Brief Genetics Report

EIF4A2 Is a Positional Candidate Gene at the 3q27 Locus Linked to Type 2 Diabetes in French Families

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One of the most replicated loci influencing type 2 diabetes–related quantitative traits (quantitative trait loci [QTL]) is on chromosome 3q27 and modulates both type 2 diabetes– and metabolic syndrome–associated phenotypes. A QTL for type 2 diabetes age of onset (logarithm of odds [LOD] score = 3.01 at D3S3686, $P = 0.0001$) was identified in a set of French families. To assess genetic variation underlying both age-of-onset QTL and our previous type 2 diabetes linkage in a 3.87-Mb interval, we explored 36 single nucleotide polymorphisms (SNPs) in two biologically relevant candidate genes for glucose homeostasis, kininogen (*KNG1*), and eukaryotic translation initiation factor 4α2 (*EIF4A2*). Analysis of 148 families showed significant association of a frequent SNP, rs266714, located 2.47 kb upstream of *EIF4A2*, with familial type 2 diabetes (family-based association test, $P = 0.0008$) and early age of onset ($P = 0.0008$). This SNP also contributes to both age-of-onset QTL (1.13 LOD score decrease $P = 0.02$) and type 2 diabetes linkage (genotype identical-by-descent sharing test, $P = 0.02$). However, no association was observed in three independent European diabetic cohorts. EIF4A2 controls specific mRNA translation and protein synthesis rate in pancreatic β-cells, and our data indicates that EIF4A2 is downregulated by high glucose in rat β-INS832/13 cells. The potential role of EIF4A2 in glucose homeostasis and its putative contribution to type 2 diabetes in the presence of metabolic stress will require further investigation. *Diabetes* 55:1171–1176, 2006

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EIF4A2, eukaryotic translation initiation factor 4α2; FBAT, family-based association test; IBD, identical by descent; LOD, logarithm of odds; MAF, minor allele frequency; MLS, maximum LOD score; SNP, single nucleotide polymorphism; QTL, quantitative trait loci.

In the French Caucasian families, chromosome 3q27-qter showed significant evidence of linkage with early-onset type 2 diabetes, defined by at least two sibs diagnosed before 45 years of age (1). Several studies have reported linkage to 3q27 with type 2 diabetes (2) and intermediate traits related to glucose and lipid metabolism (3,4) in different populations. Thus, genetic variation at this locus may modulate multiple phenotypes and increase risk for metabolic disorders. Variants of *ACDC/AdipoQ*, encoding adiponectin, are associated with type 2 diabetes in numerous populations (5) but do not contribute to the 3q27 linkage in the French families (6), suggesting that other genes may be involved. We aimed to identify gene variants underlying the early-onset type 2 diabetes linkage. As young age at diagnosis is likely to reflect an increased genetic predisposition (7), investigating such genetic variations may give additional power to detect diabetes susceptibility genes (8).

The quantitative trait loci (QTL) approach was proven powerful in the identification of genes involved in complex disease pathogenesis in both animals and humans (9,10). By reanalyzing 23 microsatellite markers from our previous genome scan in 148 French families, we identified a type 2 diabetes age-of-onset QTL (logarithm of odds [LOD] score = 3.01 for marker D3S3686; $P = 0.0001$) (online appendix Fig. 1 [available at http://diabetes.diabetesjournals.org]). After removing 81 individuals from the concordant sibpairs (age at diagnosis <45 years for both affected individuals of the sibship), the QTL remains significant (LOD score = 1.15; $P = 0.01$), suggesting that it is not entirely due to the affected pairs with earliest age at diagnosis. The QTL overlaps most of the initial type 2 diabetes linkage peak 95% CI, strengthening the probability for a type 2 diabetes gene(s) at this locus. This result also gives additional information on type 2 diabetes gene location, despite a moderate age-of-onset heritability (12%) in this sample, due to familial structure (sibpairs only) and ascertainment criteria.

Twenty-one genes map to the 3.87-Mb QTL interval (May 2004 Human Genome Assembly), including physiological candidate genes *AHSG* (11) and *SST* (12), whose genetic variations do not contribute to the 3q27 linkage (13). Two other genes are biologically relevant for glucose homeostasis: *KNG1*, which is mainly expressed in liver, encodes kininogen, and deficiency in *KNG1* leads to enhanced insulin sensitivity in rodents (14); and *EIF4A2* encoding eukaryotic translation initiation factor 4α2 (EIF4A2), an

TABLE 1
FBAT of six nonredundant SNPs according to type 2 diabetes, early-onset type 2 diabetes, and age of onset corrected for sex

SNP (major/minor allele)	MAF			T2D		EOD		Age of onset corrected for sex		Mean age of onset (years)		
	T2D subjects (n = 432)	EOD subjects (n = 168)	NGT subjects (n = 129)	Z	P	Z	P	Z	P	11	12	22
rs3806688 (T/C)	24.8	25.2	24.3	−0.97	0.33	−0.9	0.37	0.5	0.62	50.4	50.8	53.0
rs5029973 (T/C)	8.7	11.4	7.8	0.37	0.71	2.18	**0.03**	−1.55	0.12	51.1	49.6	43.2
rs1851665 (A/G)	27.5	29.4	25.6	−0.35	0.73	1.14	0.25	−1.38	0.17	51.1	49.9	53.3
rs182051 (G/T)	17.3	18.8	18.9	−0.53	0.59	0.90	0.37	−1.18	0.24	50.8	48.5	57.1
rs266714 (C/T)	23.8	28.0	23.4	3.36	**0.0008**	2.95	**0.003**	−3.37	**0.0008**	52.3	49.0	47.7
rs266720 (C/T)	47.5	46.9	51.5	−0.09	0.93	0.75	0.45	−0.64	0.53	51.6	50.7	51.4

The group-specific MAFs are given for each SNP; either all type 2 diabetic subjects from the family sample or diabetic subjects with age of onset before 45 years of age are considered. The familial association was performed in 633 individuals of 148 French families previously scanned for linkage. Association with each trait was assessed by the FBAT software. A positive Z value corresponds, for a binary trait, to an excess of allele transmitted to affected subjects, and for a quantitative trait, to a higher mean of the continuous variable for the considered allele (here, the minor allele). The mean age of onset is indicated according to each genotype status (major allele as 1 and minor allele as 2). The threshold for significance is 0.05; significant P values are indicated in bold. EOD, early-onset type 2 diabetes; NGT, normal glucose tolerance; T2D, type 2 diabetes.

ubiquitous RNA helicase involved in the protein translation initiation, is downregulated in rat β-INS832/13 cells exposed to high-glucose concentrations (online appendix Fig. 2). The remaining 16 genes in the region were not considered obvious candidates upon evaluation of biological and physiological criteria including function and expression data.

To investigate whether genetic variations in *KNG1* and *EIF4A2* (including the intergenic region and *RFC4* gene downstream of *EIF4A2*) contribute to the observed linkage, 26 common single nucleotide polymorphisms (SNPs) (minor allele frequency [MAF] >5%), including six tag-SNPs (HapMap release 8, June 2004), were selected from the dbSNP database. Ten additional frequent SNPs were identified by full gene screening in 40 diabetic probands from families contributing to the 3q27 linkage (maximum LOD score [MLS] > 0.76; see RESEARCH DESIGN AND METHODS, group 1). All of the 36 SNPs were genotyped in two groups of diabetic probands from families (groups 1 and 2 for families not contributing to the linkage; MLS = 0) in order to identify at-risk allele(s) overrepresented in diabetic individuals from families contributing to the linkage. To investigate type 2 diabetes association, 21 nonredundant variants ($r^2 < 0.8$ estimated in groups 1 and 2 separately) were genotyped in 94 normoglycemic control subjects who are spouses from additional French families not analyzed for linkage (group 3). Significant differences of allele frequencies ($P < 0.05$) were observed *1*) between groups 1 and 2 for SNP rs182051 (MAF = 18.9 vs. 7.1%, P = 0.04); *2*) between diabetic (groups 1 and 2) and control subjects for SNPs rs3806688, rs50269973, rs1851665, rs182051, and rs266720 ($0.02 \leq P \leq 0.04$); and *3*) between groups 1 and 3 for SNP rs266714 (MAF = 31 vs. 20%, P = 0.04) (online appendix Table 1). For a P value <0.05, this preliminary study based on diabetic probands from families showing linkage has sufficient power to detect SNPs with strong genetic effects (odds ratio [OR] ~2) that are expected to underlie the 3q27 linkage (97% for MAF = 0.1 and 99% for MAF = 0.2 using power for association with error; online appendix Table 2). Our study has potential limitations to be able to detect rare causative variants or variants with more modest effects (OR 1.2–1.5). This strategy allows us to test both the contribution to linkage and the association to type 2 diabetes with power at least equivalent to a moderately sized case-control study.

Six SNPs associated with type 2 diabetes in the first step of our study were investigated in the whole family sample (Table 1). Only SNP rs266714, located 2.47 kb upstream of the *EIF4A2* gene, showed a significant association with type 2 diabetes in the families (P = 0.0008 using the family-based association test [FBAT]), with the minor T allele being over-transmitted in the affected subjects. In

TABLE 2
Test for association with the evidence of linkage for rs266714

Model	GIST		Asso-Link			
	Weighted NPL score	P	Genotypes for rs266714	Pairs	LOD score	P
Dominant	4.66	**0.02**	C/T	26	4.47	**0.04**
Recessive	1.38	0.50	T/T	4	0.47	0.44
Additive	4.25	**0.05**	X/T	38	5.24	**0.02**
Combined	—	**0.03**				

Contribution to the linkage with early-onset type 2 diabetes was assessed by two different methods genotype IBD sharing test (GIST) and Asso-Link. GIST tests the correlation between the at-risk genotype frequency in a family and the nonparametric linkage (NPL) score. A new NPL score weighted by this frequency is assessed. Asso-Link tests the LOD score significance for pairs identical by status carrying the at-risk allele (genotypes column) with simulations. For rs266714, the at-risk allele is T and the protective is C. Thirty-eight pairs having at least one at-risk T allele show an LOD of 5.24, which is significantly different (P = 0.02) from what is expected. The threshold for significance is 0.05; significant P values are indicated in bold.

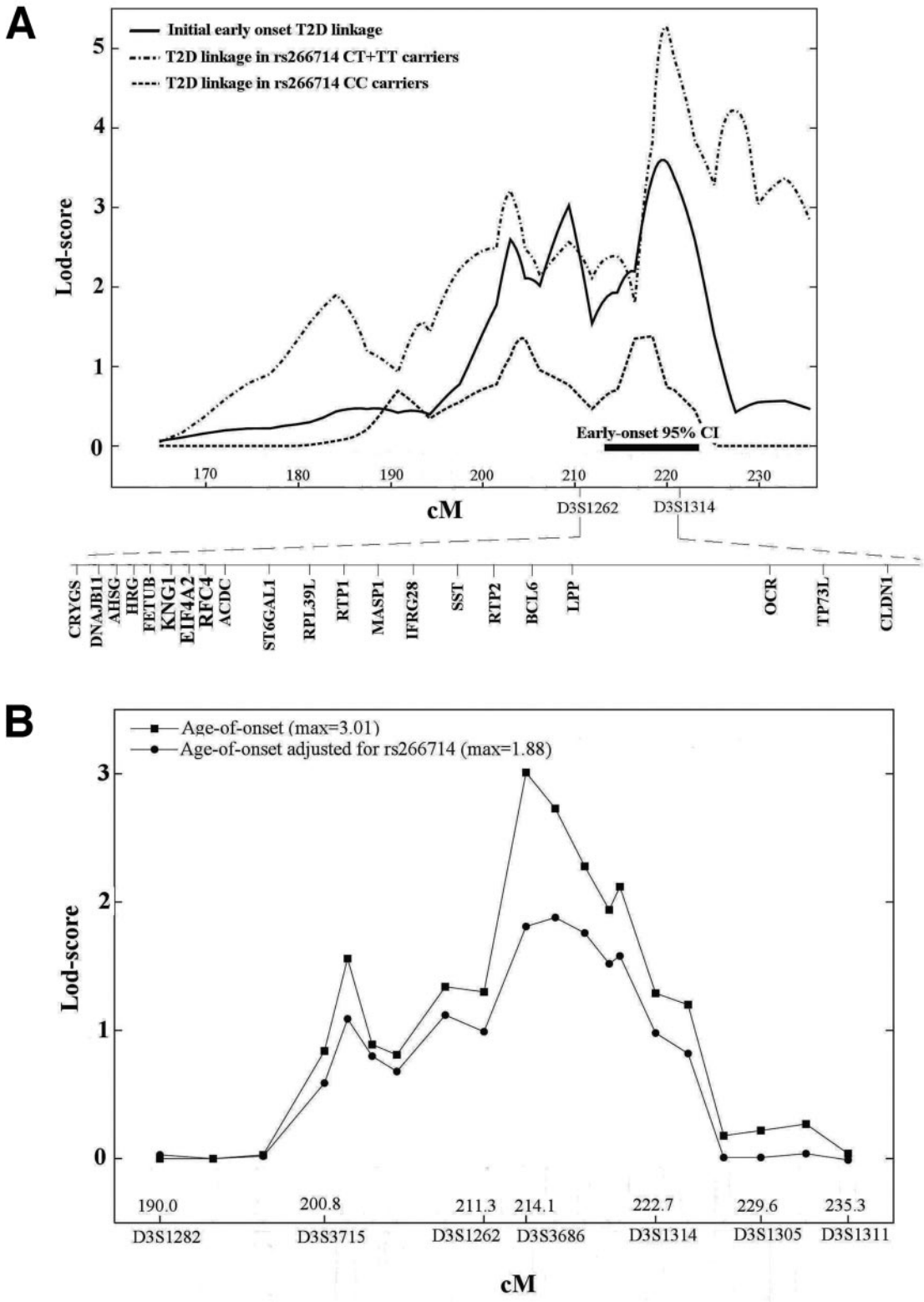

FIG. 1. Early-onset type 2 diabetes linkage, age-of-onset QTL, and the contribution of SNPs rs266714. *A*: The initial early-onset type 2 diabetes (T2D) linkage is represented by the solid line together with the 95% CI estimated by the gene location method. The 21 genes mapping in the region (NCBI build 35, May 2004) are shown below the figure. The partitioning curves illustrate the linkage in the 38 sibpairs of rs266714 CT + TT genotype carriers and in the 32 sibpairs of rs266714 CC genotype carriers. *B*: The newly identified QTL (■) spans a 3.87-Mb interval, delineated by markers D3S1262 and D3S1314, which overlaps the 95% CI of linkage to the early-onset type 2 diabetes binary trait. The effect of rs266714 was estimated by adjusting the age-of-onset quantitative trait on the carried genotype and shows a 1.13 LOD decrease (●). For both LOD-score curves, the centimorgan locations are indicated on the *x*-axis with indication of the exact microsatellite markers position on Fig. 1*B*.

TABLE 3
Characteristics of the three European populations and case-control analysis of SNP rs266714

Description of the samples	*n*	Sex ratio (M/F)	Mean age at examination (years)	Mean age at diagnosis (years)	Mean BMI (kg/m2)	Genotype frequency (%)			Allele frequency (%)		*P*
						CC	CT	TT	C	T	
French diabetic subjects from the Corbeil cohort	358	198/160	57.3 ± 11.6	45.4 ± 10.6	27.3 ± 3.3	63 (220)	31.8 (111)	5.2 (18)	78.9	21.1	0.41
French control subjects from the DESIR cohort	680	274/406	53.5 ± 5.7	—	23.2 ± 1.8	59.6 (405)	35.6 (242)	4.9 (33)	77.3	22.7	
Swiss diabetic subjects	307	198/109	60.5 ± 0.5	50.0 ± 11.8	29.6 ± 5.9	66 (223)	29.9 (101)	4.1 (14)	80.9	19.1	0.65
Swiss control subjects	420	NA	NA	NA	NA	63.9 (189)	32.1 (95)	4.1 (12)	79.9	20.1	
Danish diabetic subjects	338	170/168	61 ± 10.3	54.9 ± 9.7	29.2 ± 5	59.9 (184)	35.2 (108)	4.9 (15)	77.5	22.5	0.43
Danish control subjects	296	155/141	59.8 ± 8	—	25.4 ± 3.6	60.7 (255)	32.6 (137)	6.7 (28)	77	23	

Data are *n* and means ± SD. Genotype and allele frequencies are indicated for each group of subjects. The number of individuals is given in parenthesis for each genotype class. All genotypes are in Hardy-Weinberg equilibrium in the populations tested. χ^2 *P* values are given. A combined OR (95% CI) of 0.94 (0.81–1.08) was calculated for the three case-control studies. NA, data not available.

the families, SNP rs266714 also showed significant association with age of onset ($P = 0.0008$). The subjects with diabetes carrying the TT genotype are diagnosed earlier than the CT or CC genotype carriers (mean age of onset 47.7, 49.0, and 52.3 years, respectively). SNP rs266714 significantly associates with the early-onset type 2 diabetes status ($P = 0.003$; Table 1) in 54 families with at least two subjects presenting with diabetes before 45 years of age. When the recommended FBAT empirical variance option for a region of linkage was used, all associations remained significant (data not shown). Although a rigorous evaluation of the *P* value accounting for multiple testing is difficult to achieve from our study design, these results remain significant after a Bonferroni correction (new type I error threshold $P \leq 0.003$). Our familial study reaches sufficient power (75%) to detect a strong effect (OR ≥1.8) for SNPs with MAF ≥0.2, as evaluated by the PBAT (power calculation of FBATs) program (15). In addition, the partitioning curves show that the early-onset type 2 diabetes linkage increases in the rs266714 CT + TT genotype carriers compared with the CC group (LOD score = 5.2 vs. 1.0; Fig. 1*A*), which emphasizes stronger linkage in the "at-risk" T allele carriers. Using the genotype identical-by-descent (IBD) sharing test, a significant correlation was observed between the nonparametric linkage score and the rs266714 at-risk genotype frequency under dominant and additive models ($P = 0.02$ and $P = 0.05$, respectively, Table 2). Similarly, the simulation-based Asso-Link test showed an increased LOD score in concordant sibpairs carrying at least one at-risk allele ($P = 0.02$; Table 2). To assess the impact of SNP rs266714 on the QTL, the type 2 diabetes age of onset was adjusted for the carried genotype. By comparison to the initial QTL, a 1.13–LOD score decrease was observed (Fig. 1*B*; $P = 0.02$ estimated through simulations as described for Asso-Link in RESEARCH DESIGN AND METHODS). Residual linkage is still significant (LOD score = 1.88, $P = 0.002$), suggesting that other variant(s) may contribute to the linkage peaks. According to the wide variety of phenotypes linked to this locus in different ethnic groups, we cannot exclude a role of additional gene(s) and a more complex genetic architecture at this locus. This could now be dissected by systematic analysis of numerous variants in the entire region using HapMap2 data recently made available.

We then tested the impact of SNP rs266714 in three European case-control cohorts, but no association with type 2 diabetes was detected in the different studies nor in a meta-analysis of all populations (Table 3). As early age of onset is an important feature of the French familial cases, we also analyzed the early-onset type 2 diabetes status in the three case-control groups. The allelic frequencies of SNP rs266714 in the early-onset cases (<45 years of age) were compared with both the late-onset diabetic subjects (≥45 years of age) and the normoglycemic control subjects. No significant association was observed (data not shown). The discrepancy between case-control and family-based studies can be due to either a difference in ascertainment for familial aggregation of type 2 diabetes, clinical heterogeneity, or a false-positive result in the family sample. As previously reported for *KCNJ11*/Kir6.2 (16) and *TIEG2*/KLF11 (17) variants, higher OR values are seen in familial cases compared with case-control studies based on population cohorts. Thus, preferential detection of association can be obtained in family cases for frequent variants with moderate effect in the general population.

In conclusion, we show that SNP rs266714 is significantly associated with type 2 diabetes diagnosed before 45 years and with the age of onset in the French families. Comparable results were previously reported for *TCF1/HNF1α* gene in the Canadian Oji-Cree population, where a private coding SNP strongly influences the type 2 diabetes age of onset (18) and more recently for the Ala98Val polymorphism in Asian Indians (19). Using the Genomatix Eldorado software (http://www.genomatix.de), genetic variation at SNP rs266714 may modify a Pax5 binding site to an AP1 consensus sequence, meaning that this variant may modulate transcription factor binding affinity. Due to its role in the translation initiation process, the *EIF4A2* expression level may influence the synthesis rate of specific proteins. Our data indicate that *EIF4A2* is downregulated by high glucose in rat β-cells, suggesting that EIF4A2 may contribute to the regulation of insulin production in response to glucose. Growing data will highlight that defects in the translation initiation process in relation with stress-mediated pathways may be involved in human metabolic disorders (20). Taken together, this data may suggest a role of EIF4A2 in glucose homeostasis and possibly in the natural history of diabetes and requires further investigation to test these hypotheses.

RESEARCH DESIGN AND METHODS

For our preliminary study, three groups of subjects were selected from a previously described set of 148 French Caucasian nuclear families specifically ascertained for a strong familial aggregation of type 2 diabetes and showing linkage on 3q27 (1). Group 1 is composed of 40 unrelated type 2 diabetic probands (mean age at diagnosis 38.1 ± 5.7 years) selected in sibpairs sharing two IBD alleles from families contributing to the type 2 diabetes linkage (MLS >0.76 in a region of 10 cM centered on the most significant marker D3S3530). Group 2 is composed of 40 type 2 diabetic probands (mean age at diagnosis 54.9 ± 9.1 years) selected from families that do not contribute to the linkage (MLS = 0). Given the number of families previously scanned for linkage, no additional probands were available for this preliminary study. Group 3 includes 94 unrelated normoglycemic (fasting glycemia <5.6 mmol/l) control subjects selected among the spouses of affected sibs from additional French families recruited by the CNRS unit in Lille; these were not investigated for type 2 diabetes linkage.

The 148 nuclear families are composed of 633 individuals. According to the American Diabetes Association criteria, 432 are diabetic (mean BMI 27.9 ± 4.5 kg/m^2, mean age at diagnosis 49.5 ± 10.6 years, ratio of men to women 198:234), 72 subjects have impaired glucose tolerance or impaired fasting glucose (mean BMI 27.4 ± 4.7 kg/m^2, mean age at diagnosis 59.1 ± 9.6 years, ratio of men to women 36:36), and 129 are normoglycemic (mean BMI 25.1 ± 4.1 kg/m^2, ratio of men to women 40:89). Fifty-four families including sibships with at least two cases presenting type 2 diabetes before 45 years of age were analyzed for association with early-onset type 2 diabetes status.

Three independently ascertained European case-control cohorts (not specifically ascertained for a strong familial aggregation or early-onset type 2 diabetes) were studied (Table 3), including *1*) 349 diabetic patients from the Endocrinology-Diabetology Department of the Corbeil-Essonne Hospital and 680 control subjects from the DESIR (Epidemiologic Data on the Insulin Resistance Syndrome) cohort, *2*) 338 Danish Caucasian diabetic patients and 296 normal glucose-tolerant subjects ascertained at the Steno Diabetes Centre in Copenhagen, and *3*) 307 Swiss diabetic patients and 420 control subjects ascertained from anonymous healthy blood donors from CHUV of Lausanne.

The present study was performed in accordance with the Helsinki II declaration.

Gene screening and SNP genotyping. Screening of coding sequences, proximal promoter, 5′- and 3′-untranslated regions and exon-intron boundaries of both candidate genes, was conducted by direct sequencing in group 1. Most of the SNPs were genotyped by direct sequencing using an automated ABI Prism 3700 DNA sequencer in combination with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Other technologies were used including FRET with the LightCycler assay (Roche Diagnostics, Basel, Switzerland) based on hybridization probes labeled with fluorescent dyes, an assay based on the fluorogenic 5′ nuclease allowing simultaneous amplification and detection of specific SNP alleles (7900 HT SNP genotyping platform; Applied Biosystems), and electrophoresis of amplification products through 5% polyacrylamide gels on an ABI 377 DNA sequencer. More than 10% of genotypes were verified by resequencing resulting in >95% concordance. SNPs rs5030084 and rs5030085 showed deviation from Hardy-Weinberg equilibrium in the diabetic group 2 and were resequenced to avoid genotyping errors with a 100% concordance rate. Sequences of primers and PCR conditions are available on request.

Statistical analyses. The 95% CI of the previous early-onset type 2 diabetes linkage was determined through GeneFinder software (21). The analysis of the age of onset (adjusted by sex and normalized) quantitative trait was performed in the 148 families using a method based on generalization of allele sharing models implemented in Merlin software (22). For all analyses, age at diagnosis was considered as a marker for age of onset.

For the first step of our study, SNPs to genotype in the control group, group 3, were selected by investigating the pairwise linkage disequilibrium in the diabetic groups, groups 1 and 2, using the Haploview program (http://www.broad.mit.edu/mpg/haploview/). The Hardy-Weinberg equilibrium consistency and the comparison of the allelic frequencies between groups 1, 2, and 3 were performed through χ^2 tests, which were also used for the case-control association studies. For the statistical power evaluation of the first step, three MAF values (0.05, 0.1, and 0.2) and four ORs (1.2, 1.5, 2, and 4) were assessed to cover the spectrum of the possible single-gene genetic models. Allelic frequencies in linked (IBD = 2) and unlinked (IBD = 0) random cases and in the general population allowed power comparison between our test of group1 versus 3 and a case-control test using PAWE (http://linkage.rockefeller.edu/pawe/).

In the families, association with binary and quantitative traits was assessed through the FBAT method (23). For each SNP, the mean age at diagnosis was also calculated for each genotype group. The contribution of the SNP to the early-onset type 2 diabetes linkage was evaluated by the genotype IBD sharing test procedure (24), and a complementary method assessing the sample size decrease, referred to as Asso-Link (25), was used to determine MLS for affected sibpairs concordant for genotype categories (both sibs being diagnosed before 45 years of age). The MLS *P* value is obtained through simulation after preserving the linkage information (i.e., the IBD status for each pair). To evaluate the SNP impact on the QTL, the age-of-onset quantitative trait was corrected for the effect of the carried genotype by linear regression. The residual trait was then analyzed for linkage using Merlin, and significance of the LOD score decrease was assessed according to the same simulation procedure.

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