no evidence exists that the sex 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, despite 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 $InsAUC_{0-30}/GluAUC_{0-30}$, 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 nondiabetic 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.

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