Common Variants in Maturity-Onset Diabetes of the Young Genes and Future Risk of Type 2 Diabetes

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OBJECTIVE—Mutations in the hepatocyte nuclear factor (*HNF*)*-1α*, *HNF-4α*, glucokinase (*GCK*), and *HNF-1β* genes cause maturity-onset diabetes of the young (MODY), but it is not known whether common variants in these genes predict future type 2 diabetes.

RESEARCH DESIGN AND METHODS—We tested 14 previously associated polymorphisms in *HNF-1α*, *HNF-4α*, *GCK*, and *HNF-1β* for association with type 2 diabetes–related traits and future risk of type 2 diabetes in 2,293 individuals from the Botnia study (Finland) and in 15,538 individuals from the Malmö Preventive Project (Sweden) with a total follow-up >360,000 years.

RESULTS—The polymorphism rs1169288 in *HNF-1α* strongly predicted future type 2 diabetes (hazard ratio [HR] 1.2, $P = 0.0002$). Also, SNPs rs4810424 and rs3212198 in *HNF-4α* nominally predicted future type 2 diabetes (HR 1.3 [95% CI 1.0–1.6], $P = 0.03$; and 1.1 [1.0–1.2], $P = 0.04$). The rs2144908 polymorphism in *HNF-4α* was associated with elevated rate of hepatic glucose production during a hyperinsulinemic-euglycemic clamp ($P = 0.03$) but not with deterioration of insulin secretion over time. The SNP rs1799884 in the *GCK* promoter was associated with elevated fasting plasma glucose (fPG) concentrations that remained unchanged during the follow-up period ($P = 0.4$; SE 0.004 [−0.003–0.007]) but did not predict future type 2 diabetes (HR 0.9 [0.8–1.0], $P = 0.1$). Polymorphisms in *HNF-1β* (transcription factor 2 [*TCF2*]) did not significantly influence insulin or glucose values nor did they predict future type 2 diabetes.

CONCLUSIONS—In conclusion, genetic variation in both *HNF-1α* and *HNF-4α* predict future type 2 diabetes, whereas variation in the *GCK* promoter results in a sustained but subtle elevation of fPG that is not sufficient to increase risk for future type 2 diabetes. ***Diabetes*** **57:1738–1744, 2008**

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EGP, endogenous glucose production; FFM, fat-free mass; fPG, fasting plasma glucose; G6Pase, glucose-6-phosphatase; GCK, glucokinase; GEE, general estimation equation; HNF, hepatocyte nuclear factor; HOMA, homeostasis model assessment; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MPP, Malmö Preventive Project; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test; PCK-1, phosphoenolpyruvate carboxykinase 1; PGC-1α, peroxisome proliferator–activated receptor-γ coactivator-1α; PPAR, peroxisome proliferator–activated receptor; TCF, transcription factor.

Type 2 diabetes is a late-onset polygenic disease in which the westernized environment interacts with genetic factors to manifest the disease. More than 180 million people are reported to suffer from type 2 diabetes, with an estimated doubling within the next 15 years (1). The polygenic nature of type 2 diabetes has made the search for disease genes difficult, and the successful identification of genes causing monogenic forms of the disease, i.e., maturity-onset diabetes of the young (MODY), has been difficult to translate into type 2 diabetes with a few exceptions like peroxisome proliferator–activated receptor (*PPAR*) *G*, *KCNJ11*, *CAPN10*, and transcription factor 7L2 (*TCF7L2*) (2–5). This situation dramatically changed in 2007 with the discovery of a number of novel type 2 diabetes genes using whole-genome association studies (6–9).

MODY is an autosomal dominant form of type 2 diabetes characterized by early onset and a defect in the β-cells (10). Mutations in six genes, five of which encode for TCFs, cause MODY (11–17). It has been speculated that common variation in the MODY genes also could increase risk of late-onset type 2 diabetes. In support of this view, mutations in the genes encoding for *PPARG* and *KCNJ11* have been shown to cause rare early-onset forms of diabetes, whereas polymorphisms in these genes increase the risk for late-onset type 2 diabetes (2,5).

Hepatocyte nuclear factor-1α (*HNF-1α*; also known as *TCF1*) is a TCF part of a complex regulatory network that regulates a number of β-cell– and liver-specific genes. Common variations in this gene have been associated with impaired insulin secretion (18,19), and recently, we showed that the I27L and A98V polymorphisms in the MODY3 gene (*TCF1*) are associated with increased risk of type 2 diabetes in overweight individuals (19).

HNF-4α (also known as *TCF14*) is part of a complex regulatory network in the liver and pancreas (20) important for glucose homeostasis (21). An interaction between HNF-4α and peroxisome proliferator–activated receptor-γ coactivator-1α (*PGC-1α*) is a prerequisite for induction of the gluconeogenesis genes phosphoenolpyruvate carboxykinase 1 (*PCK-1*) and glucose-6-phosphatase (*G6Pase*) (22). *HNF-4α* is under regulation of two promoters: the liver-specific P1 promoter and the P2 promoter that mainly regulates transcription in the pancreatic β-cells. *HNF-4α* is located on chromosome 20q12-q13.1, which has shown suggestive linkage to type 2 diabetes (23). Also, several polymorphisms in the β-cell–specific

TABLE 1
Clinical characteristics of participating subjects

	Botnia study	MPP study	203 Malmö men (MPP)
Sex (male/female)	1,051/1,242	10,592/5,791	203/—
Age at onset/visit (years)	44.9 ± 13.8	45.5 ± 7.0	65.9 ± 1.9
BMI (kg/m^2)	25.3 (23.0–27.8)	24.0 (22.2–26.3)	26.4 (24.9–28.9)
Fasting plasma glucose (mmol/l)	5.6 (5.2–6.0)	5.5 ± 0.6	5.3 (4.8–6.7)*
30-min plasma glucose (mmol/l)	8.4 (7.2–9.6)	—	10.9 (9.4–12.8)†
2-h plasma glucose (mmol/l)	6.1 (5.2–7.2)	6.4 ± 1.6	8.7 (7.0–14.3)‡
Fasting serum insulin (mU/l)	4.4 (3.2–6.2)	7.0 (3.0–12.0)	10.0 (7.0–15.0)*
30-min serum insulin (mU/l)	35.7 (24.0–60.1)	—	48.0 (31.0–72.0)†
2-h serum insulin (mU/l)	25.4 (15.2–47.4)	26.0 (13.0–47.0)	50.0 (26.0–87.0)‡
A1C	5.4 (5.1–5.7)	—	5.1 (4.7–6.4)
Waist-to-hip ratio (men)	0.9 ± 0.06	—	0.97 ± 0.05
Women	0.8 ± 0.07	—	
Triglycerides (mmol/l)	1.1 (0.8–1.5)	—	1.3 (1.0–1.9)
Cholesterol (mmol/l)	5.4 (4.8–6.2)	—	5.6 ± 0.9
HDL cholesterol (mmol/l)	1.3 (1.1–1.6)	—	1.1 (1.0–1.4)
LDL cholesterol (mmol/l)	3.5 (2.9–4.2)	—	3.7 (3.2–4.3)
Systolic blood pressure (mmHg)	128.0 (116.0–140.0)	125.0 (117.5–135.0)	—
Diastolic blood pressure (mmHg)	80.0 (70.0–88.0)	82.5 (80.0–90.0)	—

Data (basal values) are means ± SD, and non-normally distributed data are median (interquartile range). *Fasting capillary blood or insulin. †40-min capillary blood. ‡120-min capillary blood.

HNF-4a P2 promoter have recently been associated with type 2 diabetes (24,25); however, results from replication studies have been inconclusive (26–30).

Heterozygous mutations in the glucokinase (*GCK*) gene, located on chromosome 7p15-p13, are known to cause MODY2 (15), and linkage to early-onset type 2 diabetes has been described to this region (12,31). MODY2 is characterized by a lifelong mild fasting hyperglycemia due to reduced glucose sensing in the β-cell (32). The −30G/A polymorphism in the *GCK* gene β-cell promoter has been associated with increased fasting plasma glucose (fPG) (33,34) and reduced β-cell function (35). In addition, it has been shown to affect birth weight (36). Even though the −30G/A polymorphism has been associated with diabetes-related traits, its contribution to type 2 diabetes is less well known. However, in a recent meta-analysis, it was shown to have a modest but significant effect on type 2 diabetes risk (37).

The MODY5-causing gene, *HNF-1β* (also known as *TCF2*), is located on chromosome 17cen-q21.3, a region that also has been linked to type 2 diabetes (38). Although *HNF-1β* is important for the development of the pancreas, mutations in *HNF-1β* show other more characteristic phenotypes like cystic kidney disease, liver dysfunction, and abnormal urogenital tract development (39–41). Recently, polymorphisms in *HNF-1β* were reported to be associated with type 2 diabetes in Caucasians (30,37,42).

A common problem with case-control studies is that case and control subjects have been ascertained differently, i.e., case subjects are selected from diabetes clinics, whereas control subjects often have not been thoroughly screened for the presence of diabetes. One way to circumvent this problem is to study whether a genetic variant predicts future type 2 diabetes in a longitudinal study where all individuals have undergone the same measurements to identify the disease.

The aim of this study was to test whether common variants in the *HNF-1α*, *HNF-4α*, *HNF-1β*, and *GCK* genes that previously have been associated with type 2 diabetes (24,25,30,33–35,37) in Caucasians could predict future type 2 diabetes in two large prospective studies and whether they influence intermediate traits.

RESEARCH DESIGN AND METHODS

Botnia study subjects. The future risk of developing type 2 diabetes and change in type 2 diabetes–related trait changes was evaluated in 2,293 (1,051 men/1,242 women) individuals from the Botnia study (43) followed for a median period of 6 years (Table 1). Of these subjects, 132 (67 men/65 women) developed type 2 diabetes, and 212 subjects developed impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (44). The Botnia study is a family-based study aimed at the identification of genes increasing susceptibility to type 2 diabetes and was initiated in 1990 in western Finland (43). Nondiabetic subjects, either family members of type 2 diabetic patients or control subjects or spouses without family history of type 2 diabetes aged between 18 and 70 years, were invited to prospective visits every 2–3 years. Subjects with clinically verified type 1 diabetes or genetically verified MODY were excluded from the study.

Malmö Preventive Project subjects. The risk of developing type 2 diabetes was also evaluated in 15,538 individuals (10,173 men/5,365 women) from the Malmö Preventive Project (MPP) study with a median follow-up of 22.8 years. Of them 1,872 individuals (1,465 men/407 women) developed type 2 diabetes during follow-up. Diabetes diagnosis was based on clinical diagnosis or measurements of fPG during re-examination (≥7.0 mmol/l). The MPP study was initiated in 1974 as a health screening of citizens in the city of Malmö, Sweden (45).

A subgroup of 203 men who had IGT at baseline in the MPP study were randomly selected to participate 20 years later in more extensive metabolic studies, including a new oral glucose tolerance test (OGTT), a euglycemic-hyperinsulinemic clamp combined with indirect calorimetry, and infusion of [3-^{3}H]-glucose to obtain estimates of glucose metabolism and substrate oxidation (46,47). The men had similar age (66 ± 2 years) but varying degree of glucose tolerance; 69 had normal glucose tolerance, 52 had IFG and/or IGT, and 82 had type 2 diabetes. Type 2 diabetic patients were treated either with diet alone (42%) or with oral hypoglycemic agents, which were withheld the day before the test.

All subjects gave their informed consent to the study, which was approved by local ethics committees.

Measurements. The participants' weight, height, waist and hip circumference, fat-free mass (Botnia study and clamp protocol), and blood pressure were measured as previously reported (43). In the MPP cohort at baseline, blood samples were drawn at 0, 40, and 120 min of the 75-g OGTT for measurements of blood glucose and serum insulin concentrations, whereas fasting samples were drawn at the follow-up visit for measurement of plasma glucose and lipid concentrations using standard techniques. In the Botnia study, blood samples were drawn at −10, 0, 30, 60, and 120 min of the OGTT for measurement of plasma glucose and serum insulin concentrations. Insulin

resistance was estimated according to the homeostasis model assessment (HOMA) index as the product of fasting glucose (millimoles per liter) and insulin (milliunits per liter) divided by the constant 22.5. β-Cell function was estimated as insulinogenic index, I/G30 (or I/G40 in MPP) (insulin 30 min − fasting insulin/glucose 30 min), and disposition index (I/G30/HOMA), which is a measure of β-cell function corrected for insulin resistance. Whole-body insulin sensitivity and basal hepatic endogenous glucose production (EGP) in the subgroup of 203 men was measured by a standard 2-h euglycemic-hyperinsulinemic clamp combined with infusion of [3-^{3}H]glucose and indirect calorimetry (47).

Genotyping. Fourteen polymorphisms previously associated with type 2 diabetes were selected for genotyping (7,9,30,37). The Botnia prospective cohort was genotyped using the allelic discrimination technique (Applied Biosystems, Foster City, CA) on an Applied Biosystems 7900HT instrument using the standard protocol (PCR primers and discrimination probes, which are detailed in online appendix 1 [available at http://dx.doi.org/10.2337/db06-1464]). The MPP cohort was genotyped by primer extension of multiplex products with detection by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the iPLEX protocol on a Sequenom platform (Sequenom, San Diego, CA). Genotyping success rate >98%; Hardy-Weinberg equilibrium ($P > 0.05$), with an error-rate <1% determined from >7% re-genotyping.

rs757210 and rs1008284 failed Applied Biosystems Taqman assay design and were not genotyped in the Botnia prospective cohort. rs2144908 and rs1884614 failed genotyping in MPP. rs757210 is tri-allelic and was analyzed as T allele carriers versus C+G allele carriers (genotype counts are given in online appendix 3). To determine the genetic model, the SNPs were analyzed using the program MODEL, and the best-fitting model was used for our calculations (Table 2). Power was estimated to ~65% for hazard ratio (HR) 1.2 and an allele frequency of 20% in MPP.

Statistical analyses. Baseline levels and rate change of phenotype residuals between different genotype carriers in the Botnia study and MPP were calculated using multiple and linear regression analyses adjusted for age at visit, BMI, sex, and family history of type 2 diabetes. Genotypes were scored as 0, 1, or 2 depending on how many copies of the rare allele each individual carried. SEs were adjusted for repeated measurements from the same individual by the general estimation equation (GEE) method (48,49). The GEE model was used because the repeated phenotype measurements were obtained at different time points in different subjects. Phenotypic data from each visit except the visit of diagnosis for the converters and all but the last visit for the nonconverters were used in the analysis. This was necessary to avoid the confounding effect of overt hyperglycemia on β-cell function, because we did not have measurements immediately before onset of diabetes.

The Cox proportional hazards model was used to estimate relative genotype and phenotype effect on the risk of developing type 2 diabetes. Data were treated as left truncated and right censored using entry data as covariates. Data were left truncated because individuals enter the study at different ages. Survival analyses were stratified for sex and adjusted for family history of diabetes and BMI (50). A robust variance estimate was used to adjust for within-pedigree dependence, treating each pedigree as an independent entity when calculating the variance of the estimates. Individuals with missing data for any of the covariates were excluded from the analyses.

Analyses in the 203 men from the MPP study (47) were adjusted for age, BMI, and level of glucose tolerance, which was dichotomized to glucose tolerant or nonglucose tolerant.

Normally distributed continuous variables of in vivo measurements are presented as mean ± SD, whereas non-normally distributed data were logarithmically transformed before analysis. Genetic models for these SNPs were evaluated in the two prospective cohorts using the program MODEL (http://pngu.mgh.harvard.edu/~purcell/model/model.html). Power calculations were performed using asymptotic normality of the estimates. A two-tailed P value of <0.05 was considered statistically significant, and nominal P values for regression analysis, GEE, and Cox proportional hazards model are presented. Multiple testing was not performed. All statistical analyses were performed using STATA (StataCorp) and/or Number Cruncher Statistical Systems (version 2000; NCSS, Kaysville, UT).

RESULTS

Genotype frequencies. Fourteen SNPs in *HNF-1α*, *HNF-4α*, *HNF-1β*, and *GCK* were genotyped in 2,293 plus 15,538 individuals from two prospective cohorts. Allele frequencies were in concordance with previously published results and in Hardy-Weinberg equilibrium (Table 2). Also, D′ and r^2 values were in the same range as previously reported (online appendix 2).

TABLE 2
Risk of future type 2 diabetes in carriers of different risk genotypes in the Botnia and MPP cohorts

SNP	Position (nucleotides)	Alleles (minor/major)	Best model*	Botnia MAF (converter)	Botnia MAF (nonconverter)	HWE (1 df)	Botnia HR (95% CI)	Botnia P value	MPP MAF (converter)	MPP MAF (nonconverter)	HWE (1 df)	MPP HR (95% CI)	MPP P value	Combined HR (95% CI)	Combined P value
HNF-4α															
rs4810424	42408437	C/G	R	0.202	0.174	0.23	1.9 (1.0–3.5)	0.04	0.177	0.170	0.61	1.2 (1.0–1.6)	0.1	1.3 (1.0–1.6)	0.03
rs1884614†	42413933	T/C	R	0.205	0.173	0.17	1.9 (1.0–3.4)	0.04	—	—	—	—	—	—	—
rs2144908†	42419131	A/G	R	0.208	0.176	0.15	1.8 (1.0–3.3)	0.06	—	—	—	—	—	—	—
rs6103716	42433044	G/T	R	0.408	0.362	0.88	1.3 (0.9–1.9)	0.2	0.331	0.329	0.52	1.0 (0.9–1.2)	0.6	1.1 (0.9–1.2)	0.3
rs2425637	42457463	G/T	A	0.480	0.472	0.63	1.0 (0.6–1.7)	0.9	0.483	0.485	0.60	1.0 (0.9–1.1)	0.9	1.0 (0.9–1.1)	0.9
rs3212198	42477776	C/T	D	0.516	0.463	0.24	1.4 (0.9–2.1)	0.1	0.420	0.412	0.87	1.1 (1.0–1.2)	0.1	1.1 (1.0–1.2)	0.04
GCK															
rs758989	44169531	G/A	R	0.457	0.502‡	0.32	1.4 (1.0–2.1)	0.06	0.478	0.476	0.15	1.0 (0.9–1.1)	0.9	1.0 (0.9–1.1)	0.9
rs2244164	44183651	T/C	M	0.56	0.49	0.02	0.7 (0.5–1.1)	0.2	0.477	0.473	0.08	1.0 (0.9–1.2)	0.6	1.0 (0.9–1.1)	0.3
rs1303722	44185599	A/G	A	0.462	0.498	0.30	1.1 (0.7–1.7)	0.7	0.477	0.473	0.12	1.0 (0.9–1.1)	0.6	1.0 (0.9–1.1)	0.7
rs1799884	44195593	A/G	D	0.118	0.119	0.42	1.8 (0.6–5.8)	0.3	0.149	0.157	0.17	0.8 (0.6–1.1)	0.2	0.9 (0.8–1.0)	0.1
HNF-1β															
rs3110641	33121530	T/C	R	0.159	0.169	0.24	0.8 (0.3–2.6)	0.7	0.195	0.200	0.27	1.1 (0.9–1.4)	0.3	1.1 (0.9–1.4)	0.4
rs1008284§	33136571	A/G	R	—	—	—	—	—	0.239	0.242	0.49	1.0 (0.9–1.1)	0.7	—	—
rs757210‡§	33170628	T/C+G	D	—	—	—	—	—	0.367	0.359	0.001	1.0 (0.9–1.1)	0.9	—	—
HNF-1α															
rs1169288	119901033	T/G	R	—	—	—	—	—	0.393	0.353	0.60	1.2 (1.1–1.3)	0.0002	—	—

Survival analyses performed in the Botnia prospective cohort, the MPP, and the combination of the two. The genetic models were evaluated using the program MODEL (http://pngu.mgh.harvard.edu/~purcell/model/model.html). *Genetic model that best fitted our data and that was used for calculations; R, recessive; A, additive; D, dominant; M, multiplicative. For the *HNF-4α* P2 promoter SNPs, a recessive model was used because this fitted our data the best ($P = 0.01$). This observation is in contrast to the genetic model used in Bonnycastle et al. (30), where a multiplicative model was used. §DNA sequence assay design was impracticable for the Applied Biosystems 7900HT system. ‡Tri-allelic 2 degrees of freedom (df) in HWE test and analyzed as T versus C + G in our analyses. †Genotypes did not pass our quality criteria, making a combined analysis impracticable. HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

HNF-1α. Genotyping the I27L polymorphism (rs1169288) in HNF-1α in the whole MPP cohort, we not only replicated our earlier finding that this SNP predicted future type 2 diabetes in elderly obese men (19) but could show that it strongly predicted type 2 diabetes in the extended cohort (HR 1.2 [95% CI 1.1–1.3], $P = 0.0002$).

HNF-4α. In the Botnia study, 132 of 2,293 individuals converted to type 2 diabetes during median follow-up of 6 years (50), and six polymorphisms previously found associated with type 2 diabetes were genotyped in this material. In accordance to the program MODEL (http://pngu.mgh.harvard.edu/~purcell/model/), a recessive model fitted the data best ($P = 0.01$) (Table 2).

Consequently, the homozygous CC (rs4810424), TT (rs1884614), and AA (rs2144908) genotypes in the *HNF-4α* P2 promoter were associated with a modestly increased risk of future type 2 diabetes in the Botnia study with HR 1.9 (95% CI 1.0–3.5) ($P = 0.04$), 1.9 (1.0–3.4) ($P = 0.04$), and 1.8 (1.0–3.3) ($P = 0.06$) (Table 2).

We also assessed whether the same genotypes in *HNF-4α* would influence glucose and insulin concentrations and measures of insulin secretion and action over time in the Botnia study.

Individuals with the rs2144908 AA risk genotype had higher 2-h glucose levels at the last visit compared with individuals with the GG/GA genotypes (6.3 ± 0.2 vs. 5.9 ± 0.04 mmol/l, $P = 0.03$). Using a GEE adjusted for age, BMI, sex, and family clustering, we observed an increase over time in measures of insulin resistance (HOMA-IR) ($P = 0.003$) in individuals with the rs2144908 AA compared with individuals with the nonrisk GG/GA genotypes. Also, in carriers of CC (rs4810424) and TT (rs1884614) genotypes of the *HNF-4α* gene, fasting insulin ($P = 0.02$), 30-min insulin ($P = 0.02$), I/G30 ($P = 0.01$), and HOMA-IR ($P = 0.01$) increased over time, suggesting worsening of insulin resistance. In contrast, there was no deterioration of insulin secretion measured as disposition index ($P > 0.8$) in carriers of these genotypes (online appendix 4).

In the MPP cohort, 1,872 of the 15,538 individuals converted to type 2 diabetes during a median follow-up of 24.5 years. None of the SNPs that predicted type 2 diabetes in the Botnia cohort predicted type 2 diabetes on their own in the MPP study (rs4810424, HR 1.2 [95% CI 1.0–1.6], $P = 0.1$; rs6103716, 1.0 [0.9–1.2], $P = 0.6$; rs2425637, 1.0 [0.9–1.1], $P = 0.9$; and rs3212198, 1.1 [1.0–1.2], $P = 0.1$) (Table 2).

Homozygous CC (rs4810424) and CC/CT genotypes (rs3212198) predicted future type 2 diabetes in the combined cohorts with HR 1.3 (1.0–1.6), $P = 0.03$, and 1.1 (1.0–1.2), $P = 0.04$, respectively.

We also assessed whether the P2 promoter polymorphisms influenced hepatic (EGP) and peripheral (insulin-stimulated glucose uptake) insulin sensitivity in the subgroup of 203 men with extensive metabolic measurements (47). All analyses were adjusted for age, BMI, and diabetes status (dichotomized variable) because the men showed a wide variation in their glucose tolerance. Individuals with the AA genotype (rs2144908) had higher rates of basal (3.4 ± 0.2 vs. 2.9 ± 0.05 mg · kg fat-free mass $[FFM]^{-1} \cdot min^{-1}$, $P = 0.05$) and residual (0.9 ± 0.2 vs. 0.3 ± 0.06 mg · kg $FFM^{-1} \cdot min^{-1}$, $P = 0.04$) EGP during clamp compared with individuals with the GG/GA genotypes (Fig. 1). As expected, there was a positive correlation between the basal rate of EGP and the fPG concentration ($r = 0.60$, $P < 0.0001$). No significant difference in insulin-stimulated glucose uptake was observed between risk and nonrisk genotype carriers for this SNP (5.7 ± 0.7 vs. 5.6 ± 0.2 mg · kg $FFM^{-1} \cdot min^{-1}$, $P = 0.9$).

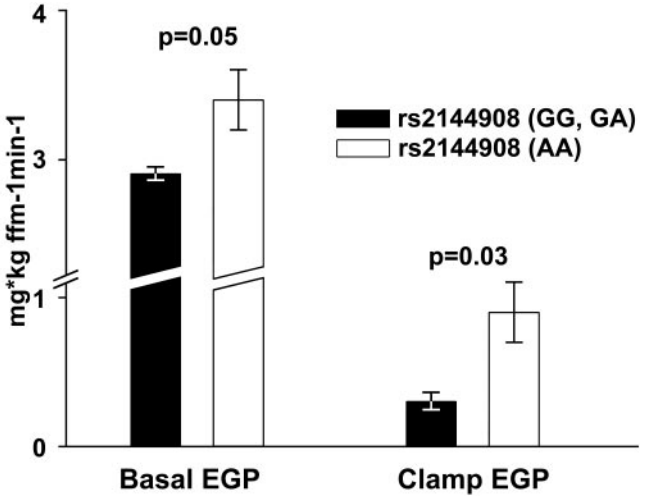

FIG. 1. The AA genotype of the rs2144908 *HNF-4α* polymorphism was associated with increased basal EGP (3.4 ± 0.2 vs. 2.9 ± 0.05 mg · kg^{-1} FFM · min^{-1}, $P = 0.05$) ($P = 0.05$) and clamp EGP (0.9 ± 0.2 vs. 0.3 ± 0.06 mg · kg^{-1} FFM · min^{-1}, $P = 0.04$) ($P = 0.03$) compared with individuals with the GG/GA genotypes. Analysis was adjusted for age and BMI, and the level of glucose homeostasis was dichotomized as non–type 2 diabetes and type 2 diabetes.

GCK. In the Botnia study, we observed a significant difference in fPG between the different rs1799884 genotypes at the basal visit (GG, 5.55 ± 0.01; GA, 5.60 ± 0.03; AA, 6.1 ± 0.1; $P = 0.000003$) and a trend toward higher fPG levels with the AA genotype at the last visit (GG, 5.30 ± 0.01; GA, 5.32 ± 0.03; AA, 5.54 ± 0.1; $P = 0.09$). Individuals with the GA/AA versus GG genotypes showed higher fPG (5.62 ± 0.02 vs. 5.55 ± 0.01, $P = 0.008$) at the basal visit (online appendix 4). None of the other tested SNPs in the *GCK* gene influenced glucose concentrations.

The same *GCK* SNP (rs1799884) influenced fasting glucose concentrations also in the MPP cohort (GG = 5.45 ± 0.005; GA = 5.49 ± 0.008; AA = 5.51 ± 0.03, $P = 0.0001$). Individuals with GA/AA had higher fPG (5.49 ± 0.008 vs. 5.45 ± 0.005, $P = 0.00002$) and 2-h glucose (6.49 ± 0.03 vs. 6.41 ± 0.02, $P = 0.02$) concentrations at baseline than individuals with GG. These subtle differences in fPG remained unchanged during the follow-up period in both the Botnia ($P = 0.4$; SE 0.004 [95% CI −0.003–0.007]) and the MPP cohorts ($P = 0.1$; SE 0.01 [−0.04–0.004]).

In addition, SNP rs2244164 and rs1303722 also influenced glucose concentrations in the MPP cohort. Individuals with the rs2244164 TT and CT genotypes (5.47 ± 0.008 vs. 5.45 ± 0.006 vs. 5.44 ± 0.009 mmol/l, $P = 0.03$) and rs1303722 GG and GA genotypes (5.48 ± 0.008 vs. 5.46 ± 0.006 vs. 5.45 ± 0.009 mmol/l, $P = 0.04$) had higher fPG than CC and AA carriers.

Despite the significant differences in fasting glucose throughout the follow-up period, none of the tested polymorphisms showed an increased risk for future risk of type 2 diabetes in the combined sample (Table 2).

HNF-1β (TCF2). None of the SNPs in *HNF-1β* gene significantly influenced future risk of type 2 diabetes nor did they influence metabolic traits (Table 2).

DISCUSSION

The key findings of the present study were that *1*) common variants in the *HNF-1α* and *-4α* genes predicted subsequent type 2 diabetes, *2*) individuals with the AA genotype

(rs2144908) had elevated rate of EGP, *3*) the A allele of rs1799884 (−30G/A) in *GCK* was associated with elevated fasting glucose concentrations, which were maintained unchanged throughout the follow-up period but not sufficient to increase risk for future type 2 diabetes, and *4*) common variants in the *HNF-1β* gene did not predict future type 2 diabetes.

Common variants in known MODY genes (30,37) have been inconclusively associated with type 2 diabetes in case-control studies (24,25,30,33–35,37). We extended these findings here to test whether the same SNPs would increase risk of future type 2 diabetes in prospective studies or whether they would influence metabolic traits.

HNF-1α. We have provided compelling evidence that a functional variant in the *HNF-1α* gene (I27L) was associated with an increased future risk of type 2 diabetes. In a previous report on a smaller subgroup from the MPP cohort (n = 4,873), we showed that the I27L variant increased future risk of type 2 diabetes only in obese elderly men (19). We interpreted this as the inability to increase insulin secretion to meet the increased insulin needs associated with aging and obesity in carriers of the risk genotype (LL) of the polymorphism. In this much larger sample, the risk of future type 2 diabetes was evident in all individuals independent of sex, age, and BMI. The I27L polymorphism thereby seems to increase future risk of type 2 diabetes with an effect size similar to the novel type 2 diabetes genes (6–9).

HNF-4α. SNPs rs4810424 and rs3212198 in the *HNF-4α* gene showed a modest association with type 2 diabetes in the combined sample (HR 1.3, P = 0.03, and 1.1, P = 0.04). This is in line with findings from some (24,25) but not all (26–30) previous studies.

We also explored potential mechanisms by which variation in the gene could lead to type 2 diabetes. Because some of these associated variants are located in the vicinity of the *HNF-4α* β-cell–specific P2 promoter, it is possible that they could influence the transcription and regulation of *HNF-4α*, which, in turn, could lead to insulin secretion defects. However, we could not demonstrate any deterioration in β-cell function in carriers of the P2 promoter SNPs. If anything, there was an increase in insulin concentrations over time in carriers of risk genotypes in *HNF-4α*. Whether this reflects an intrinsic defect in islets resulting in increased insulin secretion or is a corollary of increased insulin resistance cannot be answered by the current study.

Interestingly, carriers of the P2 promoter risks genotype showed elevated rate of basal (P = 0.05) and EGP during clamp (P = 0.04). There are many reasons to believe that this finding is real. We had a 72% power to detect a difference of this magnitude in EGP. The elevated rate of EGP was not simply a consequence of more individuals with IGT; in a multivariate analysis including both genotype and glucose tolerance, genotype was an independent predictor of EGP. We most likely increased the likelihood of finding a difference in EGP between different genotype carriers by including individuals with abnormal glucose tolerance, because there is little variation in EGP in individuals with normal glucose tolerance. We do not have sufficient explanation for differences in suppression of EGP between different genotype carriers to specifically explore mechanisms behind this elevation. Although insulin is a potent inhibitor of HGP, differences in insulin concentrations are an unlikely explanation as EGP was estimated at identical insulin concentrations during clamp. Differences in interaction between HNF-4α and the gluconeogenic pathways could be a more plausible explanation.

It is somewhat surprising that variation in the P2 promoter, which is almost exclusively expressed in the pancreas, has an effect on EGP; it should be kept in mind that HNF-4α is transcribed from the P2 promoter during development of the mouse liver (51). Increased rate of gluconeogenesis is a hallmark of type 2 diabetes (52), and the interaction between HNF-4α and PGC-1α is crucial for the expression of the gluconeogenic *PEPCK* and *G6Pase* genes (22). However, we cannot exclude that this SNP is in linkage disequilibrium with another SNP, which would influence transcription of the gene in the liver.

GCK. The −30G/A (rs1799884) variant resides in a region of β-cell–specific *GCK* promoter that shows strong homology between humans, mice, and rats (available from the Santa Cruz Genome website [http://genome.ucsc.edu]), suggesting that it is important for the regulation of *GCK*. In accordance with other studies, this variant was associated with a sustained but modest increase in fasting glucose concentrations (P = 0.4 and 0.1). Despite the higher glucose levels observed with the A allele, we did not observe an increased risk for type 2 diabetes in carriers of this allele, which is in line with results from our whole-genome–wide scan for rs1799884 (P = 0.1). Also and in keeping with some previous observations, the A allele was not associated with impaired insulin secretion (34,53). The finding of no effect on risk of future type 2 diabetes contrasts with a previous study (37). Although this is the largest prospective study to date, the power to detect an increased risk of type 2 diabetes was only 65%.

HNF-1β. A number of studies have investigated a role for common variation in *HNF-1β* with type 2 diabetes in Caucasians with contradicting results (30,37,42). The predominant phenotype of MODY5 patients with mutations in the *HNF-1β* gene is in addition to early-onset diabetes cystic kidney disease and urogenital malformations. None of the subjects included in this study reported any such problems. We investigated previously associated SNPs, but our findings do not support a role for any of these *HNF-1β* SNPs in type 2 diabetes.

Some weaknesses of the study should be emphasized. We did not correct for multiple comparisons, and despite >15,000 individuals, we only have 65% power to detect an HR of 1.2, suggesting that we might have missed some true positive associations with type 2 diabetes. However, the *HNF-1α* I27L polymorphism served as a positive control, because the previously weak association with type 2 diabetes shown in a subset from this study now became markedly stronger in the larger dataset.

Another caveat could be that we only selected SNPs that had previously been associated with type 2 diabetes in large case-control studies. Although we thereby only covered 12% of the genetic variation in the genes, this is compensated by strong linkage disequilibrium and also by information from imputed whole-genome association studies, which to some extent supported our findings (online appendix 5).

In conclusion, our data show that genetic variation in *HNF-1α* and *HNF-4α* increase future risk of type 2 diabetes. Carriers of the −30G/A polymorphism in the *GCK* gene have a sustained increase in fasting glucose concentrations that is not translated into an increased risk of future type 2 diabetes. Genetic variation in *HNF-1β* is not associated with type 2 diabetes in our cohorts. Taken together, the results indicate that common variants in the

HNF-1α and *HNF-4α* genes are associated with a modestly increased risk of future type 2 diabetes.

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REFERENCES

1. Zimmet P, Alberti KGMM, Shaw J: Global and societal implications of the diabetes epidemic. *Nature* 414:782–787, 2001
2. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80, 2000
3. Grant SFA, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K: Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38:320–323, 2006
4. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PEH, del Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI: Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163–175, 2000
5. Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Frayling TM: Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 52:568–572, 2003
6. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JRB, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney ASF, Burton PR, Clayton DG, Craddock N, Deloukas P, Duncanson A, Kwiatkowski DP, Ouwehand WH, Samani NJ, Todd JA, Donnelly P, Davison D, Easton D, Evans D, Leung H-T, Spencer CCA, Tobin MD, Attwood AP, Boorman JP, Cant B, Everson U, Hussey JM, Jolley JD, Knight AS, Koch K, Meech E, Nutland S, Prowse CV, Stevens HE, Taylor NC, Walters GR, Walker NM, Watkins NA, Winzer T, Jones RW, McArdle WL, Ring SM, Strachan DP, Pembrey M, Breen G, St. Clair D, Caesar S, Gordon-Smith K, Jones L, Fraser C, Green EK, Grozeva D, Hamshere ML, Holmans PA, Jones IR, Kirov G, Moskvina V, Nikolov I, O'Donovan MC, Owen MJ, Collier DA, Elkin A, Farmer A, Williamson R, McGuffin P, Young AH, Ferrier IN, Ball SG, Balmforth AJ, Barrett JH, Bishop DT, Iles MM, Maqbool A, Yuldasheva N, Hall AS, Braund PS, Dixon RJ, Mangino M, Stevens S, Thompson JR, Bredin F, Tremelling M, Parkes M, Drummond H, Lees CW, Nimmo ER, Satsangi J, Fisher SA, Forbes A, Lewis CM, Onnie CM, Prescott NJ, Sanderson J, Mathew CG, Barbour J, Mohiuddin MK, Todhunter CE, Mansfield JC, Ahmad T, Cummings FR, Jewell DP, Webster J, Brown MJ, Lathrop GM, Connell J, Dominiczak A, Braga Marcano CA, Burke B, Dobson R, Gungadoo J, Lee KL, Munroe PB, Newhouse SJ, Onipinla A, Wallace C, Xue M, Caulfield M, Farrall M, Barton A, Bruce IN, Donovan H, Eyre S, Gilbert PD, Hider SL, Hinks AM, John SL, Potter C, Silman AJ, Symmons DPM, Thomson W, Worthington J, Dunger DB, Widmer B, Newport M, Sirugo G, Lyons E, Vannberg F, Hill AVS, Bradbury LA, Farrar C, Pointon JJ, Wordsworth P, Brown MA, Franklyn JA, Heward JM, Simmonds MJ, Gough SCL, Seal S, Stratton MR, Rahman N, Ban M, Goris A, Sawcer SJ, Compston A, Conway D, Jallow M, Rockett KA, Bumpstead SJ, Chaney A, Downes K, Ghori MJR, Gwilliam R, Hunt SE, Inouye M, Keniry A, King E, McGinnis R, Potter S, Ravindrarajah R, Whittaker P, Widden C, Withers D, Cardin NJ, Ferreira T, Pereira-Gale J, Hallgrimsdottir IB, Howie BN, Su Z, Teo YY, Vukcevic D, Bentley D, Compston A, Ouwehand NJ, Samani MR, Isaacs JD, Morgan AW, Wilson GD, Ardern-Jones A, Berg J, Brady A, Bradshaw N, Brewer C, Brice G, Bullman B, Campbell J, Castle B, Cetnarsryj R, Chapman C, Chu C, Coates N, Cole T, Davidson R, Donaldson A, Dorkins H, Douglas F, Eccles D, Eeles R, Elmslie F, Evans DG, Goff S, Goodman S, Goudie D, Gray J, Greenhalgh L, Gregory H, Hodgson SV, Homfray T, Houlston RS, Izatt L, Jackson L, Jeffers L, Johnson-Roffey V, Kavalier F, Kirk C, Lalloo F, Langman C, Locke I, Longmuir M, Mackay J, Magee A, Mansour S, Miedzybrodzka Z, Miller J, Morrison P, Murday V, Paterson J, Pichert G, Porteous M, Rahman N, Rogers M, Rowe S, Shanley S, Saggar A, Scott G, Side L, Snadden L, Steel M, Thomas M, Thomas S, McCarthy MI, Hattersley AT: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336–1341, 2007
7. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PIW, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen M-R, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, DeFelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn G-W, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S, for the Diabetes Genetics Initiative of Broad Institute of Harvard and MIT LU, Novartis Institutes of BioMedical Research: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331–1336, 2007
8. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding C-J, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li X-Y, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345, 2007
9. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881–885, 2007
10. Fajans SS, Bell GI, Polonsky KS: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345:971–980, 2001
11. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Bell GI: Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458, 1996
12. Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, Lesage S, Vionnet N, Clement K, Fougerousse F: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:162–164, 1992
13. Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O, Kuroki H, Kasahara T, Iwamoto Y, Bell GI: Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet* 17:384–385, 1997
14. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4[alpha] gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
15. Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, Lesage S, Velho G, Iris F, Passa P, Froguel P, Cohen D: Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:721–722, 1992
16. Malecki MT, Jhala US, Antonellis A, Fields L, Doria A, Orban T, Saad M, Warram JH, Montminy M, Krolewski AS: Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. *Nat Genet* 23:323–328, 1999
17. Stoffers DA, Ferrer J, Clarke WL, Habener JF: Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 17:138–139, 1997
18. Urhammer S, Fridberg M, Hansen T, Rasmussen S, Moller A, Clausen J, Pedersen O: A prevalent amino acid polymorphism at codon 98 in the

hepatocyte nuclear factor-1α gene is associated with reduced serum C-peptide and insulin responses to an oral glucose challenge. *Diabetes* 46:912–916, 1997
19. Holmkvist J, Cervin C, Lyssenko V, Winckler W, Anevski D, Cilio C, Almgren P, Berglund G, Nilsson P, Tuomi T, Lindgren CM, Altshuler D, Groop L: Common variants in *HNF-1a* and risk of type 2 diabetes. *Diabetologia* 49:2882–2891, 2006
20. Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA: Control of pancreas and liver gene expression by HNF transcription factors. *Science* 303:1378–1381, 2004
21. Wang H, Maechler P, Antinozzi PA, Hagenfeldt KA, Wollheim CB: Hepatocyte nuclear factor 4alpha regulates the expression of pancreatic beta-cell genes implicated in glucose metabolism and nutrient-induced insulin secretion. *J Biol Chem* 275:35953–35959, 2000
22. Rhee J, Inoue Y, Yoon JC, Puigserver P, Fan M, Gonzalez FJ, Spiegelman BM: Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. *Proc Natl Acad Sci U S A* 100:4012–4017, 2003
23. Zouali H, Hani E, Philippi A, Vionnet N, Beckmann J, Demenais F, Froguel P: A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. *Hum Mol Genet* 6:1401–1408, 1997
24. Love-Gregory LD, Wasson J, Ma J, Jin CH, Glaser B, Suarez BK, Permutt MA: A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4{alpha} gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashkenazi Jewish population. *Diabetes* 53:1134–1140, 2004
25. Silander K, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, Jackson AU, Deloukas P, Hunt S, Stavrides G, Chines PS, Erdos MR, Narisu N, Conneely KN, Li C, Fingerlin TE, Dhanjal SK, Valle TT, Bergman RN, Tuomilehto J, Watanabe RM, Boehnke M, Collins FS: Genetic variation near the hepatocyte nuclear factor-4{alpha} gene predicts susceptibility to type 2 diabetes. *Diabetes* 53:1141–1149, 2004
26. Winckler W, Graham RR, de Bakker PIW, Sun M, Almgren P, Tuomi T, Gaudet D, Hudson TJ, Ardlie KG, Daly MJ, Hirschhorn JN, Groop L, Altshuler D: Association testing of variants in the hepatocyte nuclear factor 4α gene with risk of type 2 diabetes in 7,883 people. *Diabetes* 54:886–892, 2005
27. Weedon MN, Owen KR, Shields B, Hitman G, Walker M, McCarthy MI, Love-Gregory LD, Permutt MA, Hattersley AT, Frayling TM: Common variants of the hepatocyte nuclear factor-4α P2 promoter are associated with type 2 diabetes in the U.K. population. *Diabetes* 53:3002–3006, 2004
28. Vaxillaire M, Dina C, Lobbens S, Dechaume A, Vasseur-Delannoy V, Helbecque N, Charpentier G, Froguel P: Effect of common polymorphisms in the HNF4a promoter on susceptibility to type 2 diabetes in the French Caucasian population. *Diabetologia* 48:440–444, 2005
29. Bagwell AM, Bento JL, Mychaleckyj JC, Freedman BI, Langefeld CD, Bowden DW: Genetic analysis of HNF4A polymorphisms in Caucasian-American type 2 diabetes. *Diabetes* 54:1185–1190, 2005
30. Bonnycastle LL, Willer CJ, Conneely KN, Jackson AU, Burrill CP, Watanabe RM, Chines PS, Narisu N, Scott LJ, Enloe ST, Swift AJ, Duren WL, Stringham HM, Erdos MR, Riebow NL, Buchanan TA, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Boehnke M, Collins FS: Common variants in maturity-onset diabetes of the young genes contribute to risk of type 2 diabetes in Finns. *Diabetes* 55:2534–2540, 2006
31. Hattersley AT, Turner RC, Patel P, O'Rahilly S, Hattersley AT, Patel P, Wainscoat JS, Permutt MA, Tanazawa Y, Chin KC, Watkins P: Linkage of type 2 diabetes to the glucokinase gene. *Lancet* 339:1307–1310, 1992
32. Byrne MM, Sturis J, Clément K, Vionnet N, Pueyo ME, Stoffel M, Takeda J, Passa P, Cohen D, Bell GI: Insulin secretory abnormalities in subjects with hyperglycemia due to glucokinase mutations. *J Clin Invest* 98:1120–1130, 1994
33. Weedon MN, Frayling TM, Shields B, Knight B, Turner T, Metcalf BS, Voss L, Wilkin TJ, McCarthy A, Ben-Shlomo Y, Davey Smith G, Ring S, Jones R, Golding J, ALSPAC Study Team, Byberg L, Mann V, Axelsson T, Syvanen A-C, Leon D, Hattersley AT: Genetic regulation of birth weight and fasting glucose by a common polymorphism in the islet cell promoter of the glucokinase gene. *Diabetes* 54:576–581, 2005
34. Rose CS, Ek J, Urhammer SA, Glumer C, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T: A -30G>A polymorphism of the β-cell–specific glucokinase promoter associates with hyperglycemia in the general population of whites. *Diabetes* 54:3026–3031, 2005
35. Stone L, Kahn S, Fujimoto W, Deeb S, Porte D: A variation at position −30 of the β-cell glucokinase gene promoter is associated with reduced β-cell function in middle-aged Japanese-American men. *Diabetes* 45:422–428, 1996
36. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S: Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nat Genet* 19:268–270, 1998
37. Winckler W, Weedon MN, Graham RR, McCarroll SA, Purcell S, Almgren P, Tuomi T, Gaudet D, Bostrom KB, Walker M, Hitman G, Hattersley AT, McCarthy MI, Ardlie KG, Hirschhorn JN, Daly MJ, Frayling TM, Groop L, Altshuler D: Evaluation of common variants in the six known maturity-onset diabetes of the young (MODY) genes for association with type 2 diabetes. *Diabetes* 56:685–693, 2007
38. Demenais F, Kanninen T, Lindgren CM, Wiltshire S, Gaget S, Dandrieux C, Almgren P, Sjogren M, Hattersley A, Dina C, Tuomi T, McCarthy MI, Froguel P, Groop LC: A meta-analysis of four European genome screens (GIFT Consortium) shows evidence for a novel region on chromosome 17p11.2-q22 linked to type 2 diabetes. *Hum Mol Genet* 12:1865–1873, 2003
39. Nishigori H, Yamada S, Kohama T, Tomura H, Sho K, Horikawa Y, Bell G, Takeuchi T, Takeda J: Frameshift mutation, A263fsinsGG, in the hepatocyte nuclear factor-1β gene associated with diabetes and renal dysfunction. *Diabetes* 47:1354–1355, 1998
40. Lindner T, Njolstad P, Horikawa Y, Bostad L, Bell G, Sovik O: A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1beta. *Hum Mol Genet* 8:2001–2008, 1999
41. Bingham C, Ellard S, Allen L, Bulman M, Shepherd M, Frayling T, Berry PJ, Clark PM, Lindner T, Bell GI, Ryffel GU, Nicholls AJ, Hattersley AT: Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1[beta]. *Kidney Int* 57:898–907, 2000
42. Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, Manolescu A, Rafnar T, Gudbjartsson D, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Blondal T, Stacey SN, Helgason A, Gunnarsdottir S, Olafsdottir A, Kristinsson KT, Birgisdottir B, Ghosh S, Thorlacius S, Magnusdottir D, Stefansdottir G, Kristjansson K, Bagger Y, Wilensky RL, Reilly MP, Morris AD, Kimber CH, Adeyemo A, Chen Y, Zhou J, So W-Y, Tong PCY, Ng MCY, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Tres A, Fuertes F, Ruiz-Echarri M, Asin L, Saez B, van Boven E, Klaver S, Swinkels DW, Aben KK, Graif T, Cashy J, Suarez BK, van Vierssen Trip O, Frigge ML, Ober C, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Palmer CNA, Rotimi C, Chan JCN, Pedersen O, Sigurdsson G, Benediktsson R, Jonsson E, Einarsson GV, Mayordomo JI, Catalona WJ, Kiemeney LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet* 39:977–983, 2007
43. Groop L, Forsblom C, Lehtovirta M, Tuomi T, Karanko S, Nissen M, Ehrnstrom BO, Forsen B, Isomaa B, Snickars B, Taskinen MR: Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects. *Diabetes* 45:1585–1593, 1996
44. Lyssenko V, Almgren P, Anevski D, Perfekt R, Lahti K, Nissen M, Isomaa B, Forsen B, Homstrom N, Saloranta C, Taskinen M-R, Groop L, Tuomi T, for the Botnia Study Group: Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. *Diabetes* 54:166–174, 2005
45. Berglund G, Nilsson P, Eriksson K-F, Nilsson J-A, Hedblad B, Kristenson H, Lindgarde F: Long-term outcome of the Malmö Preventive Project: mortality and cardiovascular morbidity. *J Intern Med* 247:19–29, 2000
46. Eriksson K, Lindegarde F: Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise: the 6-year Malmö feasibility study. *Diabetiologia* 34:891–898, 1991
47. Tripathy D, Eriksson KF, Orho-Melander M, Fredriksson J, Ahlqvist G, Groop L: Parallel manifestation of insulin resistance and beta cell decompensation is compatible with a common defect in type 2 diabetes. *Diabetologia* 47:782–793, 2004
48. Liang K, Zeger S: Longitudinal data analysis using generalized linear models. *Biometrica* 73:13–22, 1986
49. Liang K, Zeger S: Regression analysis for correlated data. *Annu Rev Public Health* 14:43–68, 1993
50. Lyssenko V, Almgren P, Anevski D, Orho-Melander M, Sjogren M, Saloranta C, Tuomi T, Groop L, Botnia Study Group: Genetic prediction of future type 2 diabetes. *PLoS Med* 2:e345, 2005
51. Torres-Padilla ME, Fougere-Deschatrette C, Weiss MC: Expression of HNF4[alpha] isoforms in mouse liver development is regulated by sequential promoter usage and constitutive 3′ end splicing. *Mech Dev* 109:183–193, 2001
52. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI: Increased rate of gluconeogenesis in type II diabetes mellitus: A13C nuclear magnetic resonance study. *J Clin Invest* 90:1323–1327, 1992
53. Rissanen J, Saarinen L, Heikkinen S, Kekalainen P, Mykkänen L, Kuusisto J, Deeb S, Laakso M: Glucokinase gene islet promoter region variant (G→A) at nucleotide −30 is not associated with reduced insulin secretion in Finns. *Diabetes Care* 21:1194–1197, 1998