Effects of 34 Risk Loci for Type 2 Diabetes or Hyperglycemia on Lipoprotein Subclasses and Their Composition in 6,580 Nondiabetic Finnish Men

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OBJECTIVE—We investigated the effects of 34 genetic risk variants for hyperglycemia/type 2 diabetes on lipoprotein subclasses and particle composition in a large population-based cohort.

RESEARCH DESIGN AND METHODS—The study included 6,580 nondiabetic Finnish men from the population-based Metabolic Syndrome in Men (METSIM) study (aged 57 ± 7 years; BMI 26.8 ± 3.7 kg/m^2). Genotyping of 34 single nucleotide polymorphism (SNPs) for hyperglycemia/type 2 diabetes was performed. Proton nuclear magnetic resonance spectroscopy was used to measure particle concentrations of 14 lipoprotein subclasses and their composition in native serum samples.

RESULTS—The glucose-increasing allele of rs780094 in *GCKR* was significantly associated with low concentrations of VLDL particles (independently of their size) and small LDL and was nominally associated with low concentrations of intermediate-density lipoprotein, all LDL subclasses, and high concentrations of very large and large HDL particles. The glucose-increasing allele of rs174550 in *FADS1* was significantly associated with high concentrations of very large and large HDL particles and nominally associated with low concentrations of all VLDL particles. SNPs rs10923931 in *NOTCH2* and rs757210 in *HNF1B* genes showed nominal or significant associations with several lipoprotein traits. The genetic risk score of 34 SNPs was not associated with any of the lipoprotein subclasses.

CONCLUSIONS—Four of the 34 risk loci for type 2 diabetes or hyperglycemia (*GCKR*, *FADS1*, *NOTCH2*, and *HNF1B*) were significantly associated with lipoprotein traits. A *GCKR* variant predominantly affected the concentration of VLDL, and the *FADS1* variant affected very large and large HDL particles. Only a limited number of risk loci for hyperglycemia/type 2 diabetes significantly affect lipoprotein metabolism. ***Diabetes*** **60:1608–1616, 2011**

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Hyperglycemia is closely related to lipid and lipoprotein metabolism. Elevated levels of fasting and 2-h plasma glucose or the presence of type 2 diabetes are associated with an increase in total triglyceride (TG) concentrations, a decrease in HDL cholesterol (1,2), and the formation of small, dense LDL cholesterol particles (2). Several population-based studies have shown that high levels of TG and low levels of HDL cholesterol predict the development of type 2 diabetes (3–6). Therefore, hyperglycemia and dyslipidemia are likely to share similar pathophysiologic mechanisms, at least in part. One of these mechanisms is insulin resistance, which leads to hepatic overproduction of VLDL particles (especially large, TG-rich VLDL), attributable to an increased flux of free fatty acids from adipocytes into the liver, an increased rate of apolipoprotein B synthesis and degradation in the liver, and enhanced hepatic de novo lipogenesis by hyperinsulinemia (7).

Genomewide association studies (GWAS) have identified a number of gene variants reproducibly associated with hyperglycemia or type 2 diabetes (8–10). Because hyperglycemia and dyslipidemia partly share similar pathophysiologic mechanisms, the effects of hyperglycemia or type 2 diabetes single nucleotide polymorphisms (SNPs) on lipid and lipoprotein levels and composition are of great interest. However, none of the previous studies has systematically examined this question using modern methods to measure lipoprotein particle size and composition. Compared with conventional methods, the measurement of lipoprotein subclasses and particle composition provides more detailed information on the genes regulating hyperglycemia or type 2 diabetes and their effects on lipid metabolism.

We investigated the effects of the 34 risk loci for hyperglycemia or type 2 diabetes on lipoprotein subclasses and particle composition. To this aim, we determined a total of 60 lipoprotein traits, including 14 lipoprotein subclasses and their components, in 6,580 nondiabetic Finnish men.

RESEARCH DESIGN AND METHODS

Subjects. The study included 6,580 nondiabetic men from the population-based Metabolic Syndrome in Men (METSIM) study who were a mean ± SD age of 57 ± 7 years. The study design has been described in detail elsewhere (11). Glucose tolerance was evaluated according to the World Health Organization criteria (12), based on glucose levels from a 2-h oral glucose-tolerance test (OGTT) (75 g of glucose). Of these, 4,442 (67.5%) had normal glucose tolerance, 1,144 (17.4%) had isolated impaired fasting glucose, 582 (8.8%) had isolated impaired glucose tolerance, and 412 (6.3%) had impaired fasting glucose and impaired glucose tolerance. A total of 1,545 subjects (23.5%) were

receiving statin treatment, and 16 (0.2%) were being treated with a fibrate. The study was approved by the ethics committee of the University of Kuopio and Kuopio University Hospital, and was conducted 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 (kilogram) divided by height (meter) squared. Mean ± SD BMI of the cohort was 26.8 ± 3.7 kg/m^2.

Lipoprotein subclasses. Proton nuclear magnetic resonance (NMR) spectroscopy was used to measure lipid, lipoprotein subclass, and particle concentrations in native serum samples (13–15). NMR methods have been described previously in detail (16,17). Serum concentrations were determined for TG, total cholesterol (C), VLDL-TG, intermediate-density lipoprotein (IDL), LDL, and HDL cholesterol. Total lipid and particle concentrations in 14 lipoprotein subclasses were also measured. The measurements of these subclasses have been validated against high-performance liquid chromatography (18). The subclasses are as follows:

- chylomicrons (CM) and largest VLDL particles (CM/lar-VLDL), five different VLDL subclasses: very large VLDL (vl-VLDL), large VLDL (l-VLDL), medium-size VLDL (m-VLDL), small VLDL (s-VLDL), and very small VLDL (vs-VLDL);
- IDL;
- three LDL subclasses: large LDL (l-LDL), medium-size LDL (m-LDL), and small LDL (s-LDL); and
- four HDL subclasses: very large HDL (vl-HDL), large HDL (l-HDL), medium-size HDL (m-HDL), and small HDL (s-HDL).

The following components of the lipoprotein particles were measured: phospholipids (PL), TG, cholesterol, free cholesterol (FC), and cholesterol ester (CE). Average particle diameters and descriptive data for 60 traits are reported in Table 1. Particle diameters of VLDL, LDL, and HDL were also measured.

Genotyping. Genotyping of 34 SNPs, comprising 20 risk SNPs for type 2 diabetes and 14 risk SNPs for fasting and 2-h glucose in an OGTT (8–10), was performed using the TaqMan Allelic Discrimination Assay (Applied Biosystems, Carlsbad, CA) at the University of Eastern Finland *(PPARG* rs1801282, *KCNJ11* rs5219, *TCF7L2* rs7903146, *SLC30A8* rs13266634, *HHEX* rs1111875, *LOC387761* rs7480010, *CDKN2B* rs10811661, *IGF2BP2* rs4402960, *CDKAL1* rs7754840, *FTO* rs9939609, *HNF1B* rs7501939, *WFS1* rs10010131, *JAZF1* rs864745, *CDC123* rs12779790, *TSPAN8* rs7961581, *THADA* rs7578597, *ADAMTS9* rs4607103, *NOTCH2* rs10923931, *KCNQ1* rs2283228), or the iPlex Gold SBE Assay (Sequenom, San Diego, CA) at the National Human Genome Research Institute, National Institutes of Health (*MTNR1B* rs10830963, *ADRA2A* rs10885122, *FAM148A* rs11071657, *CRY2* rs11605924, *ADCY5* rs11708067, *SLC2A2* rs11920090, *FADS1* rs174550, *DGKB* rs2191349, *PROX1* rs340874, *GCK* rs4607517, *G6PC2* rs560887, *GLIS3* rs7034200, *GCKR* rs780094, *MADD* rs7944584, *GIPR* rs10423928).

The TaqMan genotyping call rate was 100%, and the discordance rate was 0% among 4.5% DNA samples genotyped in duplicate. The Sequenom iPlex call rate was 90.2–96.9%, and the discordance rate was 0% among 4.2% DNA samples genotyped in duplicate. All SNPs were consistent with Hardy-Weinberg equilibrium at the significance level corrected for multiple testing by Bonferroni method (P = 0.0015). Descriptive data for individual SNPs are reported in Supplementary Table 1.

Statistical analysis. Statistical analyses were conducted using SPSS 17 software (SPSS, Chicago, IL). All lipoprotein traits, BMI, and insulin sensitivity index (Matsuda ISI) were log-transformed to correct for their skewed distribution. Unstandardized effect sizes [B (SE)] per copy of the risk allele were estimated by linear regression adjusted for covariates, using untransformed dependent variables. Logarithmically transformed variables were used to calculate P values. The values of lipid traits equal to 0 were excluded from all analyses due to the need of log-transformation. The model included age, BMI, statin treatment (yes/no), and smoking (yes/no) as covariates. Additional adjustment for serum TG levels or Matsuda ISI was performed for selected SNPs. Hardy-Weinberg equilibrium was tested by the χ^2 test. We primarily used a conservative Bonferroni method to correct for multiple comparisons. A P value of $\leq 2.3 \times 10^{-5}$ was considered to be statistically significant given a total of 2,142 tests performed (63 traits × 34 SNPs). However, the Bonferroni correction for multiple testing might be too conservative because of high correlations between the different lipoprotein traits and subclasses. Therefore, we additionally used Benjamini-Hochberg-Yekutieli false discovery rate (FDR) method (19) to correct for multiple comparisons under dependency assumptions. In these analyses the FDR-adjusted $P_{FDR} < 0.05$ was considered statistically significant. Pearson correlations were calculated to test the association of lipoprotein traits with Matsuda ISI, calculated as [10,000/$\sqrt{}$ (fasting insulin × fasting glucose × mean insulin during OGTT × mean glucose during OGTT)] (20). The genetic risk score (GRS) was calculated as a sum of type 2 diabetes/hyperglycemia risk alleles in all 34 SNPs (GRS_{34}) in 20 type 2 diabetes risk SNPs (GRS_{T2D}) or in 17 hyperglycemia risk SNPs (GRS_{GLU}; details in Supplementary Table 6). Statistical power calculations were performed using Bioconductor's GeneticsDesign package version 1.16 (21). We had ≥80% power to detect changes in trait mean value from 1 to 28% per copy of the risk allele at the significance level of 0.05, depending on minor allele frequency (Supplementary Fig. 1).

RESULTS

The P values and effect sizes for the associations between 34 SNPs and 60 lipoprotein traits are summarized in Fig. 1. Two SNPs (*GCKR* rs780094 and *FADS1* rs174550) were significantly associated with several lipoprotein traits after adjustment for age, BMI, statin treatment, smoking, and conservative Bonferroni correction for multiple testing ($P \leq 2.3 \times 10^{-5}$). Two more SNPs (*NOTCH2* rs10923931 and *HNF1B* rs7501939) showed significant associations using a less conservative FDR correction for multiple testing ($P_{FDR} < 0.05$).

The type 2 diabetes risk (major) C allele of the intronic SNP rs780094 at the *GCKR* gene was significantly associated with low particle concentrations of VLDL subclasses (from vl- to s-VLDL, $P = 1.9 \times 10^{-8} - 7.3 \times 10^{-11}$), with effect sizes from −4 to −10% per allele (Fig. 2*A*, Supplementary Table 2). The C allele was also significantly associated with low concentrations of almost all components of (CM) and VLDL (PL, TG, C, FC, and CE). Furthermore, the C allele was nominally associated with low particle concentrations of CM/lar-VLDL ($P = 5.4 \times 10^{-4}$), vs-VLDL ($P = 1.4 \times 10^{-4}$), and the l-LDL ($P = 0.008$), m-LDL ($P = 5.0 \times 10^{-4}$), and s-LDL ($P = 3.2 \times 10^{-5}$) subclasses, and with high concentrations of vl-HDL ($P = 0.031$) and l-HDL ($P = 2.8 \times 10^{-4}$) but low concentration of s-HDL particles ($P = 8.4 \times 10^{-5}$), as well as with most of the components of these particles. Associations for vs-VLDL, s-LDL, and s-HDL were statistically significant when using the FDR correction (P_{FDR} = 0.048, 0.015, and 0.030). This SNP tended to have larger and more significant effects on the TG components of most of lipoprotein subclasses (CM, VLDL, IDL, and HDL) than on other components. The C allele was also significantly associated with VLDL particle size ($P = 4.8 \times 10^{-9}$), and nominally with LDL and HDL particle size ($P = 8.2 \times 10^{-5}$ and 2.3×10^{-4}). Almost all associations disappeared after additional adjustment for serum levels of TGs, suggesting that they were most likely secondary to the effects of rs780094 on TG synthesis or metabolism.

The type 2 diabetes risk (major) T allele of the intronic SNP rs174550 at the *FADS1* gene was significantly associated with high concentrations of vl-HDL ($P = 2.5 \times 10^{-11}$) and l-HDL ($P = 1.6 \times 10^{-8}$) particles, with effect sizes of 6.7 and 4.8% per allele, and all their components (Fig. 2*B*, Supplementary Table 3). The largest effect size (6.7% per allele) was observed for the PL component of vl-HDL particles ($P = 2.6 \times 10^{-8}$). Association with low concentration of s-HDL particles was nominally significant (P = 0.002). The T allele was also nominally associated with low concentrations vl- to s-VLDL particles, and high concentrations of vs-VLDL and IDL particles ($P = 2.8 \times 10^{-4}$ to 0.032), as well as with components of VLDL and IDL particles. Rs174550 was also significantly associated with particle size of VLDL ($P = 5.8 \times 10^{-7}$) and HDL ($P = 1.1 \times 10^{-9}$), and nominally with LDL particle size (P = 0.001). The associations with HDL subclasses and HDL particle size were not considerably affected by additional adjustment for TGs, suggesting that primary effect of the SNP might be on HDL particles.

The type 2 diabetes risk (minor) T allele of the intronic SNP rs7501939 at the *HNF1B* gene was nominally associated

TABLE 1
Descriptive statistics of 60 lipoprotein traits and their correlation with insulin sensitivity (Matsuda ISI)

Trait (μmol/L)	Particle diameter (average, nm)	n	Mean (IQR)	Correlation with Matsuda ISI	
				r	P
CM/lar-VLDL-P	≥75.0	3,970	0.0001 (0.00003–0.00013)	−0.321	5E-95
CM/lar-VLDL-PL		5,397	2.37 (1.19–3.54)	−0.365	2E-168
CM/lar-VLDL-TG		6,177	14.7 (9.3–23.8)	−0.409	4E-246
vl-VLDL-P	64.0	6,172	0.0007 (0.0005–0.0011)	−0.396	5E-230
vl-VLDL-PL		6,155	11.1 (7.1–18.3)	−0.399	3E-232
vl-VLDL-TG		6,368	46.1 (30.4–76.3)	−0.417	2E-264
l-VLDL-P	53.6	6,490	0.005 (0.004–0.008)	−0.428	4E-285
l-VLDL-PL		6,397	51.7 (37.2–86.0)	−0.414	5E-262
l-VLDL-TG		6,534	182 (132–296)	−0.450	<4E-285
l-VLDL-C		6,428	61.6 (44.8–101.0)	−0.391	9E-232
l-VLDL-FC		6,492	32.0 (23.3–52.5)	−0.407	9E-256
l-VLDL-CE		6,312	30.1 (21.5–49.6)	−0.358	3E-189
m-VLDL-P	44.5	6,580	0.018 (0.015–0.027)	−0.441	<4E-285
m-VLDL-PL		6,579	125 (108–183)	−0.421	3E-279
m-VLDL-TG		6,577	335 (270–512)	−0.447	<4E-285
m-VLDL-C		6,580	174 (152–250)	−0.383	3E-227
m-VLDL-FC		6,579	78.0 (66.6–115.5)	−0.415	9E-271
m-VLDL-CE		6,580	95.8 (83.8–135.5)	−0.338	6E-174
s-VLDL-P	36.8	6,580	0.034 (0.031–0.045)	−0.377	9E-220
s-VLDL-PL		6,580	169 (155–213)	−0.329	2E-164
s-VLDL-TG		6,580	287 (259–393)	−0.415	3E-270
s-VLDL-C		6,580	298 (262–373)	−0.239	9E-86
s-VLDL-FC		6,580	114 (103–144)	−0.301	9E-137
vs-VLDL-P	31.3	6,580	0.037 (0.033–0.046)	−0.079	2E-10
vs-VLDL-PL		6,580	154 (133–192)	0.049	8E-05
vs-VLDL-TG		6,580	130 (121–167)	−0.277	6E-116
IDL-P	28.6	6,580	0.098 (0.086–0.118)	0.041	0.0009
IDL-PL		6,580	315 (274–379)	0.072	5E-09
IDL-TG		6,580	128 (115–161)	−0.115	1E-20
IDL-C		6,580	792 (689–938)	0.082	3E-11
IDL-FC		6,579	218 (184–264)	0.171	7E-44
l-LDL-P	25.5	6,580	0.17 (0.15–0.21)	−0.002	0.856
l-LDL-PL		6,580	375 (335–439)	−0.003	0.783
l-LDL-C		6,580	1,112 (960–1334)	0.015	0.235
l-LDL-FC		6,580	292 (252–346)	0.086	3E-12
l-LDL-CE		6,580	819 (705–986)	−0.009	0.470
m-LDL-P	23.0	6,580	0.15 (0.13–0.18)	−0.051	4E-05
m-LDL-PL		6,580	246 (224–285)	−0.089	4E-13
m-LDL-C		6,580	670 (572–813)	−0.030	0.015
m-LDL-CE		6,580	493 (419–607)	−0.034	0.006
s-LDL-P	18.7	6,580	0.17 (0.15–0.20)	−0.062	6E-07
s-LDL-C		6,580	395 (340–485)	−0.018	0.145
vl-HDL-P	14.3	6,439	0.27 (0.15–0.32)	0.353	5E-187
vl-HDL-PL		6,376	152 (74–176)	0.360	9E-194
vl-HDL-TG		6,577	11.5 (9.7–15.1)	0.045	0.0003
vl-HDL-C		6,441	188 (121–229)	0.292	5E-126
vl-HDL-FC		6,432	46.2 (28.0–55.1)	0.312	2E-144
vl-HDL-CE		6,462	142 (94–173)	0.301	1E-134
l-HDL-P	12.1	6,571	0.81 (0.47–0.98)	0.412	1E-266
l-HDL-PL		6,562	301 (179–372)	0.401	1E-250
l-HDL-C		6,571	321 (175–382)	0.412	6E-266
l-HDL-FC		6,334	63.8 (27.0–75.0)	0.396	3E-235
l-HDL-CE		6,576	261 (150–309)	0.389	8E-235
m-HDL-P	10.9	6,580	1.62 (1.42–1.88)	0.114	3E-20
m-HDL-PL		6,580	395 (345–461)	0.140	9E-30
m-HDL-C		6,579	458 (384–525)	0.163	3E-40
m-HDL-FC		6,579	83.9 (69.5–99.1)	0.212	3E-67
m-HDL-CE		6,579	378 (317–430)	0.146	1E-32
s-HDL-P	8.7	6,580	4.66 (4.44–4.98)	−0.166	1E-41
s-HDL-TG		6,572	44.8 (39.9–59.7)	−0.321	4E-156

IQR, interquartile range; P, particle concentration.

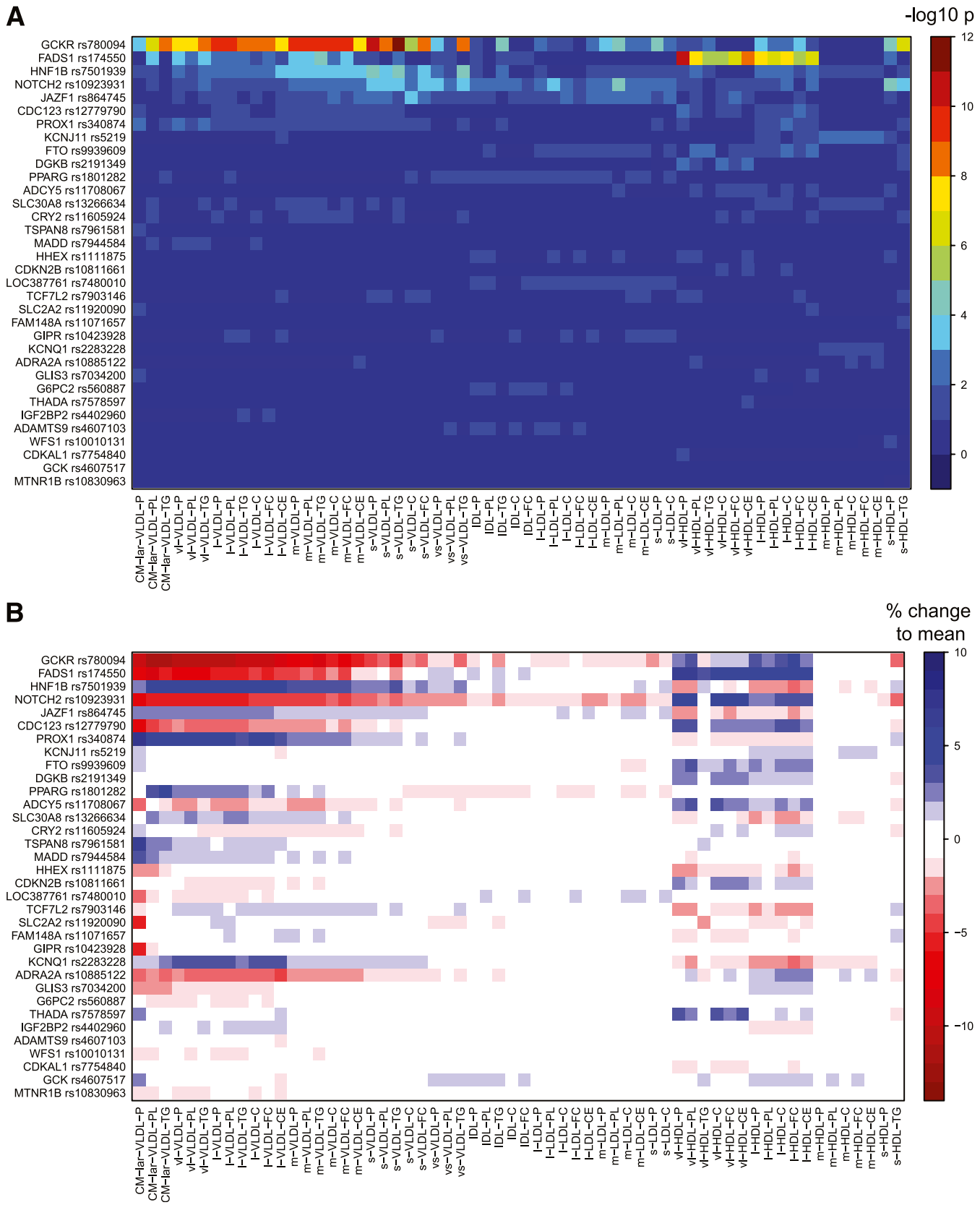

FIG. 1. Significances (*A*) and effect sizes (*B*) of associations between 34 SNP and 60 lipoprotein traits in nondiabetic men. *P* values (presented as −log10) were calculated by linear regression adjusted for age, BMI, statin treatment, and smoking, using log-transformed variables. Unstandardized effect sizes (presented as percentage from the mean) per type 2 diabetes/hyperglycemia risk allele were calculated from the same model, using untransformed variables. P, particle concentration.

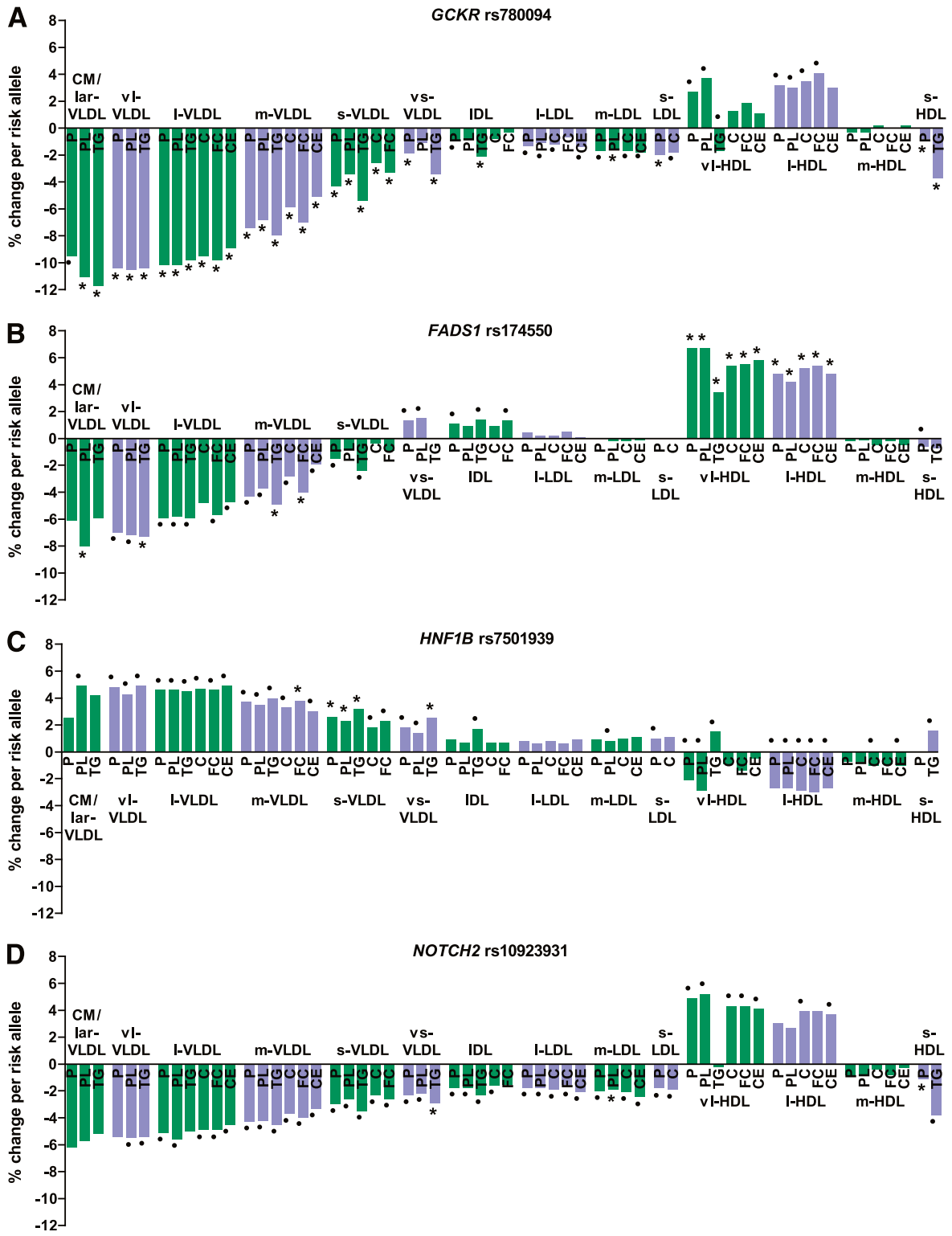

FIG. 2. Effects of *GCKR* rs780094 (*A*), *FADS1* rs174550 (*B*), *NOTCH2* rs10923931 (*C*), and *HNF1B* rs7501939 (*D*) on lipoprotein subclasses and their components in nondiabetic men. Bars represent percentage change to the mean of the trait per copy of a minor allele and were calculated by linear regression adjusted for age, BMI, statin treatment, and smoking. *Significant association after Bonferroni correction ($P < 2.3 \times 10^{-5}$) or FDR correction for multiple testing ($P_{FDR} < 0.05$). •Nominally significant associations ($P < 0.05$). P, particle concentration.

with high particle concentrations of all VLDL subclasses (P = from 8.1×10^{-5} to 0.006) and their components and low concentration of vl-HDL (P = 0.038) and l-HDL (P = 0.004) particles and their components (Fig. 2*C*, Supplementary Table 4). The association with s-VLDL was significant when using FDR correction (P_{FDR} = 0.030), as well as associations with components of m-VLDL (FC, P_{FDR} = 0.05), s-VLDL (PL, P_{FDR} = 0.045; TG, P_{FDR} = 0.020), and vs-VLDL (TG, P_{FDR} = 0.016). These associations, however, disappeared after additional adjustment for TGs.

The intronic SNP rs10923931 at the *NOTCH2* gene was nominally associated with particle concentrations and components of most of the lipoprotein subclasses (Fig. 2*D*, Supplementary Table 5). The type 2 diabetes risk (minor) *A* allele was nominally associated with low concentrations of l- to vs-VLDL, IDL, and LDL (all subclasses), and with high concentrations of vl-HDL particles ($P = 4.1 \times 10^{-4}$ to 0.039). Significant associations (using FDR correction) were observed for concentration of s-HDL particles (P_{FDR} = 0.017), the TG component of vs-VLDL (P_{FDR} = 0.044), and the PL component of m-LDL (P_{FDR} = 0.030), with effect sizes of a 1–3% decrease per *A* allele. The associations were weakened by additional adjustment for TGs.

The results for *GCKR* rs780094, *FADS1* rs174550, *NOTCH2* rs10923931, and *HNF1B* rs7501939 remained essentially similar when statin users (n = 1,545) were excluded (data not shown).

The quantile-quantile (Q-Q) plot of the association results between the 34 SNPs and the 60 lipoprotein traits (observed $-\log_{10} P$ values against theoretic expected $-\log_{10} P$ values) shows a large deviation from the null hypothesis of no association, strongly suggesting that the deviating results are true significant associations (Fig. 3). After the *P* values of two most significant SNPs (in *GCKR* and *FADS1*) were excluded from the analyses, the Q-Q plot still deviated from the null hypothesis. This suggests that some of the remaining associations could be true significant associations. However, after *P* values of *NOTCH2* and *HNF1B* SNPs were also excluded, the deviation from the null hypothesis was largely decreased; therefore, the nominally significant associations in the remaining SNPs might be false findings, such as associations of *CDC123* rs12779790 with vl- and l-HDL particles, *DGKB* rs2191349 with vl-HDL particles, *FTO* rs9939609 with l- and m-LDL and vl-HDL, *JAZF1* rs864745 with l- to s-VLDL and all LDL particles, *KCNJ11* rs5219 with m- and s-HDL, and *PROX1* rs340874 with CM/lar-VLDL and vl- to s-VLDL particles, and with vl- and l-HDL.

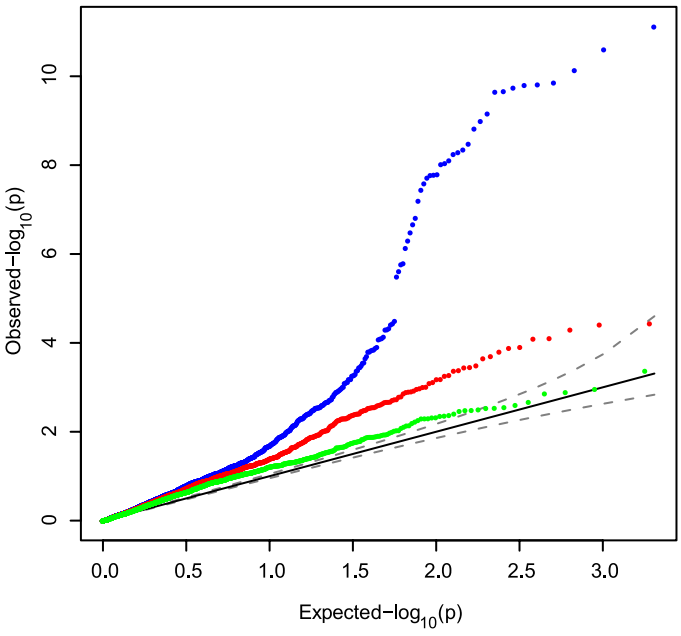

FIG. 3. Quantile-quantile (Q-Q) plot of the association results between 34 hyperglycemia and type 2 diabetes risk SNPs and 60 lipoprotein traits (observed $-\log_{10} P$ values against theoretical expected $-\log_{10} P$ values). The diagonal black line represents theoretical expected values and the gray dashed lines their 95% CI. Blue dots, all *P* values; red dots, *P* values for two leading SNPs (*GCKR* rs780094 and *FADS1* rs174550) excluded; green dots, *P* values for four SNPs showing significant (P_{FDR} < 0.05) associations (in *GCKR*, *FADS1*, *HNF1B*, *NOTCH2*) excluded.

The GRS calculated using all 34 SNPs did not show significant associations with concentrations of any of the particles, and the same was observed for GRS_{T2D}, which included type 2 diabetes risk SNPs or GRS_{GLU}, including hyperglycemia SNPs (Supplementary Table 6). Nominally significant associations of GRS_{GLU} with vl-HDL (P = 0.047) and l-HDL (P = 0.012) particle concentrations disappeared after *GCKR* and *FADS1* SNPs were excluded from the calculations. This suggests that the associations of individual SNPs with the lipoprotein traits as described are specific for these traits and are not an indication of systematic effects of type 2 diabetes/hyperglycemia risk SNPs on lipoprotein metabolism.

All lipoprotein traits, with the exception of l-LDL particles, were significantly correlated with Matsuda ISI. The strongest correlations were observed for CM/lar-VLDL and the vl- to s-VLDL subclasses, particularly with their TG components (r = −0.41 to −0.45), and with l-HDL particles and all their components (r = −0.39 to −0.41; Table 1). Of four SNPs significantly associated with lipoprotein traits, *GCKR* rs780094 and *NOTCH2* rs10923931 showed nominally significant associations with Matsuda ISI (P = 0.001 and 0.012, respectively, Supplementary Table 7). This could suggest that the associations between these two SNPs and lipoproteins are mediated through their effects on insulin sensitivity. Additional adjustment for Matsuda ISI indeed decreased the effects of *NOTCH2* rs10923931 on lipoproteins, but no such effect was observed for other SNPs. However, the effect of SNPs on lipoproteins was very small: *GCKR* and *FADS1* SNPs explained a ≤1.3% variance in their most associated lipoprotein traits compared with the effect of Matsuda ISI, which explained a 12–19% variance in the same traits.

DISCUSSION

This is the first large population–based study where the effects of 34 confirmed–risk SNPs for hyperglycemia or type 2 diabetes on lipoprotein subclasses and their composition have been systematically investigated. We found that 4 of the 34 tested SNPs, in *GCKR*, *FADS1*, *HNF1B*, and *NOTCH2* genes, were significantly associated with lipoprotein traits after correction for multiple testing. This indicates that there could be some overlap between the genes affecting both glucose and lipoprotein metabolism.

Associations of *GCKR* rs780094 with lipoprotein subclasses and composition. The most statistically significant finding was the association of the hyperglycemia/type 2 diabetes–risk *C* allele of the intronic SNP rs780094 of *GCKR* with low concentrations of all subclasses of VLDL particles (effect sizes 10–2% per allele for CM/lar-VLDL and vl- to s-VLDL) as well as with the diameter of VLDL particles. Only one previous study has examined the effects of *GCKR* on lipoprotein fractions, reporting an association ($P < 5 \times 10^{-8}$) of rs1260326 at the *GCKR* locus (in linkage disequilibrium with rs780094, r^2 = 0.89 according to HapMap CEU) with large and medium-size VLDL particles and mean particle size (22), which is in agreement with our findings. Furthermore, the *C* allele of rs780094 was significantly associated in our study with a low concentration of s-LDL particles and s-HDL particles, besides nominal associations with IDL and other subclasses of LDL and HDL

particles. The aforementioned study reported similar associations of rs780094 with s-LDL particles and s-HDL particles (22).

In addition, we observed a tendency to a stronger association of the *C* allele with the TG components of most of the lipoprotein subclasses than with other components. Previous studies and GWAS, applying only standard measurements of lipoprotein levels, have consistently shown that the *C* allele of rs780094 was associated with low TG levels (23–25). In the first GWAS performed by the Diabetes Genetics Initiative consortium, rs780094 explained 1% of the residual variance in TG levels (23). Similarly, the missense variant rs1260326 (*Leu*446*Pro*), which could be responsible for the associations of rs780094 (26), has been associated with TG levels in several studies (26–30). Our study suggests that the association between *GCKR* and TG is attributable to changes in VLDL particle concentration, especially in the largest TG-rich subclasses. Additional adjustment for serum TG levels abolished all associations of the SNP with particles and their components, suggesting that the observed effects of rs780094 on IDL, LDL, and HDL particles could be secondary to its effects on TG/VLDL metabolism rather than independent effects.

A previous study proposed that large TG-rich VLDL particles, secreted by the liver preferentially in hypertriglyceridemic conditions, are metabolized into small LDL particles (31), which could explain the stronger association of rs780094 with s-LDL particles in our study. The inverse relationship between TG and HDL levels is well documented (32) and is at least partly explained by increased catabolism of TG-enriched HDL particles (33).

The main biologic function of the glucokinase regulatory protein is to inhibit the effects of glucokinase on glycogen synthesis and glycolysis in the liver (34). SNPs at the *GCKR* locus have been associated with fasting glycemia (9,29), risk of type 2 diabetes (25,29), insulin resistance (25,29,35), and increased hepatic glucose production (26). Our study also confirmed the association of the *C* allele of rs780094 with decreased insulin sensitivity (Matsuda ISI). However, it did not seem to account for the changes in lipoprotein particle concentrations, because additional adjustment for Matsuda ISI did not attenuate the associations between rs780094 and lipoproteins. Moreover, the effects of rs780094 on insulin resistance and TG levels were in opposite directions. A recent article reporting that *Leu*446*Pro* indirectly increases GK activity proposed that the increased glycolytic flux leads to the elevation of malonyl-CoA, a substrate for de novo lipogenesis, which could explain the opposite effects of the SNP on glucose and TG levels (36).

Associations of *FADS1* rs174550 with lipoprotein subclasses and composition. The glucose-increasing (major) *T* allele of the intronic SNP rs174550 in the *FADS1* gene was significantly associated with high concentrations of vl- and l-HDL particles and all of their components, as well as with HDL particle diameter. Nominally significant associations were found with all VLDL subclasses and IDL particles. A previous study (22) found an association of two other SNPs at the *FADS1-2-3* gene cluster with medium HDL (rs174537) and large HDL particles (rs102275), and one SNP with large LDL particles (rs1535). Moreover, four recent GWAS (28,30,37,38) reported associations of the *FADS1-2-3* SNPs with HDL cholesterol, total cholesterol, LDL cholesterol, and TG. Our findings suggest that rs174550 may primarily affect vl- and l-HDL particles because the associations persisted after additional adjustment for serum TG levels. We did not observe any associations of rs174550 with LDL subclasses.

FADS1 encodes the fatty acid desaturase δ-5 (D5D), which plays a crucial role in desaturation and elongation of polyunsaturated fatty acids (PUFA). Several SNPs at the *FADS1* locus have been previously associated with PUFA concentrations in serum and tissue phospholipids (39,40). Association of the *FADS1* locus (rs174537) with serum PUFA levels was been confirmed by a GWAS in the Invecchiare in Chianti (InCHIANTI) study (41). The PL component of vl-HDL particles was one of the most strongly influenced traits by rs174550 in our study. *FADS1*, by its effects on PUFA metabolism, may influence the composition and properties of phospholipids of HDL particles (forming ~20% of a particle), which could further affect the biogenesis, maturation, and catabolism of HDL. In support for this notion, *FADS1* (rs174548) has also been shown to affect serum levels of phosphatidylcholine (42), which is the major phospholipid in HDL particles and is important in the metabolism of HDL particles (43). Furthermore, dietary PUFAs have been shown to influence lipoprotein levels, mainly HDL and LDL cholesterol (44), and variants affecting biosynthesis of PUFAs could have similar effects.

The *T* allele of rs174550 has been previously associated with increased fasting glucose levels in a GWAS by the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) (9). However, similar to our observation for the *GCKR* locus, the glucose-increasing allele was associated with high HDL and nominally with low VLDL concentrations. This may suggest that the effects of *FADS1* variants on lipoproteins and glucose levels are mediated by different mechanisms. PUFAs have an ability to potentiate insulin secretion (45), which could be one of the mechanisms by which *FADS1* variants modulate insulin secretion (35) and fasting glucose levels (9).

Associations of *HNF1B* rs7501939 and *NOTCH2* 10923931 with lipoprotein subclasses and composition. *HNF1B* rs7501939 and *NOTCH2* 10923931 showed mostly nominally significant associations with several lipoprotein traits, some of them being statistically significant using the less conservative FDR correction for multiple testing. The type 2 diabetes risk allele of the intronic SNP rs7501939 in *HNF1B* was associated with high particle concentrations of all VLDL subclasses (significantly with s-VLDL) and s-LDL particles and low concentrations of vl- and l-HDL particles. Although there are no studies on the effect of common SNPs of *HNF1B* on lipid levels, patients with maturity-onset diabetes of the young type 5 caused by mutations at the *HNF1B* locus exhibit dyslipidemia characterized by hypertriglyceridemia and low HDL cholesterol levels (46).

The type 2 diabetes risk allele of the intronic SNP rs10923931 in *NOTCH2* was nominally associated with low particle concentrations of all VLDL, IDL, and LDL subclasses, high concentrations of vl-HDL particles, and significantly with low concentration of s-HDL particles. The same allele was nominally associated with higher insulin sensitivity (Matsuda ISI), and an additional adjustment for Matsuda ISI attenuated the associations between rs10923931 and lipoproteins, indicating that these effects could be at least partly related. Associations of *NOTCH2* variants with lipid levels or insulin sensitivity have not been previously reported. The mechanisms behind the observed associations remain to be elucidated.

JAZF1, *CDC123*, *PROX1*, *KCNJ11*, *FTO*, and *DGKB* loci were nominally associated with several lipoprotein traits

in our study. Only the *FTO* locus of these loci has been previously found to affect lipid levels (47).

GRS calculated using all 34 SNPs, or type 2 diabetes SNPs and hyperglycemia SNPs separately, was not significantly associated with any of the lipoprotein subclasses. This suggests that there is no major overlap between the genetic basis of type 2 diabetes/hyperglycemia and lipoprotein metabolism. Furthermore, although insulin resistance is a major pathophysiologic link between hyperglycemia and dyslipidemia, our results do not give evidence for a role of the examined SNPs in this association. *PPARG*, as the main candidate gene for insulin resistance, was not significantly associated with any of the lipoprotein traits. In contrast, the *C* allele of rs780094 of *GCKR* was associated with decreased insulin sensitivity and low concentrations of VLDL particles, suggesting that the effects of the *C* allele on glucose and lipid metabolism could be independently regulated. The *FADS1* and *HNF1B* SNPs were not associated with insulin resistance in our study. Associations of *NOTCH2* with lipoproteins could be related to its possible effect on insulin sensitivity.

A limitation of our study is that only Finnish men were included, and therefore, we do not know whether our results are applicable to women and to different ethnic or racial groups. We had only a modest statistical power to demonstrate statistically significant associations with CM, largest to medium VLDL, and HDL particles.

In conclusion, our large population-based study shows that from the 34 loci associated with hyperglycemia or type 2 diabetes only *GCKR*, *FADS1*, *HNF1B*, and *NOTCH2* were significantly associated with several lipoprotein traits. The effects of *GCKR* were predominantly on concentrations of VLDL particles, and *FADS1* seems to mainly affect concentrations of vl- and l-HDL particles. Our findings indicate that only a limited number of risk loci for hyperglycemia or type 2 diabetes affect significantly lipoprotein metabolism.

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