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RAPID COMMUNICATION

# Analysis of novel risk loci for type 2 diabetes in a general French population: the D.E.S.I.R. study

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**Abstract** Recently, Genome Wide Association (GWA) studies identified novel single nucleotide polymorphisms (SNPs), highly associated with type 2 diabetes (T2D) in several case-control studies of European descent. However, the impact of these markers on glucose homeostasis in a population-based study remains to be clarified.

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The French prospective D.E.S.I.R. study ($N$=4,707) was genotyped for 22 polymorphisms within 14 loci showing nominal to strong association with T2D in recently published GWA analyses (*CDKAL1, IGFBP2, CDKN2A/2B, EXT2, HHEX, LOC646279, SLC30A8, MMP26, KCTD12, LDLR, CAMTA1, LOC38776, NGN3* and *CXCR4*). We assessed their effects on quantitative traits related to glucose homeostasis in 4,283 normoglycemic middle-aged participants at baseline and their contribution to T2D incidence during 9 years of follow-up.

Individuals carrying T2D risk alleles of *CDKAL1* or *SLC30A8* had lower fasting plasma insulin level (rs7756992 $P=0.003$) or lower basal insulin secretion (rs13266634 $P=0.0005$), respectively, than non-carriers. Furthermore, *NGN3* and *MMP26* risk alleles associated with higher fasting plasma glucose levels (rs10823406 $P=0.01$ and rs2499953 $P=0.04$, respectively). However, for these SNPs, only modest associations were found with a higher incidence of T2D: hazard ratios of 2.03 [1.00–4.11] for *MMP26* (rs2499953 $P=0.05$) and 1.33 [1.02–1.73] for *NGN3* (rs10823406 $P=0.03$).

We confirmed deleterious effects of *SLC30A8*, *CDKAL1*, *NGN3* and *MMP26* risk alleles on glucose homeostasis in the D.E.S.I.R. prospective cohort. However, in contrast to *TCF7L2*, the contribution of novel loci to T2D incidence seems only modest in the general middle-aged French population and should be replicated in larger cohorts.

**Keywords** Diabetes · Genetics · Metabolic · Disease

## Introduction

Recently, GWA studies revealed novel single nucleotide polymorphisms (SNPs) strongly associated with type 2 diabetes (T2D) [1–5]. In a French population, the well known rs7903146 *TCF7L2* polymorphism ranked first for its effect on T2D prevalence followed by four new loci: *SLC30A8*, *HHEX*, *LOC387761* and *EXT2* [1]. Subsequently, GWA studies in Finnish, English, Icelandic and Danish cohorts emphasized the role of *CDKAL1*, *CDKN2A/2B* and *IGFBP2* on T2D and confirmed the effect of *TCF7L2*, *SLC30A8* and *HHEX* [2–5]. In the French GWA scan, additional SNPs located in *MMP26*, *LDLR*, *KCTD12*, *CAMTA1*, *NGN*, *CXCR4* and *LOC646279* were also among the 15 first signals in joint stage I and stage II analyses, but their current status is still uncertain. Although large-scale case-control studies are very sensitive in detecting genetic effects, they are not good models for evaluating the true contribution of disease-associated genes in unselected general populations [6].

The objective of our study was to assess whether the risk alleles or risk genotypes ($N=22$) of 14 novel loci from GWA analyses modulate quantitative traits related to glucose homeostasis (fasting glucose, fasting insulin, HOMA-B and HOMA-IR) in the 9-year prospective D.E.S.I.R. cohort ($N=4,707$), a French general middle-aged population [7, 8]. As this cohort is limited in statistical power to analyze genetic variants with modest effects on T2D risk, we also examined their association with hyperglycemia (HG; individuals with T2D or impaired fasting glucose) incidence, as we recently did for *TCF7L2* [9].

## Materials and methods

### Study population

Clinical characteristics of D.E.S.I.R. participants are reported in Supplementary Table 1. A French cohort of 2,576 men and 2,636 women from a general population (aged between 30 and 65 years at inclusion) participated in the D.E.S.I.R. longitudinal study and were clinically and biologically evaluated at inclusion and at 3, 6 and 9-year visits [8]. Participants were recruited from volunteers insured by the French social security system, which offers periodic health examinations free of charge. They came from 10 health examination centers in the western-central part of France. All participants signed an informed consent. The protocol was approved by the Ethics Committee for the Protection of Subjects for Biomedical Research of Bicêtre Hospital. Among the 5,212 subjects of the D.E.S.I.R. cohort, 3,786 individuals were followed during the entire study.

Because the ethnic origin could not be legally documented at the beginning of the D.E.S.I.R. study, we estimated the proportion of subjects having non-European ancestry from a subgroup of 654 subjects selected in the D.E.S.I.R. cohort, as previously described [1]. This subgroup was genotyped for 328 SNPs which were spaced by at least 5 Mb and highly differentiated among individuals from different continents ($Fst>0.2$ based on the Perlegen dataset) [29]. Analysis using the STRUCTURE software identified only two individuals of non-European ancestry in a total of 654 individuals. From this analysis, the proportion of subjects having non-European ancestry was estimated to 0.30% in the D.E.S.I.R. cohort. Additionally, all individuals born outside France were excluded from our analysis.

Three classes of glycemic status were defined according to the 1997 American Diabetes Association criteria [30]: NG, defined as fasting glucose<6.1 mmol/l; IFG, defined as fasting plasma glucose (FPG) between 6.1 and 6.99 mmol/l; and type 2 diabetes, defined as fasting plasma glucose≥7.0 mmol/l and/or treatment by glucose lowering agents. Hyperglycemia (HG) was defined by IFG or T2D.

The study of baseline glucose homeostasis was on the 4,283 NG participants; the study of incident glucose status was on 186 incident T2D participants and 508 incident HG participants.

### Measurements

Venous blood samples were collected in the morning after subjects had fasted for 12 h. Fasting plasma glucose was assayed by the glucose oxidase method applied to fluoro-oxalated plasma, using a Technicon RA1000 (Bayer,

**Table 1** Genotypic distribution of the 22 SNPs studied according to glycemic status at baseline in 4,707 individuals: the D.E.S.I.R Study

| Whole-genome study | Gene | rs ID | $r^2$ | Genotype 1–1 | | | Genotype 1–2 | | | Genotype 2–2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NG | T2D | HG | NG | T2D | HG | NG | T2D | HG |
| Scott [3] | *CDKN2A/2B* | rs564398 | 0 | 630 (0.15) | 15 (0.13) | 63 (0.15) | 1,992 (0.47) | 52 (0.46) | 186 (0.44) | 1,626 (0.38) | 46 (0.41) | 172 (0.41) |
| Zeggini [2] | *CDKN2A/2B* | rs10811661 | | 174 (0.04) | 1 (0.01) | 16 (0.04) | 1,335 (0.31) | 25 (0.22) | 112 (0.26) | 2,742 (0.65) | 89 (0.77) | 295 (0.7) |
| Saxena [4] | *CDKAL1* | rs10946398 (a) | a–b: 0.99 | 2,005 (0.47) | 47 (0.41) | 192 (0.45) | 1,789 (0.42) | 53 (0.46) | 177 (0.42) | 442 (0.1) | 15 (0.13) | 53 (0.13) |
| Steinthorsdottir [5] | *CDKAL1* | rs7754840 (b) | a–c: 0.73 | 2,007 (0.47) | 47 (0.41) | 192 (0.46) | 1,795 (0.42) | 53 (0.46) | 178 (0.42) | 447 (0.11) | 14 (0.12) | 51 (0.12) |
| | *CDKAL1* | rs7756992 (c) | b–c: 0.73 | 2,227 (0.52) | 51 (0.44) | 203 (0.48) | 1,679 (0.4) | 49 (0.43) | 176 (0.42) | 344 (0.08) | 15 (0.13) | 44 (0.1) |
| | *IFGBP2* | rs4402960 | 0.99 | 1,970 (0.46) | 48 (0.42) | 187 (0.44) | 1,850 (0.44) | 60 (0.52) | 200 (0.47) | 421 (0.1) | 7 (0.06) | 37 (0.09) |
| | *IGFBP2* | rs1470579 | | 1,957 (0.46) | 47 (0.41) | 184 (0.44) | 1,854 (0.44) | 61 (0.53) | 201 (0.48) | 429 (0.1) | 7 (0.06) | 37 (0.09) |
| Sladek [1] | *EXT2* | rs1113132 (a) | a–b: 0.99 | 305 (0.07) | 15 (0.13) | 39 (0.09) | 1,664 (0.39) | 43 (0.37) | 166 (0.39) | 2,259 (0.53) | 57 (0.5) | 217 (0.51) |
| | *EXT2* | rs11037909 (b) | b–c: 0.99 | 303 (0.07) | 16 (0.14) | 40 (0.1) | 1,665 (0.4) | 41 (0.36) | 162 (0.39) | 2,245 (0.53) | 58 (0.5) | 216 (0.52) |
| | *EXT2* | rs3740878 (c) | a–c: 0.99 | 305 (0.07) | 16 (0.14) | 40 (0.09) | 1,673 (0.39) | 41 (0.36) | 163 (0.39) | 2,263 (0.53) | 58 (0.5) | 219 (0.52) |
| | *EXT2* | rs729287 (d) | b–d: 0.98<br>a–d: 0.97<br>c–d: 0.97 | 299 (0.07) | 16 (0.14) | 39 (0.09) | 1,669 (0.39) | 41 (0.36) | 165 (0.39) | 2,262 (0.53) | 58 (0.5) | 220 (0.52) |
| | *HHEX* | rs1111875 | 0.76 | 681 (0.16) | 10 (0.09) | 57 (0.14) | 1,968 (0.47) | 47 (0.41) | 187 (0.45) | 1,573 (0.37) | 57 (0.5) | 176 (0.42) |
| | *HHEX* | rs7923837 | | 616 (0.15) | 6 (0.05) | 47 (0.11) | 1,963 (0.46) | 50 (0.43) | 191 (0.45) | 1,667 (0.39) | 59 (0.51) | 186 (0.44) |
| | *LOC646279* | rs1256517 | na | 3,136 (0.74) | 82 (0.72) | 315 (0.75) | 1,018 (0.24) | 31 (0.27) | 99 (0.24) | 84 (0.02) | 1 (0.01) | 6 (0.01) |
| | *SLC30A8* | rs13266634 | na | 375 (0.09) | 6 (0.05) | 28 (0.07) | 1,758 (0.43) | 42 (0.37) | 165 (0.39) | 1,996 (0.48) | 67 (0.58) | 225 (0.54) |
| | *MMP26* | rs2499953 | na | 4,128 (0.97) | 113 (0.98) | 404 (0.95) | 121 (0.03) | 2 (0.02) | 20 (0.05) | 1 (0) | 0 (0) | 0 (0) |
| | *KCTD12* | rs2876711 | na | 710 (0.17) | 17 (0.15) | 71 (0.17) | 2,119 (0.5) | 56 (0.49) | 206 (0.49) | 1,419 (0.33) | 41 (0.36) | 145 (0.34) |
| | *LDLR* | rs6413504 | na | 1,062 (0.25) | 34 (0.3) | 107 (0.25) | 2,069 (0.49) | 56 (0.49) | 216 (0.51) | 1,060 (0.25) | 24 (0.21) | 100 (0.24) |
| | *CAMTA1* | rs1193179 | na | 2,372 (0.56) | 70 (0.61) | 250 (0.59) | 1,568 (0.37) | 37 (0.32) | 150 (0.35) | 282 (0.07) | 8 (0.07) | 24 (0.06) |
| | *LOC387761* | rs7480010 | na | 2,147 (0.51) | 57 (0.5) | 213 (0.5) | 1727 (0.41) | 50 (0.43) | 177 (0.42) | 360 (0.09) | 8 (0.07) | 33 (0.08) |
| | *NGN3* | rs10823406 | na | 247 (0.06) | 6 (0.05) | 25 (0.06) | 1,560 (0.37) | 41 (0.36) | 143 (0.34) | 2,400 (0.57) | 67 (0.59) | 255 (0.6) |
| | *CXCR4* | rs932206 | na | 1,017 (0.24) | 31 (0.27) | 90 (0.21) | 2,058 (0.49) | 54 (0.47) | 211 (0.5) | 1,158 (0.27) | 30 (0.26) | 123 (0.29) |

*NG* Normoglycemic, *HG* hyperglycemic (T2D+IFG), *T2D* type 2 diabetic, *Allele 2* risk allele for T2D in whole-genome association studies, $r^2$ linkage disequilibrium

**Table 2** Effects of the 22 SNPs on fasting glucose and fasting insulin in 4,283 individuals normoglycemic at baseline: the D.E.S.I.R. Study

| Whole-genome study | Gene | rs ID | Fasting glucose (mmol/l) | | | | Fasting insulin (pmol/l) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1–1 | 1–2 | 2–2 | *P* | 1–1 | 1–2 | 2–2 | *P* |
| Scott [3] | *CDKN2A/2B* | rs10811661 | 5.18±0.43 | 5.19±0.44 | 5.20±0.45 | 0.2 | 44.07±23.74 | 44.85±25.75 | 44.13±26.57 | 0.6 |
| Zeggini [2] | *CDKN2A/2B* | rs564398 | 5.20±0.45 | 5.19±0.45 | 5.21±0.45 | 0.07 | 45.92±34.98 | 44.86±25.45 | 43.33±23.49 | 0.06 |
| Saxena [4] | *CDKAL1* | rs7754840 | 5.19±0.44 | 5.20±0.45 | 5.19±0.46 | 1 | 44.90±28.38 | 43.96±23.73 | 44.20±27.51 | 0.05 |
| Steinthorsdottir [5] | *CDKAL1* | rs7756992 | 5.19±0.44 | 5.20±0.46 | 5.19±0.44 | 0.9 | 44.97±27.66 | 44.26±24.65 | 42.26±26.67 | 0.003 |
| | *CDKAL1* | rs10946398 | 5.19±0.44 | 5.20±0.45 | 5.19±0.46 | 1 | 44.98±28.4 | 43.93±23.74 | 44.17±27.56 | 0.04 |
| | *IFGBP2* | rs4402960 | 5.19±0.45 | 5.21±0.44 | 5.20±0.45 | 0.4 | 44.35±28.43 | 44.39±24.2 | 45.30±26.10 | 0.2 |
| | *IGFBP2* | rs1470579 | 5.19±0.45 | 5.21±0.44 | 5.20±0.45 | 0.5 | 44.38±28.5 | 44.4±24.19 | 45.32±25.99 | 0.2 |
| Sladek [1] | *EXT2*$^{d}$ | rs1113132 | 5.20±0.43 | 5.20±0.45 | 5.20±0.45 | 0.6 | 44.69±23.1 | 45.02±25.88 | 44.19±27.22 | 1.0 |
| | *EXT2*$^{d}$ | rs3740878 | 5.20±0.43 | 5.20±0.45 | 5.20±0.45 | 0.8 | 44.44±23.05 | 45.09±25.91 | 44.11±27.20 | 1.0 |
| | *EXT2*$^{d}$ | rs11037909 | 5.20±0.44 | 5.19±0.45 | 5.20±0.45 | 0.6 | 44.24±22.81 | 45.01±25.92 | 44.16±27.24 | 1.0 |
| | *EXT2*$^{d}$ | rs729287 | 5.21±0.43 | 5.19±0.45 | 5.20±0.45 | 0.7 | 44.90±23.16 | 45.04±26.00 | 44.15±27.17 | 0.8 |
| | *HHEX* | rs1111875 | 5.19±0.45 | 5.19±0.45 | 5.20±0.44 | 0.09 | 44.26±24.99 | 44.75±25.11 | 44.04±28.06 | 0.9 |
| | *HHEX* | rs7923837 | 5.19±0.45 | 5.20±0.45 | 5.20±0.45 | 0.09 | 45.23±25.68 | 44.58±25.29 | 44.21±27.96 | 0.6 |
| | *LOC646279* | rs1256517 | 5.20±0.45 | 5.19±0.44 | 5.25±0.47 | 1 | 44.25±26.58 | 44.90±25.94 | 45.59±22.99 | 0.3 |
| | *SLC30A8*[a] | rs13266634 | 5.13±0.46 | 5.19±0.45 | 5.22±0.44 | 0.0003 | 46.32±36.83 | 45.19±25.74 | 44.03±25.05 | 0.2 |
| | *MMP26* | rs2499953 | 5.20±0.45 | 5.22±0.44 | 5.34±na | 0.04 | 44.61±26.57 | 42.01±20.81 | 26.31±na | 0.9 |
| | *KCTD12*$^{r}$ | rs2876711 | 5.17±0.45 | 5.20±0.44 | 5.21±0.45 | 0.2 | 45.49±33.24 | 43.66±23.86 | 45.24±26.13 | 0.5 |
| | *LDLR* | rs6413504 | 5.20±0.43 | 5.20±0.46 | 5.20±0.44 | 0.4 | 44.96±30.88 | 44.29±25.56 | 44.61±23.05 | 0.7 |
| | *CAMTA1* | rs1193179 | 5.20±0.44 | 5.20±0.46 | 5.18±0.47 | 0.8 | 44.47±24.81 | 44.95±28.79 | 43.17±26.55 | 0.2 |
| | *LOC387761* | rs7480010 | 5.19±0.45 | 5.20±0.45 | 5.21±0.43 | 0.5 | 44.16±24.37 | 44.38±27.55 | 47.13±31.97 | 0.4 |
| | *NGN3* | rs10823406 | 5.10±0.45 | 5.20±0.46 | 5.21±0.44 | 0.01 | 42.97±22.84 | 44.73±29.17 | 44.68±24.98 | 0.5 |
| | *CXCR4* | rs932206 | 5.19±0.44 | 5.21±0.45 | 5.19±0.44 | 0.9 | 44.31±24.34 | 45.38±28.98 | 43.15±23.25 | 0.8 |

Data are presented as means±standard deviations

*P* values are from linear regression models after adjustments for BMI age and gender

*Allele 2* Risk allele for T2D in whole-genome association studies, *d* dominant genetic model, *r* recessive genetic model, *na* not available

[a] Remained significant after Bonferroni (multiple-testing) correction

Puteaux, France) or a Kone Automate (Evry, France). To adjust for differences between and within laboratories over the four triennial examinations, glucose concentrations were standardized by age and sex with respect to a reference population of 211,427 individuals examined in the IRSA Health Examination Centres and assayed in the IRSA laboratory. Fasting serum insulin was measured by an enzymo-immunoassay with IMX (Abbott, Rungis, France) (48). Insulin secretion was assessed by calculating the HOMA-B index, defined as (fasting insulin×20)/(fasting glucose−35), and peripheral insulin resistance was estimated by HOMA-IR, defined as (fasting insulin×fasting glucose)/22.5. Other biological and metabolic parameters were assessed as previously described [8].

### SNP genotyping

All participants were genotyped using an allelic discrimination assay-by-design TaqMan method on ABI 7900 (Applied Biosystems). There was a 97–99% genotyping success rate (Table 1). The genotyping error rate was assessed by randomly re-genotyping 384 participants. No difference was found with the first genotyping results. The genotypic distributions of all polymorphisms were in Hardy–Weinberg equilibrium.

### Statistical analysis

Polymorphism effects on quantitative traits at baseline were calculated by linear regression models adjusted for age, gender and BMI. All metabolic traits were log-transformed for linear regression analysis in order to normalize their distribution. Hazard ratios for HG and T2D incidence were assessed using the Cox model, adjusted for age, gender and BMI. For each study on quantitative traits or T2D/HG incidence, a conservative Bonferroni correction (multiplication by the number of SNPs) was applied to the *P* values for multiple comparisons. All *P* values are two-sided. SPSS (version 14.0.2) and *R* statistics (version 2.5.1) software were used for general statistics.

## Results

### Metabolic effects on glucose homeostasis

Clinical characteristics at baseline of the 4,707 participants of the D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance syndrome) study are reported in Supplementary Table 1. Physiological effects of the studied SNPs on quantitative phenotypes related to glucose

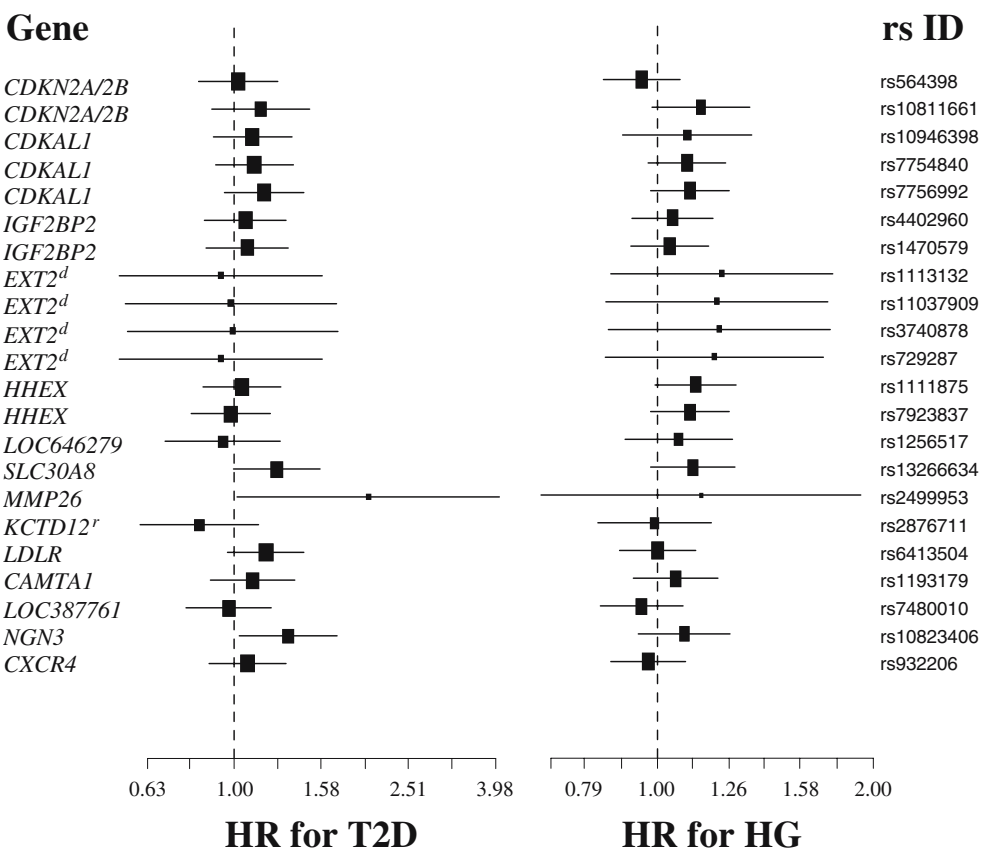


**Fig. 1** Hazards ratios for hyperglycemia (*HG*) and type 2 diabetes (*T2D*) incidence in a French general population. Hazard ratios (*HR*) were assessed by Cox survival analysis (adjusted for BMI, age, and gender) during the 9 years of follow-up. All genetic models were additive except for *EXT2* (*d*: dominant) and *KCTD12* (*r*: recessive)

homeostasis were analyzed in all participants normoglycemic (NG) at baseline ($N$=4,283). The genotypic distribution of each SNP at baseline is presented in Table 1. Fasting plasma glucose levels were higher in participants carrying risk alleles of *SLC30A8* (rs13266634 $P$=0.0003), *NGN3* (rs10823406 $P$=0.01) and *MMP26* (rs2499953 $P$=0.04) with no impact on fasting insulin (Table 2). Conversely, an association with lower fasting plasma insulin level was found in subjects carrying risk alleles of *CDKAL1* (rs7756992 $P$=0.003, rs10946398 $P$=0.04 and rs7754840 $P$=0.05) with no detectable effect on fasting glucose. In individuals carrying one of the studied risk alleles (rs564398) of *CDKN2A/2B*, trends for associations were observed with higher fasting glucose ($P$=0.07) and lower fasting insulin ($P$=0.06).

In a second analysis, effects on HOMA indices were also assessed as markers of insulin secretion (HOMA-B) and insulin resistance (HOMA-IR) (Table 3). We found lower HOMA-B value in individuals with the *SLC30A8* risk allele (rs13266634 $P$=0.0005) and a trend towards such a decrease in individuals with the *HHEX* risk alleles (rs7923837 $P$=0.06 and rs1111875 $P$=0.07). Furthermore, lower HOMA-IR value ($P$=0.04) were detected in participants carrying one of the *CDKN2A/2B* (rs564398) risk alleles. The *SLC30A8* genetic variant was the only SNP remaining significant after conservative Bonferroni corrections for its effects on fasting glucose and HOMA-B.

## Association with type 2 diabetes and hyperglycemia incidence

The genotypic distribution of incident T2D and HG cases as well as individuals remaining NG after 9 years of follow-up is presented in Table 4; in order to increase the statistical power, participants with impaired fasting glucose (IFG) or T2D were studied together as a single group of HG subjects [9, 10]. For each SNP, the best fitting genetic model was selected from our previous GWA results in French individuals [1]. In this regard, every polymorphism was analyzed using an additive genetic model except for *EXT2* (dominant model) and *KCTD12* (recessive model). For *CDKAL1*, *CDKN2A/2B*, *IGFBP2*, *HHEX* and *EXT2*, we studied more than one SNP because they have all been reported as associated with T2D in previous studies [1–5].

Only trends or nominal associations with T2D incidence were found with hazard ratios (HR) of 2.03 [1.00–4.11] (rs2499953 $P$=0.05) for *MMP26*, 1.33 [1.02–1.73] (rs10823406 $P$=0.03) for *NGN3* and 1.25 [0.99–1.58] (rs13266634 $P$=0.06) for *SLC30A8* (Fig. 1). Two other genetic variants were also associated with trends towards a

**Table 3** Effects of the 22 SNPs on HOMA indices in 4,283 individuals' normoglycemic at baseline: the D.E.S.I.R. Study

| Whole-genome study | Gene | rs ID | HOMA-B | | | | HOMA-IR | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1–1 | 1–2 | 2–2 | *P* | 1–1 | 1–2 | 2–2 | *P* |
| Scott [3] | *CDKN2A/2B* | rs10811661 | 93.89±47.15 | 90.17±54.68 | 91.86±64.67 | 0.6 | 1.73±0.95 | 1.72±1.04 | 1.70±1.00 | 0.7 |
| Zeggini [2] | *CDKN2A/2B* | rs564398 | 92.41±59.91 | 93.14±67.63 | 88.81±51.63 | 0.1 | 1.75±1.10 | 1.74±1.05 | 1.65±0.92 | 0.04 |
| Saxena [4] | *CDKAL1* | rs7754840 | 90.59±52.45 | 91.43±69.30 | 94.49±61.30 | 0.8 | 1.70±0.99 | 1.70±1.00 | 1.76±1.16 | 0.7 |
| Steinthorsdottir [5] | *CDKAL1* | rs7756992 | 90.86±53.23 | 92.65±71.42 | 89.48±53.40 | 0.4 | 1.70±0.97 | 1.72±1.04 | 1.67±1.13 | 0.1 |
| | *CDKAL1* | rs10946398 | 90.80±52.72 | 91.38±69.26 | 94.51±61.44 | 0.7 | 1.70±0.99 | 1.70±1.00 | 1.76±1.16 | 0.7 |
| | *IFGBP2* | rs4402960 | 92.54±69.85 | 90.03±51.61 | 92.60±55.87 | 0.5 | 1.69±1.01 | 1.71±1.00 | 1.76±1.08 | 0.3 |
| | *IGFBP2* | rs1470579 | 92.73±70.05 | 90.15±51.57 | 92.61±55.58 | 0.5 | 1.69±1.01 | 1.71±1.00 | 1.76±1.08 | 0.3 |
| Sladek [1] | *EXT2*[d] | rs1113132 | 89.80±48.42 | 92.30±56.86 | 91.35±65.25 | 0.8 | 1.68±0.90 | 1.73±1.00 | 1.70±1.03 | 0.5 |
| | *EXT2*[d] | rs3740878 | 89.25±48.49 | 92.27±56.95 | 91.15±65.13 | 0.7 | 1.67±0.90 | 1.73±1.00 | 1.70±1.03 | 0.4 |
| | *EXT2*[d] | rs11037909 | 88.73±47.84 | 92.31±56.93 | 91.33±65.30 | 0.7 | 1.66±0.89 | 1.73±1.00 | 1.70±1.03 | 0.4 |
| | *EXT2*[d] | rs729287 | 88.47±43.57 | 92.46±57.70 | 91.21±65.10 | 0.7 | 1.69±0.91 | 1.73±1.01 | 1.70±1.03 | 0.5 |
| | *HHEX* | rs1111875 | 91.20±53.42 | 94.12±69.94 | 88.19±51.03 | 0.07 | 1.70±0.95 | 1.73±1.03 | 1.69±1.02 | 0.8 |
| | *HHEX* | rs7923837 | 92.49±52.67 | 93.29±69.61 | 88.95±51.90 | 0.06 | 1.72±0.96 | 1.72±1.02 | 1.70±1.02 | 0.7 |
| | *LOC646279* | rs1256517 | 91.22±63.06 | 92.31±55.97 | 89.39±42.20 | 0.5 | 1.71±1.01 | 1.71±1.01 | 1.74±0.87 | 0.5 |
| | *SLC30A8*[a] | rs13266634 | 96.90±49.83 | 94.88±71.83 | 88.03±53.38 | 0.0005 | 1.74±1.04 | 1.74±1.05 | 1.70±0.98 | 0.6 |
| | *MMP26* | rs2499953 | 91.78±61.39 | 85.35±49.99 | 75.60±na | 0.2 | 1.71±1.02 | 1.67±0.88 | 0.91±na | 0.7 |
| | *KCTD12*[r] | rs2876711 | 93.09±54.16 | 88.98±50.77 | 94.94±76.98 | 0.2 | 1.74±1.09 | 1.68±1.00 | 1.73±0.99 | 0.4 |
| | *LDLR* | rs6413504 | 90.69±54.53 | 91.33±56.69 | 92.88±73.90 | 0.8 | 1.71±1.04 | 1.70±1.02 | 1.73±0.97 | 0.4 |
| | *CAMTA1* | rs1193179 | 90.75±51.72 | 92.23±60.20 | 96.15±116.18 | 0.7 | 1.71±1.00 | 1.72±1.02 | 1.69±1.13 | 0.7 |
| | *LOC387761* | rs7480010 | 91.89±67.52 | 90.30±51.95 | 93.98±58.65 | 0.8 | 1.70±0.96 | 1.71±1.02 | 1.77±1.22 | 0.9 |
| | *NGN3* | rs10823406 | 99.22±56.63 | 92.36±73.48 | 90.55±52.20 | 0.1 | 1.62±0.98 | 1.73±1.04 | 1.71±1.00 | 0.7 |
| | *CXCR4* | rs932206 | 91.77±60.39 | 92.71±56.05 | 89.14±69.92 | 0.9 | 1.72±0.99 | 1.75±1.07 | 1.63±0.91 | 0.5 |

Data are presented as means±standard deviations, and after log-transformation
*P* values are from linear regression models after adjustments for BMI age and gender
*Allele 2* Risk allele for T2D in whole-genome association studies, *d* dominant genetic model *r* recessive genetic model, *na* not available
[a] Remained significant Bonferroni (multiple-testing) correction

**Table 4** Genotypic distribution of the 22 SNPs according to incident T2D (*N*=186) or HG (*N*=508): the D.E.S.I.R. Study

| SNPs identified in whole-genome studies | Gene | rs ID | Genotype 1–1 | | Genotype 1–2 | | Genotype 2–2 | | *P* value (HR) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | T2D | HG | T2D | HG | T2D | HG | T2D | HG |
| Scott [3] | *CDKN2A/2B* | rs564398 | 27 (0.15) | 81 (0.16) | 85 (0.46) | 240 (0.48) | 74 (0.4) | 183 (0.36) | 0.9 | 0.4 |
| Zeggini [2] | *CDKN2A/2B* | rs10811661 | 6 (0.03) | 14 (0.03) | 56 (0.3) | 149 (0.3) | 124 (0.67) | 341 (0.68) | 0.3 | 0.08 |
| Saxena [4] | *CDKAL1* | rs10946398 (a) | 85 (0.46) | 219 (0.44) | 74 (0.4) | 223 (0.45) | 26 (0.14) | 59 (0.12) | 0.4 | 0.4 |
| Steinthorsdottir [5] | *CDKAL1* | rs7754840 (b) | 84 (0.46) | 219 (0.44) | 74 (0.4) | 224 (0.45) | 26 (0.14) | 59 (0.12) | 0.3 | 0.1 |
| | *CDKAL1* | rs7756992 (c) | 86 (0.46) | 240 (0.48) | 80 (0.43) | 218 (0.43) | 19 (0.1) | 44 (0.09) | 0.1 | 0.1 |
| | *IFGBP2* | rs4402960 | 80 (0.43) | 223 (0.44) | 85 (0.46) | 220 (0.44) | 20 (0.11) | 60 (0.12) | 0.6 | 0.4 |
| | *IGFBP2* | rs1470579 | 78 (0.42) | 222 (0.44) | 86 (0.47) | 219 (0.44) | 20 (0.11) | 60 (0.12) | 0.5 | 0.5 |
| Sladek [1] | *EXT2*[d] | rs1113132 (a) | 14 (0.08) | 31 (0.06) | 71 (0.38) | 192 (0.38) | 100 (0.54) | 278 (0.55) | 0.8 | 0.3 |
| | *EXT2*[d] | rs11037909 (b) | 13 (0.07) | 31 (0.06) | 71 (0.39) | 194 (0.39) | 99 (0.54) | 275 (0.55) | 0.9 | 0.3 |
| | *EXT2*[d] | rs3740878 (c) | 13 (0.07) | 31 (0.06) | 74 (0.4) | 196 (0.39) | 99 (0.53) | 279 (0.55) | 1 | 0.3 |
| | *EXT2*[d] | rs729287 (d) | 14 (0.08) | 32 (0.06) | 73 (0.39) | 197 (0.39) | 99 (0.53) | 277 (0.55) | 0.8 | 0.3 |
| | *HHEX* | rs1111875 | 29 (0.16) | 66 (0.13) | 86 (0.47) | 234 (0.47) | 67 (0.37) | 197 (0.4) | 0.8 | 0.06 |
| | *HHEX* | rs7923837 | 26 (0.14) | 56 (0.11) | 93 (0.5) | 247 (0.49) | 67 (0.36) | 203 (0.4) | 0.8 | 0.1 |
| | *LOC646279* | rs1256517 | 138 (0.75) | 366 (0.73) | 45 (0.24) | 121 (0.24) | 2 (0.01) | 15 (0.03) | 0.7 | 0.4 |
| | *SLC30A8* | rs13266634 | 12 (0.07) | 35 (0.07) | 72 (0.39) | 213 (0.43) | 100 (0.54) | 251 (0.5) | 0.06 | 0.1 |
| | *MMP26* | rs2499953 | 178 (0.96) | 492 (0.97) | 8 (0.04) | 14 (0.03) | 0 (0) | 0 (0) | 0.05 | 0.6 |
| | *KCTD12*[r] | rs2876711 | 33 (0.18) | 96 (0.19) | 95 (0.51) | 242 (0.48) | 57 (0.31) | 165 (0.33) | 0.3 | 0.9 |
| | *LDLR* | rs6413504 | 40 (0.22) | 138 (0.28) | 95 (0.51) | 238 (0.48) | 50 (0.27) | 125 (0.25) | 0.1 | 0.9 |
| | *CAMTA1* | rs1193179 | 104 (0.56) | 280 (0.56) | 71 (0.38) | 188 (0.38) | 11 (0.06) | 32 (0.06) | 0.4 | 0.4 |
| | *LOC387761* | rs7480010 | 88 (0.48) | 252 (0.5) | 83 (0.45) | 215 (0.43) | 13 (0.07) | 37 (0.07) | 0.8 | 0.5 |
| | *NGN3* | rs10823406 | 6 (0.03) | 24 (0.05) | 64 (0.35) | 189 (0.38) | 115 (0.62) | 288 (0.57) | 0.03 | 0.2 |
| | *CXCR4* | rs932206 | 39 (0.21) | 127 (0.25) | 100 (0.54) | 246 (0.49) | 46 (0.25) | 131 (0.26) | 0.5 | 0.6 |

*HG* Hyperglycemic (T2D+IFG), *T2D* type 2 diabetic, *Allele 2* risk allele for T2D in whole-genome association studies, *HR* hazard ratio for hyperglycemia (HG) or type 2 diabetes (T2D) incidence assessed by Cox survival analysis (adjusted for BMI, age, and gender), $r^2$ linkage disequilibrium, *d* dominant genetic model *r* recessive genetic model

higher incidence of HG with HRs of 1.15 [0.98–1.35] (rs10811661 $P$=0.08) for *CDKN2A/2B* and 1.13 [0.99–1.29] (rs1111875 $P$=0.06) for *HHEX* (Fig. 1). As expected, no result remained significant after conservative Bonferroni correction.

## Discussion

In the present study, we confirm that individuals from a general population of European origin carrying *CDKAL1* or *SLC30A8* T2D risk alleles had lower fasting plasma insulin level and lower basal insulin secretion, respectively, compared to non-carriers [5, 11, 12]. However, higher glycemia and T2D incidence were only detected in carriers of the *SLC30A8* risk SNP. These data are in agreement with the known function of the zinc transporter ZnT8 (*SLC30A8* protein) which is specifically expressed in pancreatic endocrine cells and may participate in insulin secretion [13, 14]. *CDKAL1* is also highly expressed in human islets [2] and shares homology with a known inhibitor of *CDK5* activation which is implicated in beta-cell function [15]. Our results further support a physiological impact of these genes on the beta-cell function.

Interestingly, the T2D risk alleles of *NGN3* or *MMP26* were found to be associated with a higher glycemia and T2D incidence in the D.E.S.I.R. cohort, confirming what was previously found in the French case/control GWA study [1]. Despite the fact that no obvious signal was found in these loci by other GWA scans [2–5, 16–19], further meta-analyses and systematic GWA phase 2 studies will be necessary to fully evaluate their contribution, if any, to T2D. These two genes are indeed good biological candidates: the transcription factor *NGN3* is essential for the differentiation of pancreatic progenitor cells into endocrine cells [20] and *MMP26* is also a target of the Wnt signaling pathway [21].

More modestly, *HHEX* and *CDKN2A/2B* SNPs showed a tendency to be associated with HG as well as with lower insulin secretion and lower insulin levels, respectively, as found previously [22, 23]. *HHEX*, known to be a target of the Wnt signaling pathway, is essential for pancreatic development [24]. The nominal effect of *CDKN2A/2B* rs564398 on lower insulin resistance estimated by HOMA-IR may be an artifact due to its association with both lower insulin level and greater glucose level. It was suggested that *CDKN2A* could be a possible biological candidate for T2D [25], as its over-expression in rodents causes a decrease in islet proliferation [26]. More recently, a new large antisense non-coding RNA named *ANRIL* was characterized [27] and constitutes another good positional candidate.

Surprisingly, we were unable to find any effect of *IGFBP2*, *EXT2*, *LOC646279*, *KCTD12*, *LDLR*, *CAMTA1*, *LOC38776* or *CXCR4* SNPs. This may be due to their modest effects on T2D incidence (or absence of true contribution) in this French non-selected general population and/or may be explained by limitations in the statistical power of the D.E.S.I.R study. Further investigations in larger prospective cohorts are therefore necessary to address this issue.

It was suggested that decreased insulin secretion, but not insulin resistance determines future glucose intolerance in non-obese subjects [28]. Similarly our data suggest that the best hits identified by the GWA studies modulate beta-cell function but not insulin resistance, at least in the D.E.S.I.R. cohort, mostly composed of non-obese participants. Additional analyses of these genetic variants in obese cohorts may therefore put new functional defect forward.

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**Duality of interest** The authors declare that there is no duality of interest.

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