Impact of Common Type 2 Diabetes Risk Polymorphisms in the DESIR Prospective Study

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OBJECTIVE—The emerging picture of type 2 diabetes genetics involves differently assembled gene variants, each modestly increasing risk with environmental exposure. However, the relevance of these genes for disease prediction has not been extensively tested.

RESEARCH DESIGN AND METHODS—We analyzed 19 common polymorphisms of 14 known candidate genes for their contribution to prevalence and incidence of glucose intolerance in the DESIR (Data from an Epidemiological Study on the Insulin Resistance syndrome) prospective study of middle-aged Caucasian subjects, including 3,877 participants (16.8% with hyperglycemia and 7.9% with diabetes after the 9-year study).

RESULTS—The *GCK* (*Glucokinase*) −30A allele was associated with increased type 2 diabetes risk at the end of the follow-up study (adjusted OR 1.34 [95% CI 1.07–1.69]) under an additive model, as supported in independent French diabetic case subjects (OR 1.22, $P = 0.007$), with increased fasting glycemia (0.85% per A allele, $P = 6 \times 10^{-5}$) and decreased homeostasis model assessment of β-cell function (4%, $P = 0.0009$). *IL6* (*Interleukin-6*) −174 G/C interacts with age in disease risk and modulates fasting glycemia according to age (1.36% decrease over 56 years, $P = 5 \times 10^{-5}$). These polymorphisms together with *KCNJ11* (Kir6.2)-E23K and *TCF7L2*-rs7903146 may predict diabetes incidence in the DESIR cohort. Each additional risk allele at *GCK*, *TCF7L2*, and *IL6* increased risk by 1.34 ($P = 2 \times 10^{-6}$), with an OR of 2.48 (95% CI 1.59–3.86), in carriers of at least four at-risk alleles compared with those with none or one risk allele.

CONCLUSIONS—Our data confirm several at-risk polymorphisms for type 2 diabetes in a general population and demonstrate that prospective studies are valuable designs to complement classical genetic approaches. ***Diabetes*** **57:244–254, 2008**

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AUC, area under the curve; DESIR, Data from an Epidemiological Study on the Insulin Resistance syndrome; FPG, fasting plasma glucose; HOMA, homeostasis model assessment; HOMA-B, HOMA of β-cell function; HOMA-IR, HOMA of insulin resistance; IFG, impaired fasting glycemia; IL, interleukin; MAF, minor allele frequency; ROC, receiver operating characteristic; SNP, single nucleotide polymorphism

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Type 2 diabetes is a complex trait where common genetic variants having modest individual effects act together and interact with environmental factors to modulate the risk of the disease (1,2). Initial efforts to identify type 2 diabetes susceptibility genes favored the genome-wide linkage approach and candidate gene association studies (3). A few common polymorphisms have been widely replicated in populations of different ethnic descents (rev. in 4), including: *PPARG*-P12A (5), the E23K single nucleotide polymorphism (SNP) in *KCNJ11* coding for Kir6.2 (6,7), two intronic polymorphisms in the protease calpain-10 (*CAPN10*, SNP-43 and -44) (8), and more recently *TCF7L2* variants, which have the most important risk effect (9,10). An association of the G-30A polymorphism in the β-cell–specific promoter of glucokinase (*GCK*) was reported with elevated fasting glycemia and birth weight (11), gestational diabetes (12), impaired glucose regulation (13), and a higher prevalence of type 2 diabetes in patients with coronary artery disease (14). Common variation in adiponectin/*ADIPOQ* is associated with insulin sensitivity, type 2 diabetes, and vascular complications of obesity (15).

Other variants in candidate genes for type 2 diabetes that showed association in at least two independent studies include the G482S coding SNP of *PPARGC1A* (encoding PGC-1α) (16,17), SNPs at the distal P2 promoter of *HNF4A* (18–20), *UCP2* (uncoupling protein-2) G-866A promoter SNP (21,22), and variants in the transcription factor *SREBF1*, which associate with obesity and type 2 diabetes in French and Austrian populations (23,24), and in *ADIPOR2* (encoding adiponectin receptor 2) (25,26). Inflammatory processes also play a pivotal role in metabolic diseases, and high plasma interleukin-6 levels were associated with increased type 2 diabetes risk (27,28). A recent well-powered joint analysis of the *IL6* (G-174C) promoter variant, showing a weaker effect on *IL6* transcription, reported a decreased risk for type 2 diabetes (29). Association with type 2 diabetes was also found for *IL6R*-D358A in Danish whites (30) and *TNF* G-308A promoter SNP in the Finnish Diabetes Prevention Study (28).

The advent of genome-wide association studies, surveying ~75% of common variation across the human genome, promises to greatly speed up the identification of novel and replicated susceptibility genes (31–35). For future application it will be important to quantify the contribution of these risk alleles to overall diabetes risk. Case-control studies investigate one primary outcome, the disease by which cases are defined, whereas complex diseases require examining genetic and lifestyle risk factors and intermediary phenotypes related to the disease

(36). Prospective studies examine aspects of disease manifestations and the preclinical phase, such as impaired β-cell function (37,38), the conversion from impaired fasting glycemia (IFG) to type 2 diabetes (39,40), or the relationship with obesity, which is not constant over time (22,41). Recently, the T allele of the noncoding rs7903146 variant in *TCF7L2* was shown to be associated with an increased incidence of hyperglycemia in the French DESIR (Data from an Epidemiological Study on the Insulin Resistance syndrome) prospective study (38). In this population-based sample with a longitudinal follow-up of 9 years, we evaluated the contribution to type 2 diabetes risk at the end of the study and to diabetes incidence of 19 common SNPs, some of which were reproducibly associated with type 2 diabetes in case-control designs (Table 1).

TABLE 1
Allele and genotype frequencies of the 19 SNPs studied in the DESIR cohort

Gene	SNP	rs-ID	Alleles	MAF	11			12			22	
					NG	IFG	T2D	NG	IFG	T2D	NG	IFG	T2D
KCNJ11	E23K	rs5219	E/K	0.39	0.37 (994)	0.37 (224)	0.35 (101)	0.48 (1287)	0.48 (294)	0.48 (137)	0.15 (403)	0.15 (89)	0.17 (49)
CAPN10	SNP-44	rs2975760	T/C	0.16	0.72 (1985)	0.71 (434)	0.74 (219)	0.25 (675)	0.26 (160)	0.24 (71)	0.03 (94)	0.03 (21)	0.02 (5)
GCK	−30 G/A	rs1799884	G/A	0.18	0.68 (1875)	0.66 (396)	0.62 (181)	0.29 (795)	0.29 (177)	0.31 (90)	0.03 (81)	0.05 (29)	0.08 (23)
HNF4A	P2 C/T	rs1884614	C/T	0.16	0.70 (1989)	0.72 (446)	0.71 (215)	0.27 (759)	0.25 (156)	0.26 (80)	0.03 (86)	0.03 (21)	0.02 (7)
HNF4A	P2 G/A	rs2144908	G/A	0.37	0.39 (1115)	0.38 (245)	0.42 (127)	0.47 (1343)	0.49 (310)	0.46 (138)	0.14 (385)	0.13 (83)	0.12 (37)
UCP2	−866 G/A	rs659366	C/T	0.37	0.43 (1183)	0.43 (262)	0.43 (127)	0.44 (1222)	0.44 (270)	0.42 (124)	0.13 (360)	0.13 (78)	0.15 (46)
PPARGC1A	G482S	rs8192678	G/A	0.35	0.32 (898)	0.29 (181)	0.36 (106)	0.48 (1369)	0.51 (315)	0.47 (142)	0.20 (558)	0.20 (123)	0.17 (52)
PPARGC1A	−1422 T/C	rs2970870	T/C	0.44	0.36 (1019)	0.37 (229)	0.39 (118)	0.48 (1351)	0.45 (283)	0.47 (142)	0.16 (470)	0.18 (113)	0.14 (42)
IL6	−174 G/C	rs1800795	G/C	0.40	0.41 (1003)	0.33 (205)	0.32 (97)	0.46 (1311)	0.51 (313)	0.55 (166)	0.18 (499)	0.16 (102)	0.13 (38)
IL6R	D358A	rs8192284	A/C	0.41	0.39 (1115)	0.38 (245)	0.42 (127)	0.47 (1343)	0.49 (310)	0.46 (138)	0.14 (385)	0.13 (83)	0.12 (37)
TNF	−308 G/A	rs1800629	G/A	0.14	0.74 (2099)	0.73 (453)	0.76 (229)	0.24 (675)	0.24 (151)	0.22 (66)	0.02 (58)	0.03 (18)	0.02 (7)
SREBF1	G952G	rs2297508	C/G	0.38	0.41 (1115)	0.36 (212)	0.38 (113)	0.45 (1212)	0.47 (276)	0.47 (137)	0.14 (389)	0.17 (103)	0.15 (45)
ADIPOR2	+33,775	rs2286380	A/T	0.12	0.79 (2212)	0.80 (493)	0.80 (238)	0.20 (568)	0.18 (112)	0.19 (57)	0.01 (34)	0.02 (12)	0.01 (4)
ADIPOR2	I290I		C/A	0.12	0.79 (2175)	0.79 (488)	0.79 (235)	0.20 (564)	0.20 (114)	0.19 (58)	0.01 (33)	0.02 (12)	0.01 (4)
TCF7L2	rs7903146	rs7903146	C/T	0.30	0.51 (1400)	0.45 (283)	0.43 (128)	0.40 (1121)	0.46 (284)	0.44 (133)	0.09 (236)	0.09 (57)	0.13 (40)
PPARG	P12A	rs1801282	P/A	0.11	0.79 (2296)	0.82 (532)	0.81 (249)	0.20 (587)	0.17 (111)	0.19 (58)	0.01 (25)	0.01 (6)	0.000 (0)
ADIPOQ	−11391G/A	rs17300539	G/A	0.10	0.81 (2358)	0.80 (520)	0.797 (245)	0.18 (510)	0.19 (123)	0.20 (61)	0.01 (32)	0.01 (6)	0.003 (1)
ADIPOQ	−11377C/G	rs266729	C/G	0.26	0.54 (1578)	0.55 (355)	0.55 (168)	0.38 (1106)	0.39 (253)	0.40 (121)	0.08 (218)	0.06 (38)	0.05 (16)
ADIPOQ	G15G	rs2241766	T/G	0.13	0.75 (2198)	0.75 (490)	0.74 (228)	0.23 (668)	0.23 (148)	0.24 (73)	0.02 (44)	0.02 (12)	0.02 (5)

Allele 1, as major allele; allele 2, as minor allele. The frequency of each genotype class is given according to status, with the number of individuals in parentheses. All genotype distributions are in Hardy-Weinberg equilibrium ($P > 0.05$). NG, normoglycemia; T2D, type 2 diabetes.

RESEARCH DESIGN AND METHODS

A cohort of 2,576 men and 2,636 women from a general population (aged 30–65 years at inclusion) participated in the DESIR longitudinal study and were clinically and biologically evaluated at inclusion, at 3-, 6-, and 9-year visits (42,43) (supplementary Table 1 [available in an online appendix at http://dx.doi.org/10.2337/db07-0615). Participants gave informed consent, and the protocol was approved by the ethics committee of Bicêtre Hospital.

Patients were categorized into three classes of glycemic status defined according to 1997 American Diabetes Association criteria: normoglycemia, defined as fasting plasma glucose (FPG) <6.1 mmol/l without hypoglycemic treatment; IFG, defined as FPG 6.1–6.99 mmol/l without hypoglycemic treatment; and type 2 diabetes, defined as FPG ≥7.0 mmol/l and/or treatment by antidiabetic agents.

Among the 5,212 subjects of the DESIR cohort, 3,877 individuals who were followed during the entire study entered the prevalence analysis at the end of the 9-year study: 2,919 normoglycemic individuals at all points of the study, 651 with IFG at least at one point during the study, and 307 with diabetes (including 120 diabetic individuals at baseline and 187 incident case subjects). Altogether, 320 individuals whose European ancestry was not established (supplemental online appendix 1) and 1,015 individuals for whom the glycemic status was not known at the end of the study were excluded from the analysis. A total of 3,442 individuals entered the incidence analysis, among whom 2,919 were censored at the end of the study and 523 were incident cases: 336 case subjects were incident for IFG and 187 for diabetes.

An additional 2,215 unrelated diabetic patients of French European ancestry were ascertained from the French family study in Lille and from the Endocrinology-Diabetology Department of the Corbeil-Essonnes Hospital, as previously described (44). The normoglycemic control subjects included 2,251 unrelated subjects (FPG <5.6. mmol/l and BMI <30 kg/m^2) selected from the current DESIR cohort. A total of 4,466 subjects, all of French Caucasian origin, were investigated for association between the *GCK* (G-30A) promoter variant and type 2 diabetes.

SNP genotyping. Two technologies were used: SNPlex and allelic discrimination TaqMan assay (Applied Biosystems, Foster City, CA) (supplemental online appendix 2). These two methods achieved 94–97% of successful genotyping in the whole cohort sample. Duplicate samples were assayed with a concordance rate ≥99%. The genotype distribution of all polymorphisms was as expected from Hardy-Weinberg equilibrium ($P > 0.05$).

Statistical analyses. Multivariate logistic regression models were used in the prevalence association study (hyperglycemia and diabetes at baseline and at the end of the 9-year study). Models included sex, age at the end of the study, and mean BMI as covariates based on all available measures during the study. A nonlinear term for age was used.

Cox proportional hazards regression models were used to estimate the genotype effect on the incidence of diabetes. Models included sex and mean BMI as covariates based on all available measures for censored individuals or for all available measures before diagnosis for incident cases. Survival time was age. Kaplan-Meier survival curves are given for the variants showing significant effects on incidence. Interaction with age on disease prevalence and on fasting glucose was tested when a genotype effect according to age was seen graphically.

In the diabetes prevalence analysis, we had a power of 75, 87, 92, and 97% for a minor allele frequency (MAF) of 0.10, 0.15, 0.20, and ≥0.30, respectively, to detect an odds ratio (OR) ≥1.35 (for $\alpha = 0.05$, additive model); for variants with MAF ≥0.35, we had 80% power to detect genotype effects with an OR of 1.25. In the incidence analysis, the power to detect hazard ratios (HRs) <1.5 is weaker (<70%) because of the small number of incident cases.

TABLE 2
Association with hyperglycemia and type 2 diabetes prevalence at the end of the 9-year follow-up study

				IFG + type 2 diabetes			Type 2 diabetes		
Gene	SNP	rs-ID	Allele	Model	*P*	OR (95% CI)	Model	*P*	OR (95% CI)
KCNJ11	E23K	rs5219	C/T	add	0.6	1.04 (0.92–1.17)	add	0.2	1.15 (0.95–1.40)
CAPN10	SNP-44	rs2975760	T/C	add	0.6	0.96 (0.82–1.11)	add	0.07	0.79 (0.61–1.02)
				rec	0.1	0.68 (0.42–1.08)	rec	0.02	0.31 (0.12–0.82)
GCK	−30 G/A	rs1799884	G/A	add	0.007	1.22 (1.06–1.41)	add	0.01	1.34 (1.07–1.69)
				rec	0.0002	2.12 (1.42–3.16	rec	0.0008	2.70 (1.51–4.83)
				dom	0.09	1.16 (0.98–1.37)	dom	0.1	1.25 (0.95–1.65)
HNF4A	P2 C/T	rs1884614	C/T	add	0.4	0.94 (0.81–1.09)	add	0.5	0.92 (0.72–1.18)
HNF4A	P2 G/A	rs2144908	G/A	add	0.5	0.95 (0.82–1.11)	add	0.8	0.96 (0.75–1.23)
UCP2	−866 G/A	rs659366	C/T	add	1	1.00 (0.89–1.12)	add	0.6	0.95 (0.79–1.16)
PPARGC1A	G482S	rs8192678	G/A	add	0.8	0.98 (0.87–1.11)	add	0.9	1.01 (0.83–1.22)
PPARGC1A	−1422 T/C	rs2970870	T/C	add	0.6	0.97 (0.87–1.09)	add	0.09	0.85 (0.70–1.03)
IL6	−174 G/C	rs1800795	G/C	add	0.2	0.92 (0.82–1.03)	add	0.01	0.79 (0.65–0.96)
							rec	0.07	0.71 (0.49–1.03)
							dom	0.03	0.75 (0.57–0.98)
IL6R	D358A	rs8192284	A/C	add	0.7	0.97 (0.87–1.09)	add	0.4	0.92 (0.76–1.12)
				rec	0.07	0.82 (0.66–1.02)	rec	0.04	0.67 (0.46–0.98)
TNF	−308 G/A	rs1800629	G/A	add	0.7	1.03 (0.88–1.21)	add	0.6	0.93 (0.71–1.21)
SREBF1	G952G	rs2297508	C/G	add	0.003	1.20 (1.07–1.35)	add	0.2	1.14 (0.94–1.38)
				rec	0.006	1.36 (1.09–1.70)	rec	0.09	1.38 (0.96–1.99)
ADIPOR2	+33,775	rs2286380	A/T	add	0.5	0.94 (0.78–1.12)	add	0.2	0.81 (0.60–1.10)
ADIPOR2	I290I		C/A	add	0.7	0.96 (0.80–1.15)	add	0.3	0.85 (0.62–1.15)
TCF7L2		rs7903146	C/T	add	0.0004	1.24 (1.10–1.41)	add	0.0002	1.45 (1.20–1.77)
				rec	0.07	1.28 (0.98–1.68)	rec	0.003	1.84 (1.23–2.75)
				dom	0.0004	1.34 (1.14–1.57)	dom	0.001	1.54 (1.18–2.00)
PPARG	P12A	rs1801282	P/A	add	0.07	0.84 (0.69–1.01)	add	0.4	0.88 (0.64–1.20)
ADIPOQ	−11391G/A	rs17300539	G/A	add	0.2	1.14 (0.95–1.37)	add	0.3	1.19 (0.88–1.61)
			G/A	dom	0.1	1.18 (0.97–1.44)	dom	0.2	1.25 (0.90–1.73)
ADIPOQ	−11377C/G	rs266729	C/G	add	0.3	0.94 (0.82–1.06)	add	0.4	0.92 (0.74–1.13)
			C/G	rec	0.08	0.75 (0.54–1.04)	rec	0.2	0.71 (0.40–1.26)
ADIPOQ	G15G	rs2241766	T/G	add	0.8	0.98 (0.83–1.16)	add	0.9	1.01 (0.77–1.32)

The prevalence study refers to all diabetic case subjects (n = 307) and hyperglycemic case subjects (n = 651) when the IFG status was analyzed, both at baseline and at the end of the study after the follow-up of 9 years. Individual SNP ORs are shown from a logistic regression model with adjustment for sex, age, and BMI. All results under an additive model are shown and those under other genetic models when significant. The *P* values indicated are nominal *P* values. add, additive; dom, dominant; rec, recessive.

Mixed models were used to estimate the genotype effects on five quantitative traits after log transformation. Repeated measures of glycemia, insulinemia, and the derived indexes homeostasis model assessment (HOMA) of β-cell function (HOMA-B) and of insulin resistance (HOMA-IR) (supplemental online appendix 3) were analyzed in normoglycemic individuals (including >12,000 observations) and BMI in all individuals. Models included, in addition to genotype variable, sex, age, and BMI as fixed effects, a random intercept, and a random time slope for each individual.

Gene-gene interactions were assessed using a logistic regression model including a variable for the number of at-risk alleles in order to quantify the risk per supplementary allele for a given set of variants. To assess gene-covariate interactions, a likelihood ratio was used for comparison of the model including the main effects with the model including the main effects and an interaction term. All genetic models were considered. To test age-genotype interaction, each interaction term was coded as the product of the genotype variable and two-class covariates. Ages were grouped into ≤56.0 and >56.0 years, since 56.0 was the median age at the end of the study.

Receiver operating characteristic (ROC) curves and areas under the curve (AUCs) were computed using logistic regression models to estimate the discriminative accuracy of multiple genetic testing. When combining three at-risk variants, three ROC curves were plotted by progressively adding each variant to the classification model.

Two-sided P values <0.05 were considered statistically significant. A permutation procedure was applied to correct for multiple testing (supplemental online appendix 4). All statistical analyses were performed using R (version 2.4.0) combined with mgcv, survival, and nlme packages (http://www.R-project.org).

RESULTS

Allele and genotype frequencies of the 19 SNPs (shown in Table 1), are in concordance with those previously reported in subjects of European ancestry, and all genotype groups were in Hardy-Weinberg equilibrium ($P > 0.05$).

Association between hyperglycemia and type 2 diabetes at the end of the 9-year study. In this middle-aged cohort, the prevalence of IFG (FPG 6.1–6.99 mmol/l) and type 2 diabetes at the end of the 9-year follow-up was 16.8 and 7.9%, respectively. The genotype frequencies for the 19 polymorphisms are shown in Table 1.

A significant association was observed between hyperglycemia and the *GCK* −30G/A variant in the whole group of IFG plus diabetic individuals (altogether 958 subjects) both at baseline and at the end of the study. The OR for hyperglycemia was 1.22 (95% CI 1.06–1.41) under an additive model ($P = 0.007$; Table 2). A stronger effect was found in individuals with diabetes only, and both the MAF (0.23 vs. 0.17) and genotype distribution differed significantly between case and control subjects with an OR of 1.34 (1.07–1.69) ($P_{\text{additive}} = 0.01$); this is supported by a recessive model (OR 2.70 [95% CI1.51–4.83], $P = 0.0008$) (Table 2). The population-attributable risk of developing type 2 diabetes, due to the A at-risk allele of *GCK* (−30G/A), is estimated to be 9% in this cohort.

The T allele at rs7903146 in *TCF7L2* was also found to be significantly associated with type 2 diabetes, as recently supported by our previous study (38), providing an OR of 1.45 (95% CI 1.20–1.77) ($P_{\text{additive}} = 0.0002$). In contrast, two variants provided a decreased risk for type 2 diabetes: *IL6* −174 G/C (OR 0.75 [95% CI 0.57–0.98]; $P_{\text{additive}} = 0.03$) and *IL6R*-D358A (0.67 [0.46–0.98]; $P_{\text{recessive}} = 0.04$). *SREBF1*-G952G was significantly associated with hyperglycemia (both IFG and type 2 diabetes, with an OR of 1.36 ($P_{\text{recessive}} = 0.006$; Table 2), as previously shown in a case-control study of the French population (23), and showed interaction with age for both diabetes only ($P_{\text{recessive}} = 0.005$) and diabetes plus IFG ($P_{\text{recessive}} = 5 \times 10^{-5}$). Trends for association with type 2 diabetes were seen for *CAPN10* SNP-44 and *PPARGC1A* −1,422 T/C variants ($P < 0.1$). The other 12 SNPs analyzed did not show any significant association, whatever the genetic model tested.

Association of the *GCK* (−30G/A) promoter variant in a larger case-control analysis. We further analyzed the effect of *GCK* −30G/A in an independent sample of 2,215 diabetic subjects compared with 2,251 normoglycemic control subjects selected from the current DESIR study (based on FPG <5.6 mmol/l throughout the study). In support of the above data, the A allele frequency was 0.19 in diabetic case subjects compared with 0.17 in control subjects ($P = 0.006$), and the A allele carriers had a 1.22 (95% CI 1.06–1.42)-fold risk of diabetes ($P_{\text{additive}} = 0.007$). A similar effect was observed when a separate analysis was done for the subgroup of patients with an affected first-degree relative (1,316 diabetic subjects, $P_{\text{additive}} = 0.014$).

When combining this independent case-control study of 4,466 samples with two previously reported European studies from Denmark (13) and Germany (14) (P for heterogeneity = 0.72), we found a very significant association between the *GCK* (−30) A allele and diabetes, with an overall OR of 1.22 (95% CI 1.13–1.32) ($P_{\text{additive}} = 10^{-6}$; after adjustment for age and sex using the Mantel-Haenszel analysis of fixed effects).

Association with hyperglycemia and type 2 diabetes incidence in the follow-up study. A total of 3,442 individuals were eligible for the incidence analysis, including 187 incident diabetic case subjects and 336 case subjects showing IFG over the 9-year follow-up.

Using Cox proportional hazards models, the *GCK* −30A allele was shown to be significant for diabetes incidence (HR 1.34 [95% CI 1.04–1.74], $P_{\text{additive}} = 0.03$, and 2.39 [1.30–4.42] for the AA genotype, $P_{\text{recessive}} = 0.005$) and hyperglycemia (both IFG and diabetic case subjects) with an HR of 1.26 and 2.17 under additive and recessive models, respectively ($P \leq 0.005$) (Table 3). Both *KCNJ11*-E23K and *IL6* −174G/C SNPs were associated with risk of type 2 diabetes ($P_{\text{additive}} < 0.01$, Table 3). The *IL6R*-D358A variant showed a borderline recessive effect (HR 0.61, $P_{\text{recessive}} = 0.04$), which is supported by a small number of homozygous AA carriers.

The individual effects of *GCK* −30G/A, *KCNJ11*-E23K, *IL6* (−174G/C), and *IL6R*-D358A variants on the risk of developing type 2 diabetes in the DESIR population are shown in Fig. 1. The Kaplan-Meier survival curves for *IL6* −174G/C and *IL6R*-D358A showed a marked effect on incidence after 56.0 years. An interaction between age and genotype was found for *IL6* −174G/C, with a stronger protective effect of the minor C allele in individuals aged >56.0 years ($P < 0.01$). Similarly, *KCNJ11*-E23K, showing an association with incidence but not with overall diabetes risk, could interact with age (since a borderline significant interaction was detected). Taking into account the association with type 2 diabetes, 5 of 19 SNPs tested were significant ($P < 0.05$), which is different from the expected proportion of replication by random effects (one-sided $P = 0.004$).

Genetic effects on quantitative metabolic parameters. The *GCK* (−30) A allele significantly modulated fasting glycemia over the 9-year study, showing an increase of 0.85% (0.44–1.27) per supplementary A allele ($P_{\text{additive}} = 6 \times 10^{-5}$, after permutations $P_{\text{corrected}} = 0.01$), and HOMA-B with a 4% decrease ($P_{\text{additive}} = 0.002$), with all other variables considered with a fixed effect (Table 4). No overall genotype effect of *IL6* −174G/C was apparent on glycemia levels over time, whereas an age-genotype

TABLE 3
Association with the risk of developing hyperglycemia and type 2 diabetes during the 9-year follow-up study

Gene	SNP	rs-ID	Allele	IFG + type 2 diabetes			Type 2 diabetes		
				Model	*P*	HR (95% CI)	Model	*P*	HR (95% CI)
KCNJ11	E23K	rs5219	C/T	add	0.4	1.06 (0.93–1.20)	add	0.009	1.34 (1.08–1.68)
							rec	0.004	1.74 (1.20–2.53)
							dom	0.1	1.30 (0.94–1.80)
CAPN10	SNP-44	rs2975760	T/C	add	0.1	0.88 (0.74–1.04)	add	0.6	0.93 (0.71–1.23)
GCK	−30 G/A	rs1799884	G/A	add	0.005	1.26 (1.07–1.47)	add	0.03	1.34 (1.04–1.74)
				rec	0.0001	2.17 (1.47–3.20)	rec	0.005	2.39 (1.30–4.42)
				dom	0.07	1.19 (0.99–1.43)	dom	0.1	1.28 (0.94–1.74)
HNF4A	P2 C/T	rs1884614	C/T	add	0.2	0.90 (0.76–1.07)	add	0.8	1.03 (0.78–1.35))
HNF4A	P2 G/A	rs2144908	G/A	add	0.3	0.92 (0.78–1.09)	add	0.6	1.07 (0.82–1.40)
UCP2	−866 G/A	rs659366	C/T	add	1	1.00 (0.88–1.14)	add	0.6	0.94 (0.75–1.18)
PPARGC1A	G482S	rs8192678	G/A	add	0.8	0.98 (0.86–1.12)	add	0.5	1.07 (0.87–1.32)
PPARGC1A	−1422 T/C	rs2970870	T/C	add	0.5	0.95 (0.84–1.08)	add	0.2	0.86 (0.70–1.06)
IL6	−174 G/C	rs1800795	G/C	add	0.3	0.94 (0.86–1.12)	add	0.002	0.70 (0.57–0.88)
							rec	0.02	0.58 (0.36–0.92)
							dom	0.006	0.66 (0.49–0.89)
IL6R	D358A	rs8192284	A/C	add	0.9	1.00 (0.89–1.14)	add	0.2	0.87 (0.70–1.07)
							rec	0.04	0.61 (0.38–0.97)
TNF	−308 G/A	rs1800629	G/A	add	0.7	1.04 (0.88–1.23)	add	0.8	0.96 (0.72–1.28)
SREBF1	G952G	rs2297508	C/G	add	0.2	1.10 (0.96–1.25)	add	0.8	0.97 (0.77–1.22)
ADIPOR2	+33,775	rs2286380	A/T	add	0.3	0.90 (0.74–1.09)	add	0.2	0.80 (0.58–1.10)
ADIPOR2	I290I		C/A	add	0.4	0.92 (0.76–1.12)	add	0.2	0.82 (0.60–1.13)
TCF7L2		rs7903146	C/T	add	0.002	1.23 (1.08–1.40)	add	0.003	1.39 (1.12–1.73)
				rec	0.3	1.17 (0.87–1.58)	rec	0.007	1.81 (1.17–2.78)
				dom	0.0008	1.35 (1.14–1.61)	dom	0.02	1.41 (1.05–1.90)
PPARG	P12A	rs1801282	P/A	add	0.3	0.89 (0.72–1.11)	add	1	1.00 (0.70–1.42)
ADIPOQ	−11391G/A	rs17300539	G/A	add	0.09	1.19 (0.98–1.44)	add	0.3	1.18 (0.84–1.66)
			G/A	dom	0.06	1.23 (1.00–1.52)	dom	0.3	1.21 (0.84–1.73)
ADIPOQ	−11377C/G	rs266729	C/G	add	0.1	0.90 (0.78–1.03)	add	0.2	0.86 (0.68–1.09)
ADIPOQ	G15G	rs2241766	T/G	add	0.4	1.07 (0.90–1.28)	add	0.09	1.29 (0.97–1.72)

In the incidence analysis, a Cox proportional hazards model was used including 2,919 normoglycemic individuals who remained normoglycemic at each examination during the 9-year follow-up study (those who were censored at the end of the study) and 523 incident case subjects who developed IFG ($n = 336$) or diabetes ($n = 187$) during follow-up visits. All results under an additive model are shown and those under other genetic models when significant. The *P* values indicated are nominal *P* values. add, additive; dom, dominant; rec, recessive.

interaction was found when analyzing normoglycemic and IFG individuals together ($P_{\text{additive}} = 0.009$) (Table 5). This effect was significant for age >56.0, explaining the 1.36% decrease of glycemia per supplementary C allele ($P_{\text{additive}} = 5 \times 10^{-5}$; Table 5). The interaction between age and *IL6R*-D358A genotype for association to fasting glycemia was also significant for all genetic models ($P_{\text{additive}} = 0.003$), with the inverse effects on glycemia depending on age (below or above 56.0 years) (Table 5).

Modest effects on fasting insulin level and HOMA indexes were observed for *PPARG*-P12A (Table 4). Both *HNF4A* rs2144908 and rs1884614 variants modulated insulin level ($P = 0.004$ under a dominant model), HOMA-B, and HOMA-IR (12 and 10% decrease, respectively, in homozygous carriers of the minor alleles, $P \leq 0.01$) (Table 4). Insulin level, HOMA-B, and HOMA-IR were highly correlated ($r^2 > 0.70$) in the study sample. A weak effect on BMI was only observed for *UCP2* −866 G/A (Table 4).

Gene-gene interactions and combined genetic effects. Pairwise interactions for all genotype combinations were tested for each SNP pair where the number of genotype pairs within disease status (IFG and type 2 diabetes) was ≥15. Potential interactions were found between *KCNJ11-E23K* and *HNF4A* rs2144908 for association with diabetes (*P* for interaction = 0.0012) and *IL6* (−174G/C)-*SREBF1*-G952G for association with IFG and diabetes (*P* for interaction = 0.0019). However, no *P* values remained significant after stringent Bonferroni correction, as 253 combinations of SNPs were tested.

An exploratory study was conducted to assess potential effects of multiple variants on diabetes and IFG at the end of the follow-up. The most significant gene combination was *GCK* −30G/A, *IL6* −174G/C, and *TCF7L2*-rs7903146 (combination 1): Each additional risk allele increased diabetes risk by 1.34 (95% CI 1.18–1.51, $P = 2 \times 10^{-6}$), with an OR of 2.48 (1.59–3.86) for carriers of at least four risk alleles compared with those with no or one risk allele ($P = 6 \times 10^{-5}$) (Fig. 2*A* and supplementary Table 2). When considering both diabetes and IFG, the most significant effect was for *GCK*, *SREBF1*-G952G, and *TCF7L2* (combination 2) (OR 1.21 [95% CI 1.12–1.31], $P = 6 \times 10^{-7}$, per supplementary allele and 2.16 [1.54–3.03] for carriers of at least four risk alleles compared with carriers of no or one risk allele, $P = 8 \times 10^{-6}$) (Fig. 2*B* and supplementary Table 2). The population-attributable risk for at least three risk alleles versus less than three was estimated to be 27% for combination 1 and 14% for combination 2. ROC curves were computed for the two gene combinations, and the area under the ROC curve, as a measure of the discriminative power of the test, was calculated for both combinations by progressively adding the three variants into the model. The AUC values were 0.56 and 0.55 when including only the genetic variants into the classification model (Fig. 3), which is significantly different from the null hypothesis

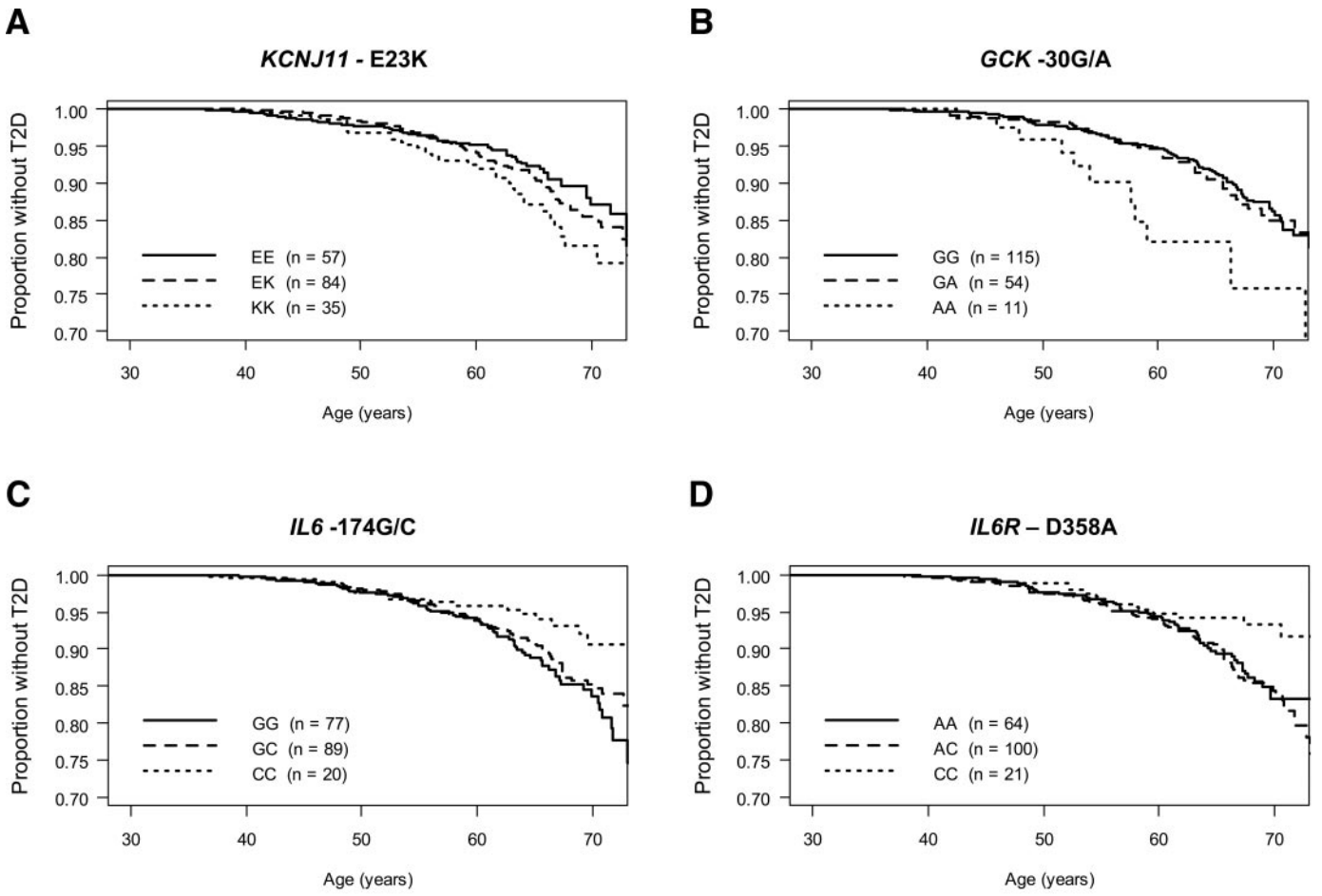

FIG. 1. Type 2 diabetes incidence in the DESIR cohort according to genotype for the *KCNJ11*-E23K (*A*), *GCK* −30G/A (*B*), *IL6* (*C*), and *IL6R* (*D*) polymorphisms. For each polymorphism, the Kaplan-Meier survival curves show the proportion of subjects without type 2 diabetes during the follow-up study depending on the age and according to genotype class. The time scale is represented by age (as a continuous scale). The number of type 2 diabetes incident cases (*n*) is indicated by genotype class along the follow-up study.

($P = 0.01$). Of note, the genetic information did not enhance the ability to predict future cases compared with using both genetic and conventional factors (i.e., age, sex, and BMI; $P = 0.26$ and 0.98 for combinations 1 and 2, respectively) (supplementary Fig. 2).

KCNJ11-E23K, *TCF7L2*, and *PPARG*-P12A, already shown to have a combined effect on type 2 diabetes prevalence (45), provided an OR of 1.27 (95% CI 1.11–1.45) ($P = 4 \times 10^{-4}$) by each additional risk allele.

DISCUSSION

The key new findings of our study are that four gene variants, Kir6.2-E23K, *GCK* −30G/A, *IL6* −174G/C, and *IL6R*-D358A, in addition to *TCF7L2*-rs7903146 (38), are associated with risk of developing type 2 diabetes in the DESIR prospective cohort. Furthermore, the significant association of *GCK* (−30A) promoter SNP with increased fasting glycemia and type 2 diabetes in both DESIR and case-control studies of North European origin extends previous reports (11) confirming that a common variant in the *MODY2* gene modulates glucose homeostasis later in life and may also predict diabetes risk in the general population.

The overall relevance of these findings for defining subgroups with higher risk for the disease must be considered with caution, given the limitations in such multiple analyses. A few studies have examined the impact of only a limited number of gene variants on the conversion to type 2 diabetes in interventional trials, such as the Finnish Diabetes Prevention Study (28,39), the STOP-NIDDM trial (40), and the Botnia prospective study (41). In the latter report, four gene variants were common with those analyzed in our study: *CAPN10*-SNP44, *UCP2* −866G/A, and *PPARG*-P12A showed association with type 2 diabetes in the Finnish population (HR 1.4–1.7), but *KCNJ11*-E23K did not. This SNP previously associated in multiple studies (4,7,45) showed a significant effect on type 2 diabetes incidence in our dataset (HR 1.34, $P = 0.009$) but not with the overall prevalence (both at baseline and at the end of the study), as this was reported in two previous prospective studies (39,41). The Botnia and DESIR studies may differ in their genetic background, as well as in lifestyle and clinical features. Notably, the Botnia family-based study was carried out in a high-risk population of first-degree relatives of patients with type 2 diabetes (41), which may increase its statistical power to detect small genetic effects but may be less representative of a general population. In this regard, the recent FUSION study, which investigated >100 SNPs putatively associated with type 2 diabetes through a staged case-control design (46), highlighted reasons for discordant effects among heterogeneous populations.

Although nominally significant effects on type 2 diabetes risk are seen for several variants from our study, they did

TABLE 4
Effect of individual genotypes on quantitative traits using mixed models

			BMI		Fasting glucose		Fasting insulin		HOMA-B		HOMA-IR	
Gene	SNP	Model	*P*	Δ (95% CI)	*P*	Δ (95% CI)	*P*	Δ (95% CI)	*P*	Δ (95% CI)	*P*	Δ (95% CI)
KCNJ11	E23K	add	0.5	−0.00 (−0.01 to 0.01)	0.7	−0.07 (−0.40 to 0.26)	0.4	−0.73 (−2.61 to 1.18)	0.8	−0.23 (−2.23 to 1.82)	0.4	−0.84 (−2.82 to 1.18)
CAPN10	SNP-44	add	0.8	−0.00 (−0.02 to 0.01)	1	0.00 (−0.42 to 0.42)	0.7	−0.50 (−2.90 to 1.95)	0.7	−0.44 (−2.96 to 2.15)	0.7	−0.50 (−3.03 to 2.11)
GCK	−30 G/A	add	0.3	−0.01 (−0.02 to 0.01)	6.10^{-5}	0.85 (0.44 to 1.27)	0.6	−0.67 (−3.02 to 1.73)	0.002	−4.00 (−6.40 to −1.54)	0.9	0.24 (−2.28 to 2.83)
		dom	0.6	0.00 (−0.02 to 0.01)	0.0002	0.90 (0.42 to 1.39)	0.4	−1.12 (−3.82 to 1.66)	0.0009	−4.82 (−7.56 to −2.00)	0.9	−0.13 (−3.02 to 2.85)
		rec	0.1	−0.03 (−0.08 to 0.01)	0.01	1.67 (0.38 to 2.98)	0.7	1.59 (−5.64 to 9.37)	0.4	−3.59 (−10.87 to −4.28)	0.4	3.29 (−4.55 to 11.77)
HNF4A	P2 C/T	add	0.5	0.00 (−0.02 to 0.01)	0.9	0.02 (−0.39 to 0.43)	0.004	−3.49 (−5.79 to −1.13)	0.003	−3.81 (−6.23 to −1.32)	0.009	−3.34 (−5.79 to −0.83)
		dom	0.7	0.00 (−0.02 to 0.01)	0.7	−0.10 (−0.58 to 0.38)	0.02	−3.24 (−5.92 to −0.49)	0.02	−3.36 (−6.18 to −0.46)	0.04	−3.07 (−5.91 to −0.15)
		rec	0.3	−0.02 (−0.07 to 0.02)	0.2	0.89 (−0.39 to 2.19)	0.006	−9.99 (−16.44 to −3.04)	0.001	−12.04 (−18.71 to −4.81)	0.01	−9.84 (−16.71 to −2.40)
HNF4A	P2 G/A	add	0.6	0.00 (−0.02 to 0.01)	1	0.00 (−0.41 to 0.41)	0.004	−3.51 (−5.79 to −1.16)	0.004	−3.62 (−6.04 to −1.15)	0.01	−3.29 (−5.72 to −0.79)
		dom	0.7	0.00 (−0.02 to 0.01)	0.7	−0.11 (−0.59 to 0.37)	0.02	−3.23 (−5.89 to −0.49)	0.04	−3.12 (−5.94 to −0.22)	0.05	−2.94 (−5.77 to −0.02)
		rec	0.5	−0.02 (−0.06 to 0.03)	0.2	0.78 (−0.48 to 2.06)	0.004	−10.20 (−16.56 to −3.35)	0.001	−11.93 (−18.54 to −4.78)	0.007	−10.16 (−16.92 to −2.84)
UCP2	−866 G/A	add	0.04	0.01 (0.00–0.02)	0.3	0.18 (−0.15 to 0.50)	0.3	1.05 (−0.82 to 2.96)	1	−0.00 (−1.98 to 2.01)	0.2	1.21 (−0.78 to 3.24)
		dom	0.05	0.02 (0.00–0.03)	0.3	0.25 (−0.20 to 0.71)	0.7	0.60 (−1.99 to 3.25)	0.7	−0.49 (−3.21 to 2.31)	0.5	0.95 (−1.79 to 3.78)
PPARGC1A	G482S	add	0.8	0.00 (−0.01 to 0.01)	0.1	−0.26 (−0.59 to 0.06)	0.7	−0.32 (−2.19 to 1.58)	0.3	1.10 (−0.90 to 3.15)	0.7	−0.39 (−2.37 to 1.63)
		rec	0.8	0.00 (−0.03 to 0.02)	0.06	−0.62 (−1.28 to 0.04)	0.7	0.64 (−3.07 to 4.50)	0.04	4.34 (0.17–8.67)	0.9	0.17 (−3.84 to 4.35)
PPARGC1A	−1422 T/C	add	0.8	0.00 (−0.01 to 0.01)	0.7	−0.06 (−0.37 to 0.25)	0.7	−0.33 (−2.12 to 1.49)	0.4	0.88 (−0.97 to 2.77)	0.9	−0.16 (−2.06 to 1.77)
IL6	−174 G/C	add	0.6	0.00 (−0.01 to 0.01)	0.1	−0.23 (−0.54 to 0.08)	0.2	−1.07 (−2.86 to 0.76)	0.3	−0.93 (−2.83 to 1.00)	0.3	−1.12 (−3.02 to 0.82)
		rec	0.1	−0.02 (−0.04 to 0.00)	0.07	−0.55 (−1.14 to 0.04)	0.5	−1.20 (−4.53 to 2.25)	0.7	−0.63 (−4.18 to 3.04)	0.4	−1.55 (−5.07 to 2.10)
IL6R	Asp358A	add	0.4	−0.00 (−0.02 to 0.01)	0.7	−0.06 (−0.37 to 0.26)	0.8	−0.18 (−1.98 to 1.66)	0.8	−0.28 (−2.18 to 1.66)	0.7	−0.41 (−2.31 to 1.53)
TNF	−308 G/A	add	0.7	0.00 (−0.01 to 0.02)	0.3	0.21 (−0.23 to 0.65)	0.9	−0.24 (−2.77 to 2.35)	0.3	−1.32 (−3.96 to 1.38)	0.9	−0.10 (−2.78 to 2.65)
SREBF1	G952G	add	0.1	−0.01 (−0.02 to 0.00)	0.6	−0.09 (−0.42 to 0.23)	0.2	1.22 (−0.67 to 3.15)	0.05	2.01 (−0.01 to 4.08)	0.4	0.93 (−1.07 to 2.97)
		dom	0.2	−0.01 (−0.02 to 0.01)	0.3	−0.24 (−0.69 to 0.22)	0.3	1.46 (−1.21 to 4.20)	0.03	3.13 (0.26–6.07)	0.5	1.01 (−1.80 to 3.90)
ADIPOR2	3′UTR A/T	add	0.3	0.01 (−0.01 to 0.02)	0.08	0.45 (−0.05 to 0.94)	0.7	0.54 (−2.29 to 3.44)	0.4	−1.21 (−4.14 to 1.80)	0.5	0.99 (−2.03 to 4.09)
		rec	0.5	−0.02 (−0.08 to 0.04)	0.3	1.09 (−0.85 to 3.07)	0.07	10.99 (−0.81 to 24.18)	0.2	7.60 (−4.43 to 21.14)	0.04	13.26 (0.51 to 27.63)
ADIPOR2	I290I	add	0.2	0.01 (−0.01 to 0.03)	0.07	0.45 (−0.04 to 0.95)	0.6	0.78 (−2.08 to 3.72)	0.5	−1.14 (−4.09 to 1.90)	0.4	1.20 (−1.84 to 4.34)
		rec	0.6	−0.02 (−0.08 to 0.05)	0.2	1.24 (−0.73 to 3.25)	0.07	11.22 (−0.77 to 24.65)	0.2	7.76 (−4.46 to 21.55)	0.04	13.61 (0.64 to 28.26)
TCF7L2		add	0.4	0.00 (−0.01 to 0.02)	0.8	0.05 (−0.29 to 0.40)	0.03	−2.19 (−4.13 to 0.20)	0.03	−2.31 (−4.36 to −0.21)	0.06	−1.99 (−4.06 to 0.12)
		dom	0.8	0.00 (−0.01 to 0.02)	0.6	0.12 (−0.32 to 0.57)	0.09	−2.24 (−4.74 to 0.32)	0.05	−2.64 (−5.27 to 0.05)	0.2	−1.97 (−4.62 to 0.76)
PPARG	P12A	add	0.9	0.00 (−0.02 to 0.02)	0.2	−0.32 (−0.83 to 0.19)	0.4	−1.14 (−4.00 to 1.80)	0.9	−0.13 (−3.19 to 3.03)	0.3	−1.65 (−4.66 to 1.45)
		rec	0.7	−0.02 (−0.10 to 0.06)	0.4	−0.98 (−3.37 to 1.48)	0.02	18.22 (2.73 to 36.06)	0.03	18.61 (2.08–37.81)	0.08	14.15 (−1.75 to 32.62)
ADIPOQ	−11391G/A	add	0.3	0.01 (−0.01 to 0.03)	1	−0.01 (−0.52 to 0.51)	0.2	−2.05 (−4.92 to 0.89)	0.2	−1.94 (−4.98 to 1.19)	0.2	−2.24 (−5.27 to 0.88)
ADIPOQ	−11377C/G	add	0.2	0.01 (−0.00 to 0.02)	0.9	−0.02 (−0.37 to 0.32)	0.8	0.27 (−1.71 to 2.30)	0.8	0.29 (−1.81 to 2.44)	0.8	0.30 (−1.81 to 2.45)
ADIPOQ	G15G	add	0.6	0.00 (−0.01 to 0.02)	0.2	−0.32 (−0.78 to 0.14)	0.3	1.32 (−1.34 to 4.05)	0.1	2.39 (−0.46 to 5.33)	0.6	0.81 (−2.00 to 3.69)

For each quantitative trait, the relative variation (Δ) explained by the genotypes indicates the % of decrease or increase in the continuous trait per one supplementary minor allele (additive model) or when passing from normal to at-risk genotype (dominant or recessive model) or when the minor allele decreases the risk from normal to protective genotype. The *P* values indicated are nominal *P* values. add, additive; dom, dominant; rec, recessive.

TABLE 5
Interaction between age and genotype on fasting glycemia for *IL6* and *IL6R* polymorphisms

Gene	SNP	rs-ID	Model	*P* for interaction (age × genotype)*	Age ≥56 years		Age <56 years	
					P for genotype†	ΔGlycemia (95% CI)	*P* for genotype†	ΔGlycemia (95% CI)
IL6	−174G/C	rs1800795	add	**0.009**	**5×10^{-5}**	−1.36 (−2.00 to −0.71)	0.80	0.06 (−0.42 to 0.754)
			dom	**0.023**	**0.0002**	−1.82 (−2.76 to −0.87)	0.90	0.05 (−0.64 to 0.75)
			rec	**0.025**	**0.004**	−1.78 (−2.98 to −0.57)	0.80	0.12 (−0.80 to 1.504)
IL6R	D358A	rs8192284	add	**0.003**	0.10	−0.54 (−1.20 to 0.12)	0.30	0.26 (−0.22 to 0.74)
			dom	**0.005**	0.30	−0.55 (−1.52 to 0.43)	0.10	0.55 (−0.15 to 1.26)
			rec	**0.023**	0.10	−0.97 (−2.17 to 0.25)	1.00	0.02 (−0.87 to 0.91)

*The significance of an age-genotype interaction term used in the mixed model regression analysis is given by the first *P* value. † Significance of the genotype term in the model without interaction, including measures at age ≥56 or <56 years. ΔGlycemia is indicated as the % of decrease or increase per one supplementary minor allele (additive model) or from normal to protective genotype (dominant or recessive model). The *P* values indicated in bold face are nominal *P* values. add, additive; dom, dominant; rec, recessive.

not remain significant after correction for multiple testing. Such attempts at correction or adjustment have not been systematically done in the previous prospective studies (22,39,41). Given the small number of incident cases at the end of the follow-up, our study has a limited power to detect modest genetic effects, particularly in assessing risk prediction for low-frequency alleles (<15%); this is also documented by the ROC analysis and AUC estimates for several at-risk alleles (Fig. 3). In our nested case-control analysis, at the end of the follow-up study, we had a good power (>80%) to detect effects with ORs >1.3. However, 523 converters (including 187 diabetic case subjects) were analyzed from the prospective design, and further studies with a greater sample size and possibly a longer follow-up are required to definitively conclude type 2 diabetes incidence. Variable effects depending on age were found in our study for several variants, which is important to consider when studies with different ascertainments are compared or combined.

Our data show that the *GCK* −30A allele is a true risk factor for the development of both IFG and type 2 diabetes, having a significant impact on β-cell function impairment. The most significant effect is seen on the modulation of fasting glycemia and HOMA-B in the 2,919 individuals who were normoglycemic over the entire period of the study. No allele dosage effect was found (11). Importantly, the association of *GCK* (−30A) with FPG is still significant ($P_{corrected} = 0.01$) after 500 permutations, while accounting for the total number of variants and traits analyzed and the number of models tested. In addition, the *GCK* (−30A) promoter variant is associated with type 2 diabetes in independently ascertained French Caucasian diabetic patients. Our findings are further supported by a recent meta-analysis of previous association results for *GCK* (−30A) with type 2 diabetes showing an overall OR of 1.08 ($P = 0.004$) in populations of mostly European origin (47). Haploinsufficiency in glucokinase enzyme activity cause *MODY2* characterized by lifelong mild fasting hyperglycemia (usually between 5.5 and 8.5 mmol/l) (48). The exact mechanism by which the *GCK* −30A allele causes hyperglycemia is uncertain, but its effect seems constant throughout the lifespan, although insulin secretion is known to decrease with age in the general population. This is in accordance with the constant effect of the *GCK* −30A allele on fasting glucose reported in several groups of normoglycemic subjects whose median age varied from 8 to 72 years (11).

There is evidence that interleukin (IL)-6 adipokine signaling is involved in type 2 diabetes physiopathology (49) and possibly related to diabetes risk. Our study confirms a decreased risk for the minor C allele of *IL6* −174G/C, both in prevalence and incidence analysis, as reported with a lower effect (OR 0.91, $P = 0.037$) in a large joint analysis of 21 case-control studies (29). A protective effect of the C allele on diabetes incidence is more apparent in older individuals (aged >56 years). This interaction with age is even much more significant on fasting glycemia with a 1.36% decrease provided by each minor protective allele ($P = 5 \times 10^{-5}$). Functional relevance was attributed to *IL6* (−174G/C) by in vitro data, which indicated that the C allele affects promoter strength with a weaker effect on *IL6* transcription (50). A direct relationship between proinflammatory mediators and diabetogenic mechanisms is not completely established, although IL-6 and tumor necrosis factor-α may act directly on β-cell survival and secretory function (49). Besides mechanisms relevant to β-cell function, IL-6R was shown to localize to the pancreatic α-cells, and IL-6 was shown to regulate α-cell mass and glucagon secretion in human and mouse islets and to decrease glucose-stimulated insulin secretion (51).

Furthermore, the combination of *GCK* (−30G/A), *IL6* −174G/C, and *TCF7L2* SNPs is associated with type 2 diabetes risk in the DESIR cohort through a multiplicative allelic effect, supporting the view that several defects in β-cell function, even modest individually, exacerbate insulin secretion deficiency and may provoke overt diabetes in the context of aging and overweight. However, the ability of this combined effect to predict diabetes in the general population is low compared with using conventional factors, like age, sex, and BMI, as indicated by the ROC curves. The estimation of combined effects of several genes must be interpreted with caution and only for SNPs whose role in type 2 diabetes pathogenesis is well established. Therefore, our results, together with others recently published (41,45), should be viewed as a first attempt to assess more global genetic effects. Such genetic information when completed will help to identify people at higher risk for type 2 diabetes. As genome-wide association studies are delivering new variants in novel genes, like in *SLC30A8* and *HHEX* (31), or *CDKAL1*, *CDKN2A/2B*, and *IGF2BP2* (32–35), whose physiological role in type 2 diabetes etiology must be further characterized, similar prospective studies and methodological designs are valuable tools to complement more classical genetic approaches.

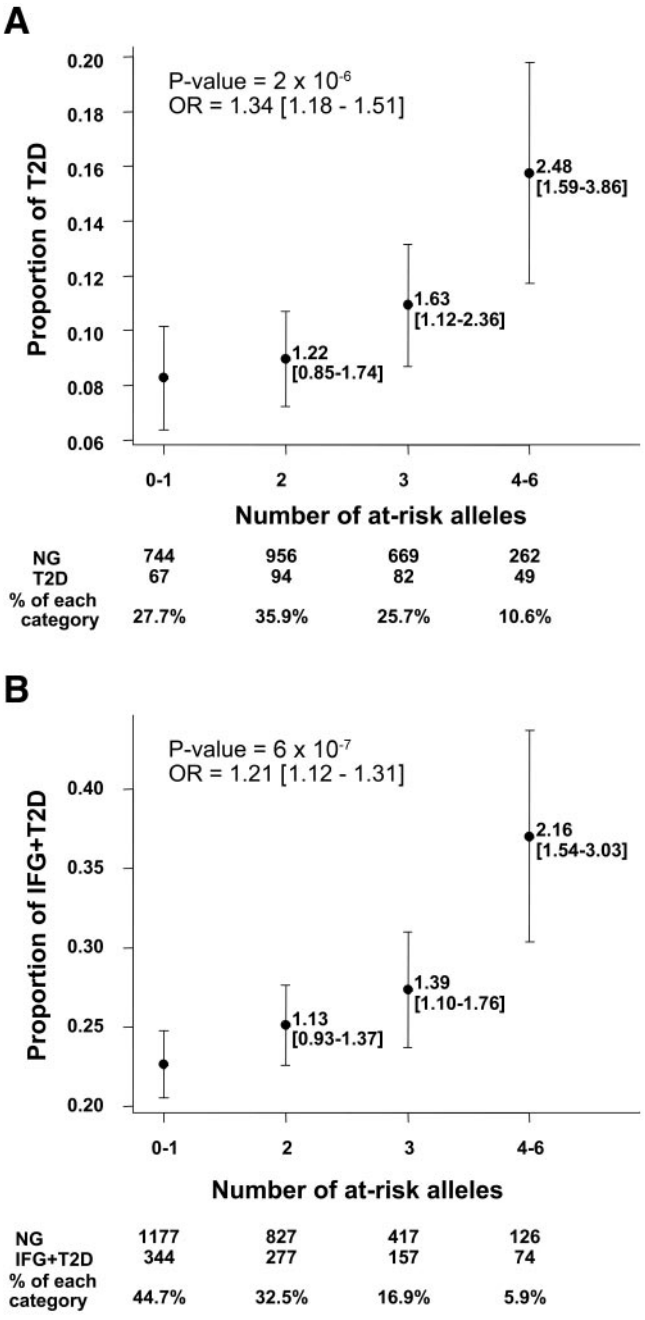

FIG. 2. Additive effects of multiple at-risk polymorphisms on diabetes and IFG at the end of the follow-up. *A*: Combination of *GCK* −30G/A, *TCF7L2*-rs7903146, and *IL6* −174G/C. *B*: Combination of *GCK* −30G/A, *TCF7L2*-rs7903146, and *SREBF1*-G952G. ORs and 95% CIs are shown for each class of the number of at-risk alleles as a variable in the logistic regression model. The OR and *P* value corresponding to each additional allele are indicated at the top left side. The number of normoglycemic (NG) and diabetic (T2D) or hyperglycemic (IFG) plus diabetic individuals, with the percentage of each category of the number of at-risk alleles, are indicated at the bottom of each graph. The G allele of *IL6* variant was considered at-risk. The population attributable risk was calculated for individuals carrying at least three at-risk alleles versus those carrying less than three at-risk alleles; it was evaluated to 27% for the combination of *GCK* −30G/A, *TCF7L2*-rs7903146, and *IL6* −174G/C and to 14% for the combination of *GCK* −30G/A, *TCF7L2*-rs7903146, and *SREBF1*-G952G.

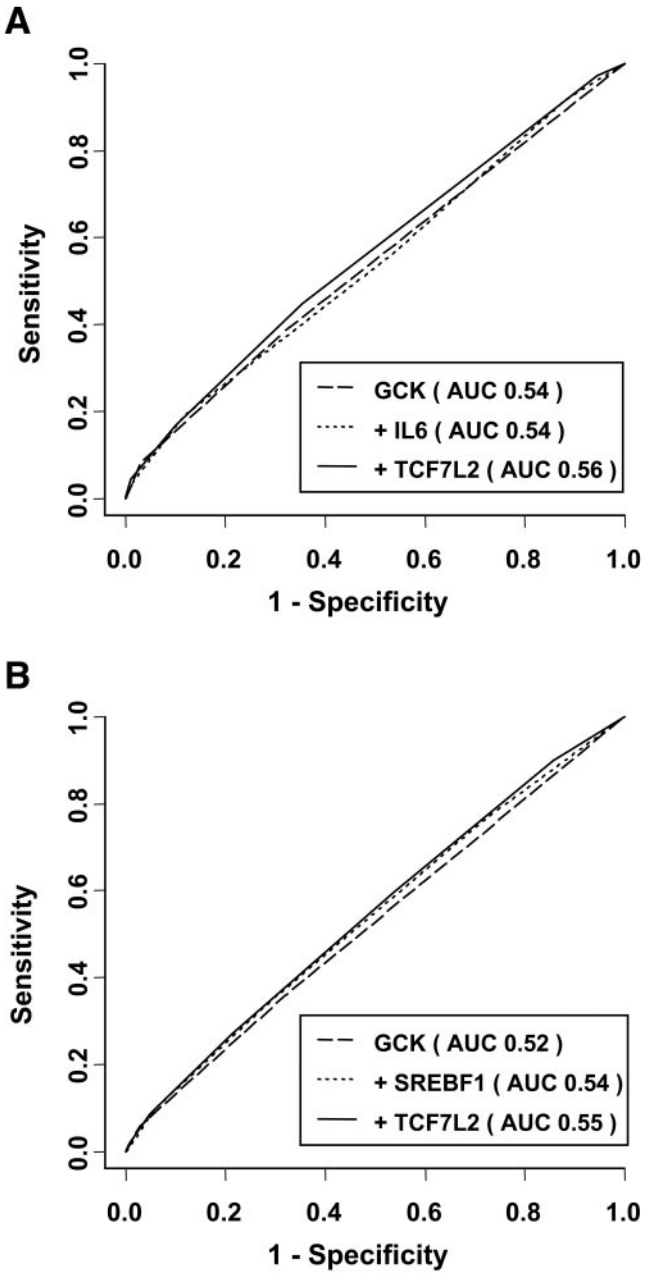

FIG. 3. ROC curves including the genetic information for two combinations of at-risk polymorphisms. *A*: Combination of *GCK* −30G/A, *IL6* −174G/C, and *TCF7L2*-rs7903146. *B*: Combination of *GCK* −30G/A, *SREBF1*-G952G, and *TCF7L2*-rs7903146. The ROC curves and AUC were generated after fitting a logistic regression model including only the genetic factors.

APPENDIX

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