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/m²). Genotyping of 34 single nucleotide polymorphisms (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, 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.

Hyperglycemia is closely related to lipid and lipoprotein metabolism. Elevated levels of fasting and 2-h postprandial 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 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 age of 57 ± 7 (SD) years. The study design has been described in detail elsewhere (11). Glucose tolerance was evaluated according to the World Health Organization criteria (10), 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

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Kaupio 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/m².

Lipoprotein subclasses. Proton nuclear magnetic resonance (NMR) spectroscopy was used to measure lipid, lipoprotein subclass, and particle concentrations in native serum samples (12–14). NMR methods have been described previously in detail (15,16). Serum concentrations were determined for TG, total cholesterol (TC) together with VLDL-TG, and intermediate-density lipoprotein (IDL). LDL, and HDL cholesterol. Total lipids and particle concentrations in 14 lipoprotein subclasses were also measured. The measurements of these subclasses have been validated against high-performance liquid chromatography (17). The subclasses are as follows:

- chylomicrons 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
- large LDL classes (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 (PPABG rs1801282, KCNJ11 rs2319, TCF7L2 rs7903146, SLC30A8 rs12366364, HHEX rs1118757, LOC387761 rs4801010, CDKN2B rs10010131, CDKAL1 rs7774540, FTO rs8050131, HNF1B rs7501939, WFS1 rs10010131, JAZF1 rs1864745, CDC123 rs1277970, TSPAN5 rs7601851, THADA rs7578597, ABAMT2 rs4067103, NOTCH2 rs10923931, CDK8 rs2382292, or the iPLEX Gold SNP Assay (Sequenom, San Diego, CA) at the National Human Genome Research Institute, National Institutes of Health (MTNR1B rs10830963, ADRA2A rs10885122, FAM148A rs11071567, CR1Y rs11060524, ADCT5 rs1178067, SLC2A2 rs19200090, FADS1 rs174550, DGKB rs2101934, PROX1 rs5043874, GCK rs600517, G6PC2 rs500857, GLIS3 rs7034290, GCKR rs780094, MADD rs8744584, GIPR rs10423982).

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 99.2–96.0%, 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 Index) 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 effect sizes. 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 Index was performed for selected SNPs. Hardy-Weinberg equilibrium was tested by the χ² test. We primarily used a conservative Bonferroni method to correct for multiple comparisons. A P value of ≤2.3 × 10⁻⁵ was considered to be statistically significant given a total of 344 traits 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–Yekutiel false discovery rate (FDR) method (18) to correct for multiple comparisons under dependency assumptions. In these analyses the FDR-adjusted P value < 0.05 was considered statistically significant. Pearson correlations were calculated to test the association of lipoprotein traits with Matsuda Index, calculated as [10,000/(fasting insulin × fasting glucose × mean insulin during OGTT × mean glucose during OGTT)] (19). The genetic risk score (GRS) was calculated as a sum of type 2 diabetes/hyperglycemia risk alleles in all 34 SNPs (GRSAD) in 20 type 2 diabetes risk SNPs (GRSt2d) or in 17 hyperglycemia risk SNPs (GRSglu; details in Supplementary Table 6). Statistical power calculations were performed using Bioconductor’s GeneticsDesign package version 1.16 (20). 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 ≤ 2.3 × 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 × 10⁻⁸ to 7.3 × 10⁻¹¹), with effect sizes from -4 to -10% per allele (Fig. 2A, Supplementary Table 2). The C allele was also significantly associated with low concentrations of all components of chylomicrons and VLDL (PL, TG, C, FC, and CE). Furthermore, the C allele was nominally associated with low particle concentrations of chylomicrons and l-VLDL (P = 5.4 × 10⁻⁵), vs-VLDL (P = 1.4 × 10⁻⁴), and the l-LDL (P = 0.008), m-LDL (P = 5.0 × 10⁻⁴), and s-LDL (P = 3.2 × 10⁻⁵) subclasses, and with high concentrations of h-VLDL (P = 0.031) and 1-HDL (P = 2.8 × 10⁻⁴) but low concentration of s-HDL particles (P = 8.4 × 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 lipoprotein subclasses (chylomicrons, VLDL, IDL, and HDL) than on other lipoproteins. The C allele was also significantly associated with VLDL particle size (P = 4.8 × 10⁻⁹), and nominally with LDL and HDL particle size (P = 8.2 × 10⁻⁵ and 2.3 × 10⁻⁴). Almost all associations disappeared after additional adjustment for serum levels of TGs, suggesting that they were mostly 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 × 10⁻¹¹) and l-HDL (P = 1.6 × 10⁻⁵) particles, with effect sizes of 6.7 and 4.8% per allele, and all their components (Fig. 2B, Supplementary Table 3). The largest effect size (6.7% per allele) was observed for the PL component of vL-HDL particles (P = 2.6 × 10⁻⁷). Association with low concentration of s-HDL particles was nominally significant (P = 0.002). The T allele was also nominally associated with low concentrations of vL- to s-VLDL particles, and high concentrations of vs-VLDL and IDL particles (P = 2.8 × 10⁻⁴ to 0.032), as well as of VLDL and IDL particles. Rs174550 was also significantly associated with particle size of VLDL (P = 5.8 × 10⁻⁵) and HDL (P = 1.1 × 10⁻⁵), 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 with high particle concentrations of all VLDL
TABLE 1
Descriptive statistics of 60 lipoprotein traits and their correlation with insulin sensitivity (Matsuda ISI)

| Trait (μmol/L) (average) | Particle diameter (nm) | n  | Mean (IQR) | Correlation with Matsuda ISI |
|--------------------------|------------------------|----|------------|-------------------------------|
|                          |                        |    |            |                               |
| CM/HDL-P, CM/HDL-PL, CM/HDL-TG | ≥75.0                  | 3,970 | 0.0001 (0.00003-0.00013) | -0.321 5E-95 |
| vl-VLDL-P, vl-VLDL-PL, vl-VLDL-TG | 64.0                   | 6,172 | 0.0007 (0.0005-0.0011) | -0.396 5E-230 |
| l-VLDL-P, l-VLDL-PL, l-VLDL-TG   | 53.6                   | 6,490 | 0.005 (0.004-0.008) | -0.428 4E-285 |
| m-VLDL-P, m-VLDL-PL, m-VLDL-TG | 44.5                   | 6,580 | 0.018 (0.015-0.027) | -0.441 5E-285 |
| s-VLDL-P, s-VLDL-PL, s-VLDL-TG | 36.8                   | 6,580 | 0.034 (0.031-0.045) | -0.377 9E-220 |
| vs-VLDL-P, vs-VLDL-PL, vs-VLDL-TG | 31.3                   | 6,580 | 0.037 (0.033-0.046) | -0.079 2E-10 |
| IDL-P, IDL-PL, IDL-TG         | 28.6                   | 6,580 | 0.098 (0.086-0.118) | 0.041 0.0009 |
| m-LDL-P, m-LDL-PL, m-LDL-TG   | 23.0                   | 6,580 | 0.15 (0.13-0.18) | -0.051 4E-05 |
| s-LDL-P, s-LDL-PL, s-LDL-TG   | 18.7                   | 6,580 | 0.17 (0.15-0.20) | -0.062 6E-07 |
| vl-HDL-P, vl-HDL-PL, vl-HDL-TG | 14.3                   | 6,499 | 0.27 (0.15-0.32) | 0.353 5E-187 |
| vl-HDL-C, vl-HDL-FC, vl-HDL-CE | 12.1                   | 6,562 | 0.301 (0.279-0.322) | 0.292 5E-14 |
| m-HDL-P, m-HDL-PL, m-HDL-TG   | 10.9                   | 6,580 | 1.62 (1.42-1.88) | 0.114 3E-20 |
| m-HDL-C, m-HDL-FC, m-HDL-CE   | 8.7                    | 6,580 | 4.66 (4.44-4.98) | -0.166 4E-11 |

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. CM, chylomicrons; P, particle concentration.
subclasses ($P = \text{from } 8.1 \times 10^{-5} \text{ to } 0.006$) and their components and low concentration of vL-HDL ($P = 0.038$) and l-HDL ($P = 0.004$) particles and their components (Fig. 2C, Supplementary Table 4). The association with s-VLDL was significant when using FDR correction ($P_{\text{FDR}} = 0.030$), as well as associations with components of m-VLDL (FC, $P_{\text{FDR}} = 0.05$), s-VLDL (PL, $P_{\text{FDR}} = 0.045$; TG, $P_{\text{FDR}} = 0.020$), and vs-VLDL (TG, $P_{\text{FDR}} = 0.016$). 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. 2D, 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 \( (P = 4.1 \times 10^{-4} \text{ to } 0.039) \). Significant associations (using FDR correction) were observed for concentration of s-HDL particles \( (P_{\text{FDR}} = 0.017) \), the TG component of vs-VLDL \( (P_{\text{FDR}} = 0.044) \), and the PL component of m-LDL \( (P_{\text{FDR}} = 0.030) \), 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 rs8939609 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 chylomicrons and l- to s-VLDL particles, and with vl- and l-HDL.

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 \(_{\text{T2D}}\), which included type 2 diabetes risk SNPs or GRS \(_{\text{GLU}}\), including hyperglycemia SNPs (Supplementary Table 6). Nominally significant associations of GRS \(_{\text{GLU}}\) 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 chylomicrons and the l- to s-VLDL subclasses, particularly with their TG components \( (r = -0.41 \text{ to } -0.45) \), and with l-HDL particles and all their components \( (r = -0.39 \text{ to } -0.41) \); Table 1). Of four SNPs significantly associated with lipoprotein traits, GCKR rs780094 and NOTCH2 rs10923931 showed nominally significant associations with Matsuda ISI \( (P = 0.001 \text{ and } 0.012, \text{ respectively, Supplementary Table 7}) \). 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 l- 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 \( (P < 5 \times 10^{-5}) \) of rs1260326 at the GCKR locus (in linkage disequilibrium with rs780094, \( r^2 = 0.89 \) according to HapMap CEU) with l- and m-VLDL particles and mean particle size \((21)\), 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 (21).

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 (22–24). In the first GWAS performed by the Diabetes Genetics Initiative consortium, rs780094 explained 1% of the residual variance in TG levels (22). Similarly, the missense variant rs1260326 (Leu446Pro), which could be responsible for the associations of rs780094 (25), has been associated with TG levels in several studies (25–29). Our study suggests that the association between GCKR and TG is attributable to changes in VL DL 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 TG-rich l-VLDL particles, secreted by the liver preferentially in hypertriglyceridemic conditions, are metabolized into s-LDL particles (30), 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 (31) and is at least partly explained by increased catabolism of TG-enriched HDL particles (32).

The main biologic function of the glucokinase regulatory protein is to inhibit the effects of glucokinase (GCK) on glycogen synthesis and glycolysis in the liver (33). SNPs at the GCKR locus have been associated with fasting glycemia (9,28), risk of type 2 diabetes (24,28), insulin resistance (24,28,34), and increased hepatic glucose production (25). 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 Leu446Pro 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 (35).

**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 (21) found an association of two other SNPs at the FADS1–2–3 gene cluster with m-HDL (rs174537) and l-HDL particles (rs102275), and one SNP with l-LDL particles (rs1535). Moreover, recent GWAS (27,29,36,37) reported associations of the FADS1–2–3 SNPs with HDL cholesterol, C, 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 PL (38,39). Association of the FADS1 locus (rs174537) with serum PUFA levels was confirmed by a GWAS in the Invecchiare in Chianti (InCHIANTI) study (40). 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 PL 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 (41), which is the major phospholipid in HDL particles and is important in the metabolism of HDL particles (42). Furthermore, dietary PUFAs have been shown to influence lipoprotein levels, mainly HDL and LDL cholesterol (43), 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 (44), which could be one of the mechanisms by which FADS1 variants modulate insulin secretion (34) 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 5 caused by mutations at the HNF1B locus exhibit dyslipidemia characterized by hypertriglyceridemia and low HDL cholesterol levels (45).

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.
in our study. Only the FTO locus of these loci has been previously found to affect lipid levels (46).

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 chylomicrons, l- to m-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 v- and i-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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