Impact of Common Type 2 Diabetes Risk Polymorphisms in the D.E.S.I.R. Prospective Study

Martine Vaxillaire PharmD, PhD1, Jacques Veslot1, Christian Dina1, Christine Proença1, Stéphane Cauchi PhD1, Guillaume Charpentier MD2, Jean Tichet MD3, Frédéric Fumeron PhD4,5, Michel Marre MD4,5,6, David Meyre PhD1, Beverley Balkau PhD7,8, and Philippe Froguel MD, PhD1,9, for the D.E.S.I.R. Study Group.

1 UMR8090 and Institute of Biology, Lille 2 University, CNRS and Pasteur Institute, Lille, France,
2 Department of Endocrinology-Diabetology, Centre Hospitalier Sud-Francilien, Corbeil-Essonnes, France
3 the Regional Institut for Health, Tours, France
4 INSERM U695, Paris, France,
5 Université Paris Diderot - Paris 7, Paris, France
6 Department of Endocrinology-Diabetology and Nutrition, Bichat Claude Bernard Hospital, Paris, France,
7 INSERM U780-IFR69, Villejuif, France
8 University of Paris-Sud, Villejuif, France
9 Genomic Medicine, Hammersmith Hospital, Imperial College London, United Kingdom.

Running title: Type 2 Diabetes Genetics and lifetime Risk

Corresponding author:
Dr Martine Vaxillaire
CNRS UMR8090, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 245, 59019 Lille, France
martine.vaxillaire@good.ibl.fr

Received for publication 28 June 2007 and accepted in revised form 15 October 2007.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

Copyright American Diabetes Association, Inc., 2007
ABSTRACT

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 D.E.S.I.R. 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 [1.07-1.69] under an additive model, as supported in independent French diabetic cases (OR 1.22, \( p = 0.007 \)), with increased fasting glycemia (0.85% per A-allele, \( p = 6.10^{-5} \)) and decreased HOMA-B (4%, \( p = 0.0009 \)). IL6 (Interleukine 6) -174 G/C interacts with age in the disease risk and modulates fasting glycemia according to age (1.36% decrease above 56 years, \( p = 5.10^{-5} \)). These polymorphisms together with KCNJ11(Kir6.2)-E23K and TCF7L2-rs7903146 may predict diabetes incidence in the D.E.S.I.R. cohort. Each additional risk allele at GCK, TCF7L2 and IL6 increased risk by 1.34 (\( p = 2.10^{-6} \)), with OR of 2.48 [1.59-3.86] in carriers of at least 4 at-risk alleles compared to those with 0 or 1 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.

ABBREVIATIONS

DESIR, Data from an Epidemiological Study on the Insulin Resistance syndrome
FPG, fasting plasma glucose
HOMA, homeostasis model assessment
HOMA-B, HOMA of \( \beta \)-cell function
HOMA-IR, HOMA of insulin resistance
HR, Hazard Ratio
IFG, impaired fasting glycemia
MAF, minor allele frequency
OR, odds ratio
SNP, single nucleotide polymorphism
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 favoured 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 (reviewed 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 having 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), with 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, which showed association in at least two independent studies, include the G482S coding SNP of PPARC1A (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), 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 (GWA) studies, surveying about 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 non coding rs7903146 variant in TCF7L2 was shown to be associated with an increased incidence of hyperglycemia in the French D.E.S.I.R. 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).

**RESEARCH DESIGN AND METHODS**

**Study Population.** A cohort of 2,576 men and 2,636 women from a general population (aged between 30-65 years at inclusion) participated in the D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance syndrome) longitudinal study and were clinically and biologically evaluated at inclusion, at 3, 6 and 9-year
visits (42, 43; and supplementary Table 1). Participants signed an informed consent, and the protocol was approved by the ethics committee of Bicêtre Hospital.

Three classes of glycemic status were 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 between 6.1 and 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 D.E.S.I.R. 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 of the study, and 307 diabetic cases (including 120 diabetic individuals at baseline and 187 incident cases). 320 individuals whose European ancestry was not established (supplemental Appendix 1 [available at http://diabetes.diabetesjournals.org]), and the 1,015 remaining for whom the glycemic status was not known at the end of the study were excluded from the analysis. 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 cases were incident for IFG, and 187 for diabetes.

**Additional Cases.** 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 controls were 2,251 unrelated subjects (FPG < 5.6. mmol/l and BMI < 30 kg/m²) selected from the current D.E.S.I.R. cohort. 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 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 as covariates: gender, age at the end of the study and mean BMI based on all available measures during the study. A non-linear term for age was used.

Cox proportional hazards regression models were used to estimate the genotype effect on the incidence of diabetes. Models included as covariates: gender and mean BMI 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 MAF of 0.10, 0.15, 0.20 and ≥ 0.30, respectively, to detect an odds ratios (ORs) ≥ 1.35 (for α=0.05, additive model); for variants with MAF ≥ 0.35 we had 80% power to detect genotype effects with ORs of 1.25. In the incidence analysis, the power to detect Hazard Ratios (HRs) lower than 1.5 is weaker (< 70%) because of the small number of incident cases.

Mixed models were used to estimate the genotype effects on five quantitative traits after log-transformation. Repeated measures of glycemia, insulinenia, and the derived indexes HOMA-B and HOMA-IR
Type 2 Diabetes Genetics and lifetime Risk

(supplemental Appendix 3) were analyzed in normoglycemic individuals (including >12,000 observations), and BMI in all individuals. Models included, in addition to genotype variable, gender, 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 only 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 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 (AUC) 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 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 nineteen 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 with hyperglycemia and type 2 diabetes at the end of the 9-years study.** In this middle-aged cohort, the prevalence of IFG (FPG between 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 cases and controls 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 [1.51-4.83], \( p = 0.0008 \) (Table 2). The population attributable risk (PAR) to develop 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 significantly associated with type 2 diabetes, as recently supported by our previous study (38), providing an OR of 1.45 [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 of 0.75 [0.57-0.98] \( (p_{\text{additive}} = 0.03) \), and IL6R-D358A, OR of 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 (\( p_{\text{recessive}} = 5.10^{-2} \)). Trends for association with type 2 diabetes were seen for CAPN10 SNP-44 and PPARGC1A -1422 T/C variants (\( p < 0.1 \)). The other twelve 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 analyzed further the effect of GCK -30G/A in an independent sample of 2,215 diabetic subjects compared to 2,251 normoglycemic controls selected from the current D.E.S.I.R. study (based upon FPG < 5.6 mmol/l at all times of the study). In support of the above data, the A-allele frequency was 0.19 in diabetic cases compared to 0.17 in controls ($p = 0.006$), and the A-allele carriers had a 1.22 [1.06-1.42]-fold risk of diabetes ($p_{\text{additive}} = 0.007$). A similar effect was observed when analyzing apart 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 [1.13-1.32] ($p_{\text{additive}} = 10^{-6}$; after adjustment on age and gender using the Mantel-Haenszel analysis of fixed effect).

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

Using Cox proportional hazards models, the GCK -30A allele was shown significant for diabetes incidence (HR 1.34 [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 cases) with HR 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 D.E.S.I.R. population are shown in Figure 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 association with incidence but not with the overall diabetes risk could interact with age (since a borderline significant interaction was detected). Taking association with type 2 diabetes, 5 out of 19 SNPs tested were significant ($p < 0.05$), that is different from the expected proportion of replication by random effect (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 ($p_{\text{additive}} = 6.10^{-5}$, after permutations $p_{\text{corrected}} = 0.01$), and HOMA-B with a 4% decrease ($p_{\text{additive}} = 0.002$), all other variables being considered with a fixed effect (Table 4). No overall genotype effect of IL6 -174G/C was apparent on glycemia levels over the time, whereas an age-genotype interaction was found when analyzing together normoglycemic and IFG individuals ($p_{\text{additive}} = 0.009$) (Table 5). This effect was significant for age > 56.0, explaining 1.36%-decrease of glycemia per supplementary C-allele ($p_{\text{additive}} = 5.10^{-5}$; Table 5). The interaction between age and IL6R-D358A genotype on fasting glycemia was also significant under all genetic models ($p_{\text{additive}} = 0.003$) with inverse effects on glycemia depending on age (below or above 56.0) (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 not lower than 15. Potential interactions were found between $KCNJ11$-E23K and $HNF4A$ rs2144908 for association with diabetes ($p(interaction) = 0.0012$) and $IL6$ (-174G/C)-$SREBF1$-G952G for association with IFG and diabetes ($p(interaction) = 0.0019$). However, no $p$-values would remain 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): the OR by each additional risk allele increased diabetes risk by 1.34 [1.18-1.51], $p = 2.10^{-6}$, with an OR of 2.48 [1.59-3.86] for carriers of at least 4 risk alleles compared to those with 0-1 risk allele ($p = 6.10^{-5}$) (Figure 2A and supplementary Table 2). When considering both diabetes and IFG, the most significant effect was for $GCK$, $SREBF1$-G952G and $TCF7L2$ (combination 2): OR of 1.21 [1.12-1.31] ($p = 6.10^{-7}$) per supplementary allele and 2.16 [1.54-3.03] for carriers of at least 4 risk alleles compared to carriers of 0-1 risk allele ($p = 8.10^{-6}$) (Figure 2B and supplementary Table 2). The PAR for at least three risk alleles versus less than 3 was estimated to 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 (AUC), 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 (Figure 3), which is significantly different from the null hypothesis ($p = 0.01$). Of note, the genetic information did not enhance the ability to predict future cases compared to using both genetic and conventional factors (i.e. age, gender and BMI; $p$-value = 0.26 and 0.98 for combination 1 and 2 respectively) (supplementary Figure 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 [1.11-1.45] ($p = 4.10^{-4}$) 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, are associated with risk of developing type 2 diabetes in the D.E.S.I.R. prospective cohort, in addition to $TCF7L2$-rs7903146 (38). Furthermore, the significant association of $GCK$ (-30A) promoter SNP with increased fasting glycemia and type 2 diabetes, in both D.E.S.I.R. and case-control studies of North European origin extends the 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 papers 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) and the STOP-NIDDM trial (40), and in 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 showing association with type 2 diabetes in the Finnish population (HR 1.4-1.7), whereas 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 D.E.S.I.R. studies may differ in their genetic background as well as 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 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 (Figure 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 cases) were analyzed from the prospective design, and further studies with a greater sample size and possibly a longer follow-up are required to definitely conclude on type 2 diabetes incidence. Variable effects depending on age were found in our study for several variants, which is important to address when studies with different ascertainment are compared or combined.

Our data show that the GCK-30A allele is a true risk factor for development of both IFG and type 2 diabetes, having a significant impact on \( \beta \)-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 time 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 a 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 IL6 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
Type 2 Diabetes Genetics and lifetime Risk

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). This interaction with age is even much more significant on fasting glycemia with a 1.36% decrease provided by each minor protective allele (\( p = 5.10^{-5} \)). Functional relevance was attributed to \textit{IL6} (-174G/C) by \textit{in vitro} data which indicated that the C-allele affects promoter strength with a weaker effect on \textit{IL6} transcription (50). A direct relationship between proinflammatory mediators and diabetogenic mechanisms is not completely established, although IL6 and TNF-\( \alpha \) may directly act on \( \beta \)-cell survival and secretory function (49). Besides mechanisms relevant to \( \beta \)-cell function, IL-6R was shown to localize to the pancreatic \( \alpha \)-cells, and IL-6 to regulate \( \alpha \)-cell mass and glucagon secretion in human and mouse islets, along with a decrease in glucose-stimulated insulin secretion (51).

Furthermore, the combination of \textit{GCK} (-30G/A), \textit{IL6} -174G/C and \textit{TCF7L2} SNPs is associated with type 2 diabetes risk in the D.E.S.I.R. cohort through a multiplicative allelic effect, supporting the view that several defects in \( \beta \)-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 to using conventional factors, like age, gender 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 \textit{SLC30A8} and \textit{HHEX} (31), or \textit{CDKAL1}, \textit{CDKN2A/2B} and \textit{IGF2BP2} (32-35), whose physiological role in type 2 diabetes aetiology has to be further characterized, similar prospective studies and methodological designs are valuable tools to complement more classical genetic approaches.

ACKNOWLEDGMENTS
We thank Stefan Gaget and Céline Lange for their participation in the genotype and phenotype database management; Aurélie Dechaume and Emmanuel Vaillant for their technical assistance in SNP genotyping; Frédéric Allegaert, Marianne Deweider and Franck Péan for DNA samples management. We are also indebted to all study participants.

This study was supported in part by a grant from the French association ALFEDIAM and MERCK-LIPHA Santé. The D.E.S.I.R. study has been supported by INSERM, CNAMTS, Lilly, Novartis Pharma and Sanofi-Aventis, by INSERM (Réseaux en Santé Publique, Interactions entre les determinants de la santé), by the Association Diabète Risque Vasculaire, the Fédération Française de Cardiologie, La Fondation de France, ALFEDIAM, ONIVINS, Ardix Medical, Bayer Diagnostics, Becton Dickinson, Cardionics, Merck Santé, Novo Nordisk, Pierre Fabre, Roche, Topcon.
APPENDIX

The D.E.S.I.R. Study Group is composed of: INSERM U258: B. Balkau, P. Ducimetière, and E. Eschwège; INSERM U367: F. Alhenc-Gelas; CHU D’Angers: Y. Gallois and A. Girault; Bichat Hospital: F. Fumeron and M. Marre; Medical Examination Services: Alençon, Angers, Caen, Chateauroux, Cholet, Le Mans, and Tours; Research Institute for General Medicine: J. Cogneau; General practitioners of the region; Cross-Regional Institute for Health: C. Born, E. Caces, M. Cailleau, J.G. Moreau, F. Rakotozafy, J. Tichet, and S. Vol.
REFERENCES

1. Zimmet P, Alberti KG, Shaw J: Global and societal implications of the diabetes epidemic. Nature 414:782–787, 2001
2. Stumvoll M, Goldstein BJ, van Haeften TW: Type 2 diabetes: principles of pathogenesis and therapy. Lancet 365:1333-1346, 2005
3. Permutt MA, Wasson J, Cox N: Genetic epidemiology of diabetes. J Clin Invest 115:1431-1439, 2005
4. Barroso I: Genetics of Type 2 diabetes. Diabet Med 22:517-535, 2005
5. 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 MJ, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 1:76-80, 2000
6. 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 K-ATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with increased risk of Type 2. Diabetes 52:568–572, 2003
7. Love-Gregory L, Wasson K, Lin J, Skolnick G, Suarez B, Permutt MA: An E23K single nucleotide polymorphism in the islet ATP-sensitive potassium channel gene (Kir6.2) contributes as much to the risk of type II diabetes in Caucasians as the PPARgamma. Diabetologia 46:136–137, 2003
8. Tsuchiya T, Schwarz PE, Bosque-Plata LD, Geoffrey Hayes M, Dina C, Froguel P, Wayne Towers G, Fischer S, Temelkova-Kurtschiev T, Rietzsch H, Graessler J, Vcelak J, Palyzova D, Selisko T, Bendlova B, Schulze J, Julius U, Hanefeld M, Weedon MN, Evans JC, Frayling TM, Hattersley AT, Orho-Melander M, Groop L, Malecki MT, Hansen T, Pedersen O, Fingerlin TE, Boehnke M, Hanis CL, Cox NJ, Bell GI: Association of the calpain-10 gene with type 2 diabetes in Europeans: Results of pooled and meta-analyses. Mol Genet Metab 89:174-184, 2006
9. Grant SF, 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
10. Cauchi S, El Achhab Y, Choquet H, Dina C, Kremling F, Weitgasser R, Nejjar C, Patsch W, Chikri M, Meyre D, Froguel P: TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. J Mol Med in press, 2007
11. Weedon MN, Clark VJ, Qian Y, Ben-Shlomo Y, Timpson N, Ebrahim S, Lawlor DA, Pembrey ME, Ring S, Wilkin TJ, Voss LD, Jeffery AN, Metcalf B, Ferrucci L, Corsi AM, Murray A, Melzer D, Knight B, Shields B, Smith GD, Hattersley AT, Di Rienzo A, Frayling TM: A common haplotype of the glucokinsase gene alters fasting glucose and Birth Weight: Association in Six Studies and Population-Genetics Analyses. Am J Hum Genet 79:991-1001, 2006
12. Shaat N, Karlsson E, Lernmark A, Ivarsson S, Lynch K, Parikh H, Almgren P, Berntorp K, Groop L: Common variants in MODY genes increase the risk of gestational diabetes mellitus. Diabetologia 49:1545-1551, 2006
13. Rose CS, Ek J, Urhammer SA, Glumer C, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T: A -30G>A polymorphism of the beta-cell-specific glucokinsase promoter
associates with hyperglycemia in the general population of whites. *Diabetes* 54:3026-3031, 2005

14. Marz W, Nauck M, Hoffmann MM, Nagel D, Boehm BO, Koenig W, Rothenbacher D, Winkelmann BR: G(-30)A polymorphism in the pancreatic promoter of the glucokinase gene associated with angiographic coronary artery disease and type 2 diabetes mellitus. *Circulation* 109:2844-2849, 2004

15. Vasseur F, Meyre D, Froguel P: Adiponectin, type 2 diabetes and the metabolic syndrome: lessons from human genetic studies. *Expert Rev Mol Med* 8:1-12, 2006

16. Oberkofler H, Linnemayr V, Weitgasser R, Klein K, Xie M, Iglseder B, Krempler F, Paulweber B, Patsch W: Complex haplotypes of the PGC-1alpha gene are associated with carbohydlate metabolism and type 2 diabetes. *Diabetes* 53:1385-1393, 2004

17. Barroso I, Luan J, Sandhu MS, Franks PW, Crowley V, Schafer AJ, O'rahilly S, Wareham NJ: Meta-analysis of the Gly482Ser variant in PPARGC1A in type 2 diabetes and related phenotypes. *Diabetologia* 49:501-505, 2006

18. Silander K, Mohlke KL, Scott LJ, Peck EC, Holstein 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-4alpha Gene Predicts Susceptibility to Type 2 Diabetes. *Diabetes* 53:1141-1149, 2004

19. 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-4alpha P2 promoter are associated with type 2 diabetes in the U.K. population. *Diabetes* 53:3002-3006, 2004

20. Winckler W, Graham RR, de Bakker PI, 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 4alpha gene with risk of type 2 diabetes in 7,883 people. *Diabetes* 54:886-892, 2005

21. Sasahara M, Nishi M, Kawashima H, Ueda K, Sakagashira S, Furuta H, Matsumoto E, Hanabusa T, Sasaki H, Nanjo K: Uncoupling protein 2 promoter polymorphism -866G/A affects its expression in beta-cells and modulates clinical profiles of Japanese type 2 diabetic patients. *Diabetes* 53:482-485, 2004

22. Gable DR, Stephens JW, Cooper JA, Miller GJ, Humphries SE: Variation in the UCP2-UCP3 Gene Cluster Predicts the Development of Type 2 Diabetes in Healthy Middle-Aged Men. *Diabetes* 55:1504-1511, 2006

23. Eberle D, Clement K, Meyre D, Sahbatou M, Vaxillaire M, Le Gall A, Ferre P, Basdevant A, Froguel P, Foufelle F: SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. *Diabetes* 53:2153-2157, 2004

24. Felder TK, Oberkofler H, Weitgasser R, Mackevics V, Krempler F, Paulweber B, Patsch W. The SREBF-1 locus is associated with type 2 diabetes and plasma adiponectin levels in a middle-aged Austrian population. *Int J Obes* 31(7):1099-103, 2007

25. Vaxillaire M, Dechaume A, Vasseur-Delannoy V, Lahmidi S, Vatin V, Lepretre F, Boutin P, Hercberg S, Charpentier G, Dina C, Froguel P: Genetic analysis of ADIPOR1 and ADIPOR2 candidate polymorphisms for type 2 diabetes in the Caucasian population. *Diabetes* 55:856-861, 2006

26. Damcott CM, Ott SH, Pollin TI, Reinhart LJ, Wang J, O’connell JR, Mitchell BD, Shuldiner AR: Genetic variation in adiponectin receptor 1 and adiponectin receptor 2 is associated with type 2 diabetes in the Old Order Amish. *Diabetes* 54:2245-2250, 2005

27. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Inflammatory cytokines and the risk to develop type 2 diabetes: results of the
Kubaszek A, Pihlajamaki J, Komarovsky V, Lind V, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M: Finnish Diabetes Prevention Study. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *Diabetes* 52:1872-1876, 2003

Kubaszek A, Pihlajamaki J, Komarovsky V, Lind V, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M: Finnish Diabetes Prevention Study. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *Diabetes* 52:1872-1876, 2003

Huth C, Heid IM, Vollmert C, Gieger C, Grallert H, Wolford JK, Langer B, Thorand B, Klopp N, Hamid YH, Pedersen O, Hansen T, Lyssenko V, Group L, Meisinger C, Doring A, Lowel H, Lieb W, Hengstenberg C, Rathmann W, Martin S, Stephens JW, Ireland H, Mather H, Miller GJ, Stringham HM, Boehnke M, Tuomilehto J, Boehing H, Mohlig M, Spranger J, Pfeiffer A, Wernstedt I, Niklason A, Lopez-Bermejo A, Fernandez-Real JM, Hanson RL, Gallart L, Vendrell J, Tsiavou A, Hatzigelaki E, Humphries SE, Wichmann HE, Herder C, Illig T: IL6 gene promoter polymorphisms and type 2 diabetes: joint analysis of individual participants' data from 21 studies. *Diabetes* 55:2915-2921, 2006

Hamid YH, Urhammer SA, Jensen DP, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O: Variation in the interleukin-6 receptor gene associates with type 2 diabetes in Danish whites. *Diabetes* 53:3342-3345, 2004

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(7130):881-885, 2007

Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsrson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorradottir S, Bjarnason H, Ng MC, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatay A, So WY, Ma RC, Andersen G, Borch-Johnsen K, Jorgensen T, van Vliet-Ostapchouk J, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulleher JR, Thorsteinsdottir U, Kong A, Stefansson K: A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* 39(6):770-775, 2007

Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316(5829):1331-1336, 2007

Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruijm R, Xiao R, Li XY, Connelly 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(5829):1341-1345, 2007

Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447(7145):661-678, 2007

Hattersley AT, McCarthy MI: What makes a good genetic association study? *Lancet* 366:1315-1323, 2005

Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D: Diabetes Prevention Program Research Group. TCF7L2...
polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355:241-250, 2006

38. Cauchi S, Meyre D, Choquet H, Dina C, Born C, Marre M, Balkau B, Froguel P: TCF7L2 Variation Predicts Hyperglycemia Incidence in a French General Population: The Data From an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) Study. *Diabetes* 55:3189-3192, 2006

39. Laukkanen O, Pihlajamaki J, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M: Finnish Diabetes Prevention Study Group. Polymorphisms of the SUR1 (ABCC8) and Kir6.2 (KCNJ11) genes predict the conversion from impaired glucose tolerance to type 2 diabetes. The Finnish Diabetes Prevention Study. *J Clin Endocrinol Metab* 89:6286-6290, 2004

40. Andrulionyte L, Peltola P, Chiasson JL, Laakso M; STOP-NIDDM Study Group: Single nucleotide polymorphisms of PPARD in combination with the Gly482Ser substitution of PGC-1A and the Pro12Ala substitution of PPARG2 predict the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial. *Diabetes* 55:2148-2152, 2006

41. Lyssenko V, Almgren P, Anevski D, Orho-Melander M, Sjögren M, Saloranta C, Tuomi T, Groop L: Genetic Prediction of Future Type 2 Diabetes. *PLoS Med* 2:1299-1308, 2005

42. Jaziri R, Lobbens S, Aubert R, Pean F, Lahmidi S, Vaxillaire M, Porchay I, Bellini N, Tichet J, Balkau B, Froguel P, Marre M, Fumeron F: The PPARG Pro12Ala Polymorphism Is Associated With a Decreased Risk of Developing Hyperglycemia Over 6 Years and Combines With the Effect of the APM1 G-11391A Single Nucleotide Polymorphism: The Data From an Epidemiological Study on the Insulin Resistance Syndrome (DESIR) Study. *Diabetes* 55:1157-1162, 2006

43. Balkau B, Eschwege E, Tichet J, Marre M: Proposed criteria for the diagnosis of diabetes: evidence from a French epidemiological study (D.E.S.I.R.). *Diabetes Metab* 23:428-434, 1997

44. Cheyssac C, Lecoeur C, Dechaume A, Bibi A, Charpentier G, Balkau B, Marre M, Froguel P, Gibson F, Vaxillaire M: Analysis of common PTPN1 gene variants in type 2 diabetes, obesity and associated phenotypes in the French population. *BMC Med Genet* 7:44, 2006

45. Weedon MN, McCarthy MI, Hitman G, Walker M, Groves CJ, Zeggini E, Rayner NW, Shields B, Owen KR, Hattersley AT, Frayling TM: Combining Information from Common Type 2 Diabetes Risk Polymorphisms Improves Disease Prediction. *PLoS Med* 3:e374, 2006

46. Willer CJ, Bonnycastle LL, Conneely KN, Duren WL, Jackson AU, Scott LJ, Narisu N, Chines PS, Skol A, Stringham HM, Petrie J, Erdos MR, Swift AJ, Enloe ST, Sprau AG, Smith E, Tong M, Doheny K, Pugh EW, Watanabe RM, Buchanen TA, Valle TT, Bergman RN, Tuomilehto J, Mohlke KL, Collins FS, Boehnke M: Screening of 134 Single Nucleotide Polymorphisms (SNPs) previously associated with Type 2 Diabetes replicates association with 12 SNPs in nine genes. *Diabetes* 56:256-264, 2007

47. Stride A, Vaxillaire M, Tuomi T, Barbetti F, Njolstad PR, Hansen T, Costa A, Conget I, Pedersen O, Sovik O, Lorini R, Groop L, Froguel P, Hattersley AT, for the Gift Mody Consortium: The genetic abnormality in the beta-cell determines the response to an oral glucose load. *Diabetologia* 45:427-435, 2002

48. Donath MY, Elhesa JA, Maedler K, Schumann DM, Ellingsgaard H, Eppler E, Reinecke M: Mechanisms of beta-cell death in type 2 diabetes. *Diabetes* 54:S108-113, 2005
50. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P: The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 102:1369-1376, 1998

51. Ellingsgaard H, Ehres JA, Van Lommel L, Schuit FC, Donath MY: IL-6 regulates alpha-cell mass and function (Abstract). *Diabetologia* 49(S1):A463, 2006
| 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   | 0.37   | 0.35   | 0.48   | 0.48   | 0.48   | 0.15   | 0.15   | 0.17   |
|      | CAPN10 | SNP-44 | T/C     | 0.16 | 0.72   | 0.71   | 0.74   | 0.25   | 0.26   | 0.24   | 0.03   | 0.03   | 0.02   |
|      | GCK   | -30 G/A | rs1799884 | 0.18 | 0.68   | 0.66   | 0.62   | 0.29   | 0.29   | 0.31   | 0.03   | 0.05   | 0.08   |
|      | HNF4A | P2 C/T | rs1884614 | 0.16 | 0.70   | 0.72   | 0.71   | 0.27   | 0.25   | 0.26   | 0.03   | 0.03   | 0.02   |
|      | HNF4A | P2 G/A | rs2144908 | 0.16 | 0.70   | 0.71   | 0.70   | 0.27   | 0.25   | 0.27   | 0.03   | 0.03   | 0.03   |
|      | UCP2  | -866 G/A | rs659366 | 0.37 | 0.39   | 0.38   | 0.42   | 0.47   | 0.49   | 0.46   | 0.14   | 0.13   | 0.12   |
|      | PPARC1A | G482S | G/A     | 0.35 | 0.43   | 0.43   | 0.43   | 0.44   | 0.44   | 0.42   | 0.13   | 0.13   | 0.15   |
|      | PPARC1A | -1422 T/C | rs2970870 | 0.44 | 0.32   | 0.29   | 0.36   | 0.48   | 0.51   | 0.47   | 0.20   | 0.20   | 0.17   |
|      | IL6   | -174 G/C | rs1800795 | 0.40 | 0.36   | 0.37   | 0.39   | 0.48   | 0.45   | 0.47   | 0.16   | 0.18   | 0.14   |
|      | IL6R  | D358A | A/C     | 0.41 | 0.36   | 0.33   | 0.32   | 0.46   | 0.51   | 0.55   | 0.18   | 0.16   | 0.13   |
|      | TNF   | -308 G/A | rs1800629 | 0.14 | 0.74   | 0.73   | 0.76   | 0.24   | 0.24   | 0.22   | 0.02   | 0.03   | 0.02   |
|      | SREBF1 | G952G | C/G     | 0.38 | 0.41   | 0.36   | 0.38   | 0.45   | 0.47   | 0.47   | 0.14   | 0.14   | 0.15   |
|      | ADIPOR2 | +33,775 | rs2286380 | 0.12 | 0.79   | 0.80   | 0.80   | 0.20   | 0.18   | 0.19   | 0.01   | 0.02   | 0.01   |
|      | ADIPOR2 | I290I | C/A     | 0.12 | 0.79   | 0.79   | 0.79   | 0.20   | 0.20   | 0.19   | 0.01   | 0.02   | 0.01   |
|      | TCF7L2 | rs7903146 | C/T     | 0.30 | 0.51   | 0.45   | 0.43   | 0.40   | 0.46   | 0.44   | 0.09   | 0.09   | 0.13   |
|      | PPARC1A | P12A | rs1801282 | 0.11 | 0.79   | 0.82   | 0.81   | 0.20   | 0.17   | 0.19   | 0.01   | 0.01   | 0.00   |
| Gene       | SNP         | Allele | MAF | NG (n) | IFG (n) | T2D (n) | NG (n) | IFG (n) | T2D (n) | NG (n) | IFG (n) | T2D (n) |
|------------|-------------|--------|-----|--------|---------|---------|--------|---------|---------|--------|---------|---------|
| ADIPOQ     | -11391G/A  | G     | 0.10| 0.81   | 0.80    | 0.18    | 0.19   | 0.20    | 0.01    | 0.01   | 0.003   |
| ADIPOQ     | -11377C/G  | C     | 0.26| 0.54   | 0.55    | 0.55    | 0.39   | 0.40    | 0.08    | 0.06   | 0.05    |
| ADIPOQ     | G15G       | T     | 0.13| 0.75   | 0.75    | 0.74    | 0.23   | 0.24    | 0.02    | 0.02   | 0.02    |

MAF, minor allele frequency. Allele 1, as major allele; and Allele 2, as minor allele.
The frequency of each genotype class is given according to the status with the number of individuals in parenthesis.
All genotype distributions are in Hardy-Weinberg equilibrium (p > 0.05).
NG, normoglycemia; IFG, impaired fasting glycemia; T2D, type 2 diabetes.
### TABLE 2. Association with hyperglycemia and type 2 diabetes prevalence at the end of the 9-year follow-up study

| Gene   | SNP     | rs-ID | Allele | IFG + T2D | T2D |
|--------|---------|-------|--------|-----------|-----|
|        |         |       | 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) |
| Gene/SNP          | SNP Position | rs Number | Model | P-value | OR (95% CI) | OR (95% CI) |
|------------------|--------------|-----------|-------|---------|-------------|-------------|
| TNF              | -308 G/A     | rs1800629 | add   | 0.7     | 1.03 (0.88-1.21) | add 0.6 0.93 (0.71-1.21) |
| SREBF1           | G952G        | rs2297508 | C/G   | 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   | 0.5     | 0.94 (0.78-1.12) | add 0.2 0.81 (0.60-1.10) |
|                 | I290I        |           | C/A   | 0.7     | 0.96 (0.80-1.15) | add 0.3 0.85 (0.62-1.15) |
| TCF7L2           | rs7903146    |           | C/T   | 0.0004  | 1.24 (1.10-1.41) | add 0.002 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   | 0.07    | 0.84 (0.69-1.01) | add 0.4 0.88 (0.64-1.20) |
| ADIPOQ           | -11391G/A    | rs17300539| G/A   | 0.2     | 1.14 (0.95-1.37) | add 0.3 1.19 (0.88-1.61) |
|                  |              |           | G/A   | 0.1     | 1.18 (0.97-1.44) | dom 0.2 1.25 (0.90-1.73) |
| ADIPOQ           | -11377C/G    | rs266729  | C/G   | 0.3     | 0.94 (0.82-1.06) | add 0.4 0.92 (0.74-1.13) |
|                  |              |           | C/G   | 0.08    | 0.75 (0.54-1.04) | rec 0.2 0.71 (0.40-1.26) |
| ADIPOQ           | G15G         | rs2241766 | T/G   | 0.8     | 0.98 (0.83-1.16) | add 0.9 1.01 (0.77-1.32) |

The prevalence study refers to all diabetic cases (n = 307) and hyperglycemic cases (n = 651), when the IFG status is 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 gender, 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. IFG, impaired fasting glycemia; T2D, type 2 diabetes.
### 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 + T2D Model | P  | HR (95% CI)  | T2D 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)   |
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 were censored at the end of the study), and 523 incident cases who developed IFG (336 cases) or diabetes (187 cases) at one time of the 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. IFG, impaired fasting glycemia; T2D, type 2 diabetes.

| Gene      | SNP       | Allele | Model | *p*-value | OR (95% CI)  |
|-----------|-----------|--------|-------|-----------|--------------|
| TNF       | -308 G/A  | rs1800629 G/A | add   | 0.04      | 0.61 (0.38-0.97) |
| SREBF1    | G952G     | rs2297508 C/G | add   | 0.03      | 1.04 (0.88-1.23) |
| ADIPO2    | +33,775 A/T | rs2286380 A/T | add   | 0.002     | 1.23 (1.08-1.40) |
| ADIPO2    | I290I C/A | rs2286380 A/T | add   | 0.3       | 0.90 (0.74-1.09) |
| TCF7L2    | rs7903146 C/T | add   | 0.0002 | 1.23 (1.08-1.40) |
| PPAR      | P12A      | rs1801282 P/A | add   | 0.0008    | 1.35 (1.14-1.61) |
| ADIPOQ    | -11391G/A | rs17300539 G/A | add   | 0.09      | 1.19 (0.98-1.44) |
| ADIPOQ    | -11377C/G | rs266729 C/G | add   | 0.01      | 0.90 (0.78-1.03) |
| ADIPOQ    | G15G      | rs2241766 T/G | add   | 0.04      | 1.07 (0.90-1.28) |
### TABLE 4. Effect of individual genotypes on quantitative traits using mixed models

| Gene     | SNP       | Model | BMI   | Fasting glucose | Fasting insulin | HOMA-B | HOMA-IR |
|----------|-----------|-------|-------|-----------------|-----------------|--------|---------|
|          |           |       | $P$   | $\Delta$ (95% CI) | $P$   | $\Delta$ (95% CI) | $P$   | $\Delta$ (95% CI) | $P$   | $\Delta$ (95% CI) |
| KCNJ11   | E23K      | add   | 0.5   | -0.00 (-0.01-0.01) | 0.7   | -0.07 (-0.40-0.26) | 0.4   | -0.73 (-2.61-1.18) | 0.8   | -0.23 (-2.23-1.82) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
| CAPN10   | SNP-44    | add   | 0.8   | -0.00 (-0.02-0.01) | 1    | 0.00 (-0.42-0.42) | 0.7   | -0.50 (-2.90-1.95) | 0.7   | -0.44 (-2.96-2.15) |
| GCK      | -30 G/A   | add   | 0.3   | -0.01 (-0.02-0.01) | $6.10^{-5}$ | 0.85 (0.44-1.27) | 0.6   | -0.67 (-3.02-1.73) | 0.002 | -4.00 (-6.40-1.54) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | dom   | 0.6   | 0.00 (-0.02-0.01) | 0.002  | 0.90 (0.42-1.39) | 0.4   | -1.12 (-3.82-1.66) | 0.0009| -4.82 (-7.56-2.00) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | rec   | 0.1   | -0.03 (-0.08-0.01) | 0.01  | 1.67 (0.38-2.98)  | 0.7   | 1.59 (-5.64-3.97)  | 0.4   | -3.59 (-10.87-4.28) |
| HNF4A    | P2 C/T    | add   | 0.5   | 0.00 (-0.02-0.01) | 0.9   | 0.02 (-0.39-0.43) | 0.004 | -3.49 (-5.79-1.13) | 0.003 | -3.81 (-6.23-1.32) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | dom   | 0.7   | 0.00 (-0.02-0.01) | 0.7   | -0.10 (-0.58-0.38) | 0.02  | -3.24 (-5.92-0.49) | 0.02  | -3.36 (-6.18-0.46) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | rec   | 0.3   | -0.02 (-0.07-0.02) | 0.2   | 0.89 (-3.92-1.19) | 0.006 | -9.99 (-16.44-3.04) | 0.001 | -12.04 (-18.71-4.81) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
| HNF4A    | P2 G/A    | add   | 0.6   | 0.00 (-0.02-0.01) | 1    | 0.00 (-0.41-0.41) | 0.004 | -3.51 (-5.79-1.16) | 0.004 | -3.62 (-6.04-1.15) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | dom   | 0.7   | 0.00 (-0.02-0.01) | 0.7   | -0.11 (-0.59-0.37) | 0.02  | -3.23 (-5.89-0.49) | 0.04  | -3.12 (-5.94-0.22) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | rec   | 0.5   | -0.02 (-0.06-0.03) | 0.2   | 0.78 (-0.48-2.06) | 0.004 | -10.20 (-16.56-3.35) | 0.001 | -11.93 (-18.54-4.78) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
| UCP2     | -866 G/A  | add   | 0.04  | 0.01 (0.00-0.02) | 0.3   | 0.18 (-0.15-0.50) | 0.3   | 1.05 (-0.82-2.96)  | 1     | -0.00 (-1.98-2.01) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | dom   | 0.05  | 0.02 (0.00-0.03) | 0.3   | 0.25 (-0.20-0.71) | 0.7   | 0.60 (-1.99-3.25)  | 0.7   | -0.49 (-3.21-2.31) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
| PPARC1A  | G482S     | add   | 0.8   | 0.00 (-0.01-0.01) | 0.1   | -0.26 (-0.59-0.06) | 0.7   | 0.32 (-2.19-1.58)  | 0.3   | 1.10 (-0.90-2.35) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | rec   | 0.8   | 0.00 (-0.03-0.02) | 0.06  | -0.62 (-1.28-0.04) | 0.7   | 0.64 (-3.07-4.50)  | 0.04  | 4.34 (0.17-8.67) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
| PPARC1A  | -1422 T/C | add   | 0.8   | 0.00 (-0.01-0.01) | 0.7   | -0.06 (-0.37-0.25) | 0.7   | -0.33 (-2.12-1.49) | 0.4   | 0.88 (-0.97-2.77) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
| IL6      | -174 G/C  | add   | 0.6   | 0.00 (-0.01-0.01) | 0.1   | -0.23 (-0.54-0.08) | 0.2   | -1.07 (-2.86-0.76) | 0.3   | -0.93 (-2.83-1.00) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
|          |           | rec   | 0.1   | -0.02 (-0.04-0.00) | 0.07  | -0.55 (-1.14-0.04) | 0.5   | -1.20 (-4.53-2.25) | 0.7   | -0.63 (-4.18-3.04) |
|          |           |       |       |                 |                 |        |         |         |                 |         |
| IL6R     | Asp358Ala | add   | 0.4   | -0.00 (-0.02-0.01) | 0.7   | -0.06 (-0.37-0.26) | 0.8   | -0.18 (-1.98-1.66) | 0.8   | -0.28 (-2.18-1.66) |
|          |           |       |       |                 |                 |        |         |         |                 |         |

Type 2 Diabetes Genetics and lifetime Risk
| Gene       | SNP                  | Model | p-value (95% CI) | p-value (95% CI) | p-value (95% CI) | p-value (95% CI) |
|------------|----------------------|-------|-----------------|-----------------|-----------------|-----------------|
| TNF        | -308 G/A             | add   | 0.7             | 0.00 (-0.01-0.02) | 0.3             | 0.21 (-0.23-0.65) | 0.9             | -0.24 (-2.77-2.35) | 0.3             | -1.32 (-3.96-1.38) | 0.9             | -0.10 (-2.78-2.65) |
| SREBF1     | G952G                | add   | 0.1             | -0.01 (-0.02-0.00) | 0.6             | -0.09 (-0.42-0.23) | 0.2             | 1.22 (-0.67-3.15) | 0.05            | 2.01 (-0.01-4.08) | 0.4             | 0.93 (-1.07-2.97)  |
|           |                      | dom   | 0.2             | -0.01 (-0.02-0.01) | 0.3             | -0.24 (-0.69-0.22) | 0.3             | 1.46 (-1.21-4.20) | 0.03            | 3.13 (0.26-6.07)  | 0.5             | 1.01 (-1.80-3.90)  |
| ADIPO2     | 3'UTR A/T            | add   | 0.3             | 0.01 (-0.01-0.02) | 0.08            | 0.45 (-0.05-0.94) | 0.7             | 0.54 (-2.29-3.44) | 0.4             | -1.21 (-4.14-1.80) | 0.5             | 0.99 (-2.03-4.09)  |
|           |                      | rec   | 0.5             | -0.02 (-0.08-0.04) | 0.3             | 1.09 (-0.85-3.07) | 0.07            | 10.99 (-0.81-24.18) | 0.2             | 7.60 (-4.43-21.14) | 0.04            | 13.26 (0.51-27.63) |
| ADIPO2     | I290I                | add   | 0.2             | 0.01 (-0.01-0.03) | 0.07            | 0.45 (-0.04-0.95) | 0.6             | 0.78 (-2.08-3.72) | 0.5             | -1.14 (-4.09-1.90) | 0.4             | 1.20 (-1.84-4.34)  |
|           |                      | rec   | 0.6             | -0.02 (-0.08-0.05) | 0.2             | 1.24 (-0.73-3.25) | 0.07            | 11.22 (-0.77-24.65) | 0.2             | 7.76 (-4.46-21.55) | 0.04            | 13.61 (0.64-28.26) |
| TCF7L2     | add                  |       | 0.4             | 0.00 (-0.01-0.02) | 0.8             | 0.05 (-0.29-0.40) | 0.03            | -2.19 (-4.13-0.20) | 0.03            | -2.31 (-4.36-0.21) | 0.06            | -1.99 (-4.06-0.12) |
|           |                      | dom   | 0.8             | 0.00 (-0.01-0.02) | 0.6             | 0.12 (-0.32-0.57) | 0.09            | -2.24 (-4.74-0.32) | 0.05            | -2.64 (-5.27-0.05) | 0.2             | -1.97 (-4.62-0.76) |
| PPARG      | P12A                 | add   | 0.9             | 0.00 (-0.02-0.02) | 0.2             | -0.32 (-0.83-0.19) | 0.4             | -1.14 (-4.00-1.80) | 0.9             | -0.13 (-3.19-3.03) | 0.3             | -1.65 (-4.66-1.45) |
|           |                      | rec   | 0.7             | -0.02 (-0.10-0.06) | 0.4             | -0.98 (-3.37-1.48) | 0.02            | 18.22 (2.73-36.06) | 0.03            | 18.61 (2.08-37.81) | 0.08            | 14.15 (-1.75-32.62) |
| ADIPOQ     | -11391G/A            | add   | 0.3             | 0.01 (-0.01-0.03) | 1               | -0.01 (-0.52-0.51) | 0.2             | -2.05 (-4.92-0.89) | 0.2             | -1.94 (-4.98-1.19) | 0.2             | -2.24 (-5.27-0.88) |
| ADIPOQ     | -11377C/G            | add   | 0.2             | 0.01 (-0.00-0.02) | 0.9             | -0.02 (-0.37-0.32) | 0.8             | 0.27 (-1.71-2.30) | 0.8             | 0.29 (-1.81-2.44) | 0.8             | 0.30 (-1.81-2.45)  |
| ADIPOQ     | G15G                 | add   | 0.6             | 0.00 (-0.01-0.02) | 0.2             | -0.32 (-0.78-0.14) | 0.3             | 1.32 (-1.34-4.05) | 0.1             | 2.39 (-0.46-5.33)  | 0.6             | 0.81 (-2.00-3.69)  |

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

| Gene | SNP     | rs-ID   | Model | p-val for interaction$^§$ | p-val genotype$^#$ | Δ Glycemia (95% CI) | p-val genotype$^#$ | Δ Glycemia (95% CI) |
|------|---------|---------|-------|---------------------------|-------------------|---------------------|-------------------|---------------------|
|      |         |         |       | age*genotype              | genotype          |                     | genotype          |                     |
|      |         |         |       |                           |                   |                     |                   |                     |
|      |         |         |       |                           |                   |                     |                   |                     |
| *IL6*| -174G/C | rs1800795| add   | 0.009                     | 0.00005           | -1.36 (-2.00/-0.71) | 0.80              | 0.06 (-0.42/0.754) |
|      |         |         |       |                           |                   |                     |                   |                     |
|      |         |         |       |                           |                   |                     |                   |                     |
|      |         |         |       |                           |                   |                     |                   |                     |
|      |         |         |       |                           |                   |                     |                   |                     |
| *IL6R*| D358A  | rs8192284| add   | 0.003                     | 0.10              | -0.54 (-1.20/0.12)  | 0.30              | 0.26 (-0.22/0.74)  |
|      |         |         |       |                           |                   |                     |                   |                     |
|      |         |         |       |                           |                   |                     |                   |                     |
|      |         |         |       |                           |                   |                     |                   |                     |

$^§$The significance of an age-genotype interaction term used in the mixed model regression analysis is given by the first p-value.

$^#$The significance of the genotype term in the model without interaction, including measures at age ≥ 56 or < 56 years, is given by the second p-value.

Δ Glycemia 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 are nominal p-values.
FIGURE LEGENDS

Figure 1. Type 2 diabetes incidence in the D.E.S.I.R. cohort according to genotype for the \textit{KCNJ11} -E23K, \textit{GCK} -30G/A, \textit{IL6} and \textit{IL6R} 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.

Figure 2. Additive effects of multiple at-risk polymorphisms on diabetes and IFG at the end of the follow-up.

The top graph corresponds to the combination of \textit{GCK} -30G/A, \textit{TCF7L2}-rs7903146 and \textit{IL6} -174G/C; and the bottom graph to the combination of \textit{GCK} -30G/A, \textit{TCF7L2}-rs7903146 and \textit{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 \textit{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 \textit{IL6} variant was considered at-risk.

The Population Attributable Risk (PAR) was calculated for individuals carrying at least 3 at-risk alleles versus those carrying less than 3 at-risk alleles; it was evaluated to 27 \% for the combination of \textit{GCK} -30G/A, \textit{TCF7L2}-rs7903146 and \textit{IL6} -174G/C, and to 14 \% for the combination of \textit{GCK} -30G/A, \textit{TCF7L2}-rs7903146 and \textit{SREBF1}-G952G.

Figure 3. ROC curves including the genetic information for two combinations of at-risk polymorphisms.

The top graph corresponds to \textit{GCK} -30G/A, \textit{IL6} -174G/C and \textit{TCF7L2}-rs7903146 combination and the bottom graph to \textit{GCK} -30G/A, \textit{SREBF1}-G952G and \textit{TCF7L2}-rs7903146 combination. The ROC curves and the area under the curve (AUC) were generated after fitting a logistic regression model including only the genetic factors.
FIGURE 2

**Type 2 Diabetes Genetics and lifetime Risk**

**FIGURE 2**

**P-value = 2E-06**  
**OR = 1.34 (1.18 - 1.51)**

**Number of at-risk alleles**

| NG  | T2D  | % of each category |
|-----|------|--------------------|
| 744 | 67   | 27.7%              |
| 956 | 94   | 35.9%              |
| 669 | 82   | 25.7%              |
| 262 | 49   | 10.6%              |

**P-value = 6E-07**  
**OR = 1.21 (1.12 - 1.31)**

**Number of at-risk alleles**

| NG  | IFG+T2D | % of each category |
|-----|---------|--------------------|
| 1177| 344     | 44.7%              |
| 827 | 277     | 32.5%              |
| 417 | 157     | 16.9%              |
| 126 | 74      | 5.9%               |

27
