Brief Genetics Report

Decreased Fasting and Oral Glucose Stimulated C-peptide in Nondiabetic Subjects With Sequence Variants in the Sulfonylurea Receptor 1 Gene

S. John Weisnagel,[1] Tuomo Rankinen,[2] André Nadeau,[3] D.C. Rao,[4] Yvon C. Chagnon,[1] Louis Pérusse,[1] and Claude Bouchard[2]

The high-affinity sulfonylurea receptor 1 (SUR1) plays an important role in regulating insulin secretion. In the Québec Family Study, we genotyped 731 individuals (685 nondiabetic [ND] subjects) for the SUR1 gene IVS15-3c→t and exon 18 Thr759(ACC→ACT) polymorphisms using polymerase chain reaction–restriction fragment-length polymorphism analysis. Phenotypes measured were fasting plasma glucose (GLU), fasting plasma insulin (INS), and fasting C-peptide (CPEP), as well as oral glucose tolerance test (OGTT) responses; they were adjusted for age, sex, waist circumference, and the sum of six skinfold thicknesses. In ND subjects, exon 18 Thr759(ACC→ACT) T allele carriers (T^+) had lower CPEP ($P = 0.022$, −12.8%) and acute C-peptide responses (area above basal in first 30 min [CP30]) ($P = 0.051$, −12.4%) than noncarriers (T^-). Also, in those with the cT/tC haplotype (from both IVS15-3c→t and exon 18 Thr759[ACC→ACT] polymorphisms), CPEP ($P = 0.005$, −21.2%), CP30 ($P = 0.034$, −19.2%), and total C-peptide responses ($P = 0.016$, −20.2%) were lower than that in cT^- subjects. In overweight individuals (BMI >25 kg/m^2), differences between carriers and noncarriers of the T or cT alleles were greater for GLU ($P = 0.023–0.034$), CPEP ($P = 0.021–0.015$), acute OGTT insulin response ($P = 0.014–0.019$), and CP30 ($P = 0.034–0.019$). These results suggest that the T and cT allele variants are associated with lower insulin secretion parameters, particularly in female and overweight subjects, adding evidence to the role of SUR1 sequence variants in decreased insulin secretion. *Diabetes* 50:697–702, 2001

From the [1]Physical Activity Sciences Laboratory, Laval University, Ste-Foy, Québec, Canada; the [2]Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana; the [3]Diabetes Research Unit, Laval University Health Center, Ste-Foy, Québec, Canada; and the [4]Division of Biostatistics and Departments of Psychiatry and Genetics, Washington University Medical School, St. Louis, Missouri.

Address correspondence and reprint requests to S. John Weisnagel, M.D., FRCPC, Physical Activity Sciences Laboratory, Kinesiology, PEPS 0234, Laval University, Ste-Foy, Québec, Canada G1K 7P4. E-mail: john.weisnagel@kin.msp.ulaval.ca.

Received for publication 4 May 2000 and accepted in revised form 7 December 2000.

Additional information can be found in an online appendix at www.diabetes.org/diabetes/appendix.asp.

CP30, OGTT C-peptide area above basal in first 30 min; CPEP, fasting plasma C-peptide; CPtot, OGTT C-peptide area above basal over 180 min; GLU, fasting plasma glucose; IN30, OGTT insulin area above basal in first 30 min; INS, fasting plasma insulin; IRI, immunoreactive insulin; ND, nondiabetic; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; PI, proinsulin; QFS, Québec Family Study; SF6; sum of six skinfold thicknesses; SUR1, sulfonylurea receptor 1; WC, waist circumference.

Type 2 diabetes has been shown to have a strong hereditary basis (1,2); however, the major genes for the common late-onset type have not yet been clearly identified (3). Because impaired insulin secretion plays a critical role in the pathogenesis of the disease (4), genes that encode major elements involved in insulin secretion are good candidates for evaluating genetic susceptibility to type 2 diabetes (3).

A central component in glucose-induced insulin secretion is the ATP-sensitive potassium (K_{ATP}) channel of the β-cell. Increasing ATP generated by intracellular glucose metabolism leads to the closing of these channels, membrane depolarization, the opening of voltage-gated calcium channels with influx of calcium, and exocytosis of insulin (5). The K_{ATP} channel is composed of the high-affinity sulfonylurea receptor (SUR1) and Kir6.2, a member of the inwardly rectifying ion channel family (6). Pathological regulation of insulin secretion has been demonstrated in studies of persistent hyperinsulinemic hypoglycemia of infancy, a rare autosomal recessive disease in which mutations in the nucleotide-binding fold-2 region of the SUR1 gene have been found (7).

In type 2 diabetes, association studies in diabetic populations have been positive, with the IVS15-3c→t and exon 18 Thr759(ACC→ACT) variants of the SUR1 gene being found more frequently in Danish (8), U.S., U.K. (9), and French Caucasian (10) diabetic individuals, and the IVS15-3c→t variant alone found more frequently in Dutch Caucasian diabetic subjects (11). However, results in Japanese populations have been negative (12). Few studies have reported on the physiological impact of SUR1 gene sequence variants. A Danish group demonstrated decreased tolbutamide-stimulated insulin secretion in normal glucose-tolerant carriers of the above intron 15 and exon 18 variants (8). Another group recently reported an association of the IVS15-3c→t variant with lower second-phase insulin secretion in Dutch nondiabetic (ND) individuals (13).

The present study was thus undertaken to examine the role of the SUR1 IVS15-3c→t and exon 18 Thr759 (ACC→ACT) polymorphisms and their haplotypes on glucose metabolism in French-Canadian Caucasians of the Québec Family Study (QFS).

TABLE 1
Glucose tolerance status in QFS

	Sex		
	Men	Women	Total
All subjects			
Nondiabetic	294 (40.4)	391 (53.7)	685 (94.1)
Diabetic	22 (3.0)	21 (2.9)	43 (5.9)
Total	316 (43.4)	412 (56.6)	728 (100)
OGTT subjects			
Normal glucose tolerance	194 (34.2)	260 (45.9)	454 (80.0)
Impaired glucose tolerance and impaired fasting glucose	41 (7.2)	48 (8.5)	89 (15.7)
Diabetic	15 (2.6)	9 (1.6)	24 (4.2)
Total	250 (44.1)	317 (55.9)	567 (100)

Data are *n* (%).

There were only 43 diabetic subjects in QFS (Table 1). Unadjusted baseline data for the 685 ND subjects are presented in Table 2. In unrelated individuals from the parental generation ($n = 259$), the IVS15-3c→t polymorphism frequencies were 56% for the c allele and 44% for the t variant; and for the exon 18 Thr759(ACC→ACT) polymorphism, the frequencies were 95.8% for the C allele and 4.2% for the T variant. These frequencies are similar to those reported in ND Caucasians (8,9,11). No carriers of the rarer exon 18 Thr759(ACC→ACT) T allele (T^+) were found among diabetic subjects, and IVS15-3c→t polymorphism frequencies were similar in diabetic and ND subjects (data not shown). Genotypes were in Hardy-Weinberg equilibrium and the two polymorphisms were in strong linkage disequilibrium ($\chi^2 = 28.4$, $P < 0.0001$).

In ND subjects, the T^+ group had significantly lower values than noncarriers (T^-) with regard to fasting plasma C-peptide (CPEP) and acute oral glucose tolerance test (OGTT) C-peptide response, defined as the area above basal in the first 30 minutes (CP30) (Fig. 1). The cT/tC double heterozygotes had even lower values than all cT^- haplotypes with regard to CPEP, CP30, and total OGTT C-peptide response (area above basal over 180 min) (CPtot). Trends were seen for lower values in the T^+ group than in the T^- group with regard to acute OGTT insulin response (IN30), the area above basal in the first 30 min ($P = 0.063$, −14.4%). Results were independent of age, sex, and adiposity.

In women, CPEP and CP30 were significantly decreased in the T^+ group compared with the T^- group and in the cT/tC group compared with the cT^- group (Table 3). CPtot followed the same pattern in cT/tC carriers. In men, fasting

TABLE 2
Baseline values in nondiabetic QFS population

Phenotype	*n*	Mean (range)
Age (years)	685	42.0 (17–92)
BMI (kg/m^2)	677	26.1 (16.8–64.9)
Waist circumference (cm)	656	84.8 (57.9–164)
Sum of six skinfolds (cm)	649	105 (23.5–448)
Waist-to-hip ratio	648	0.90 (0.56–1.19)
Fasting glucose (mmol/l)*	684	4.96 (3.20–6.90)
Fasting insulin (pmol/l)*	612	54.4 (1.0–350)
Fasting C-peptide (pmol/l)*	573	646 (178–3143)

* Fasting plasma measurements.

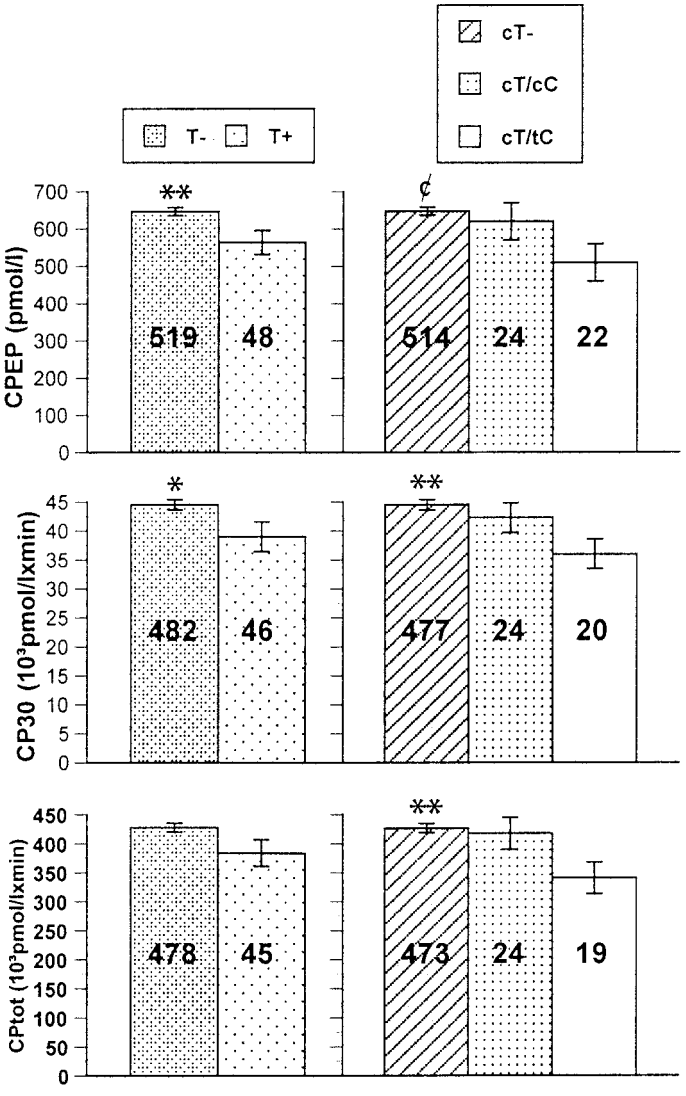

FIG. 1. Fasting plasma C-peptide and OGTT C-peptide responses in relation to exon 18 Thr759(ACC→ACT) genotype and IVS15-3c→t/exon 18 Thr759(ACC→ACT) haplotype in ND subjects overall in QFS. Results given are back-transformed least-square means + SE from the general linear model multiple regression procedure, adjusting for age, age^2, age^3, sex, WC, and SF6; *0.06 > *P* > 0.05; **0.05 > *P* > 0.01; ¢ 0.01 > *P* > 0.001 for comparisons with exon 18 Thr759(ACC→ACT) T allele carriers or the cT/tC haplotype. Numbers given are those for each subgroup.

plasma glucose (GLU) was higher ($P = 0.034$, +4.2%), and IN30 tended to be lower ($P = 0.074$, −19.6%) in the T^+ group than in T^- subjects.

In younger subjects (≤45 years old), CPEP was lower in the T^+ group than in the T^- group ($P = 0.026$, −16.1%) and in the cT/tC group compared with the cT^- group ($P = 0.045$, −22.4%). IN30 tended to be lower in the T^+ group than in T^- subjects ($P = 0.080$, −17.6%). CP30 was lower in T^+ subjects than in T^- subjects ($P = 0.058$, −15.6%), and CPtot was decreased in the cT/tC carriers when compared with the cT^- group ($P = 0.034$, −25.0%).

With higher BMI, differences between genotype groups increased (Fig. 2). In those with BMI >25 kg/m^2, the T^+ subjects had higher GLU (+4.6%) and lower CPEP (−18.1%), IN30 (−26.6%), and CP30 (−19.2%) than T^- subjects. The cT/tC group had even higher GLU (+5.9%)

TABLE 3
Results in nondiabetic men and women for exon 18 Thr759(ACC→ACT) genotypes and IVS15-3c→t/exon 18 Thr759(ACC→ACT) haplotypes

	Men					Women				
Phenotype	T^-	T^+	cT^-	cT/cC	cT/tC	T^-	T^+	cT^-	cT/cC	cT/tC
n	207–266	21–24	203–262	10	9–12	271–340	24–27	270–338	14	10–13
GLU (mmol/l)	**4.97 ± 0.04**	**5.18 ± 0.10***	4.96 ± 0.04	5.05 ± 0.15	5.22 ± 0.14	4.96 ± 0.03	4.96 ± 0.09	4.96 ± 0.03	4.93 ± 0.12	5.00 ± 0.13
INS (pmol/l)	51.2 ± 3.0	48.5 ± 7.0	51.1 ± 3.0	46.9 ± 9.9	51.5 ± 10.8	55.0 ± 2.6	51.4 ± 6.7	54.8 ± 2.5	48.7 ± 8.6	54.5 ± 10.3
CPEP (pmol/l)	621 ± 23	588 ± 53	621 ± 23	626 ± 82	557 ± 72	**666 ± 20**	**542 ± 44***	**667 ± 20**	**615 ± 67**	**471 ± 55†**
GL30 (mmol/l × min)	212 ± 2.5	208 ± 6.0	212 ± 2.5	207 ± 8.4	215 ± 8.7	200 ± 1.9	193 ± 5.2	199 ± 1.9	192 ± 6.6	194 ± 7.8
GLtot (mmol/l × min)	1,180 ± 20	1,120 ± 50	1,170 ± 20	1,080 ± 60	1,180 ± 70	1,140 ± 20	1,080 ± 40	1,141 ± 20	1,110 ± 60	1,050 ± 60
IN30 (10^3 pmol/l × min)	8.29 ± 0.43	6.67 ± 0.83	8.34 ± 0.40	6.72 ± 1.22	6.95 ± 1.25	8.29 ± 0.35	7.45 ± 0.89	8.24 ± 0.34	7.75 ± 1.17	7.02 ± 1.26
INtot (10^3 pmol/l × min)	66.0 ± 3.0	56.2 ± 6.1	65.6 ± 3.0	49.1 ± 7.8	61.2 ± 9.6	64.4 ± 2.4	61.1 ± 6.1	64.3 ± 2.4	69.1 ± 9.2	51.3 ± 8.1
CP30 (10^3 pmol/l × min)	43.9 ± 1.9	40.9 ± 4.1	44.0 ± 1.9	44.7 ± 6.6	39.2 ± 5.7	**45.0 ± 1.6**	**37.2 ± 3.6***	**44.9 ± 1.6**	**40.6 ± 5.0**	**32.9 ± 4.8***
CPtot (10^3 pmol/l × min)	409 ± 16	370 ± 35	406 ± 16	370 ± 49	356 ± 49	442 ± 14	394 ± 34	**442 ± 14**	**455 ± 51**	**324 ± 43***

Data are least-square means ± SE. GL30, OGTT glucose area above basal in the first 30 min; GLtot, OGTT glucose area above basal over 180 min; INtot, OGTT insulin area above basal over 180 min. * $P < 0.05$ and † $P < 0.01$ for comparisons between T^+ and T^- genotype or cT/tC and cT^- haplotype subgroups.

and lower CPEP (−24.8%), IN30 (−33.9%), and CP30 (−28.6%) values than the cT^- groups.

No significant differences between IVS15-3c→t genotypes were seen, and fasting plasma insulin (INS), OGTT glucose responses, and total insulin response were similar among the different genotypes (data not shown).

After taking into account independent family effects by adding a covariate for family membership in the analysis of variance for the above phenotypes, we found that the results were only modestly modified. For the overall cohort, *P* values for differences between T^+ and T^- subjects were 0.072 for CP30, and for differences between cT/tC and non-cT haplotypes, the values were 0.119 for CP30 and 0.063 for CPtot. For all CPEP and glucose data and for all results in sex and BMI subgroups, differences between T^+ and T^- and between cT/tC and cT^- groups remained significant at the same level (results not shown).

In this study, we have shown that the T allele of the exon 18 Thr759(ACC→ACT) polymorphism of the SUR1 gene was associated with lower CPEP and acute OGTT C-peptide responses in ND Canadians of French descent. Even lower CPEP and OGTT C-peptide responses were seen with the cT/tC haplotype, obtained from both the IVS15-3c→t and exon 18 Thr759(ACC→ACT) polymorphisms. These greater differences in the haplotype comparisons suggest that using two variants in the SUR1 gene more clearly defines the allele associated with the phenotypes mentioned. This supports findings in Danish Caucasians (8) of lower insulin and C-peptide secretion after intravenous tolbutamide in ND carriers of both the exon 18 C/T or T/T and the IVS15-3c→t c/t or t/t genotypes. However, in that study haplotypes could not be clearly identified; in the QFS they could be determined because family structures were known. Moreover, we could adjust metabolic parameters for important covariates such as waist circumference (WC) and the sum of six skinfold thicknesses (SF6).

Although we focused our study on ND subjects, for which interpretation of OGTT data are straightforward, analysis was also performed on all subjects combined. Similar results were obtained for C-peptide and insulin parameters (data not shown). Therefore, we do not believe that restricting analysis to ND subjects biased the results. There were no T^+ subjects among the diabetic subjects. This should not be unexpected because QFS was not specifically designed to study type 2 diabetes and the numbers of diabetic subjects was small.

Lower OGTT responses could result either from decreased β-cell secretion or higher insulin sensitivity. Several arguments point to a relationship with secretion. First, SUR1 has been described mainly in pancreatic β-cells (6). Second, INS levels, which are variably correlated with insulin resistance, were unaffected by genotype status, yet CPEP levels were significantly changed. C-peptide is secreted in an equimolar fashion with insulin, has a longer plasma half-life, displays smaller oscillations in plasma concentration, and undergoes minimal hepatic extraction; therefore, it has been suggested to be a better reflection of insulin secretion overall (14). Third, OGTT glucose responses were not significantly different between the genotypes, whereas we would have expected a significant decrease in GLU and OGTT glucose responses in the case

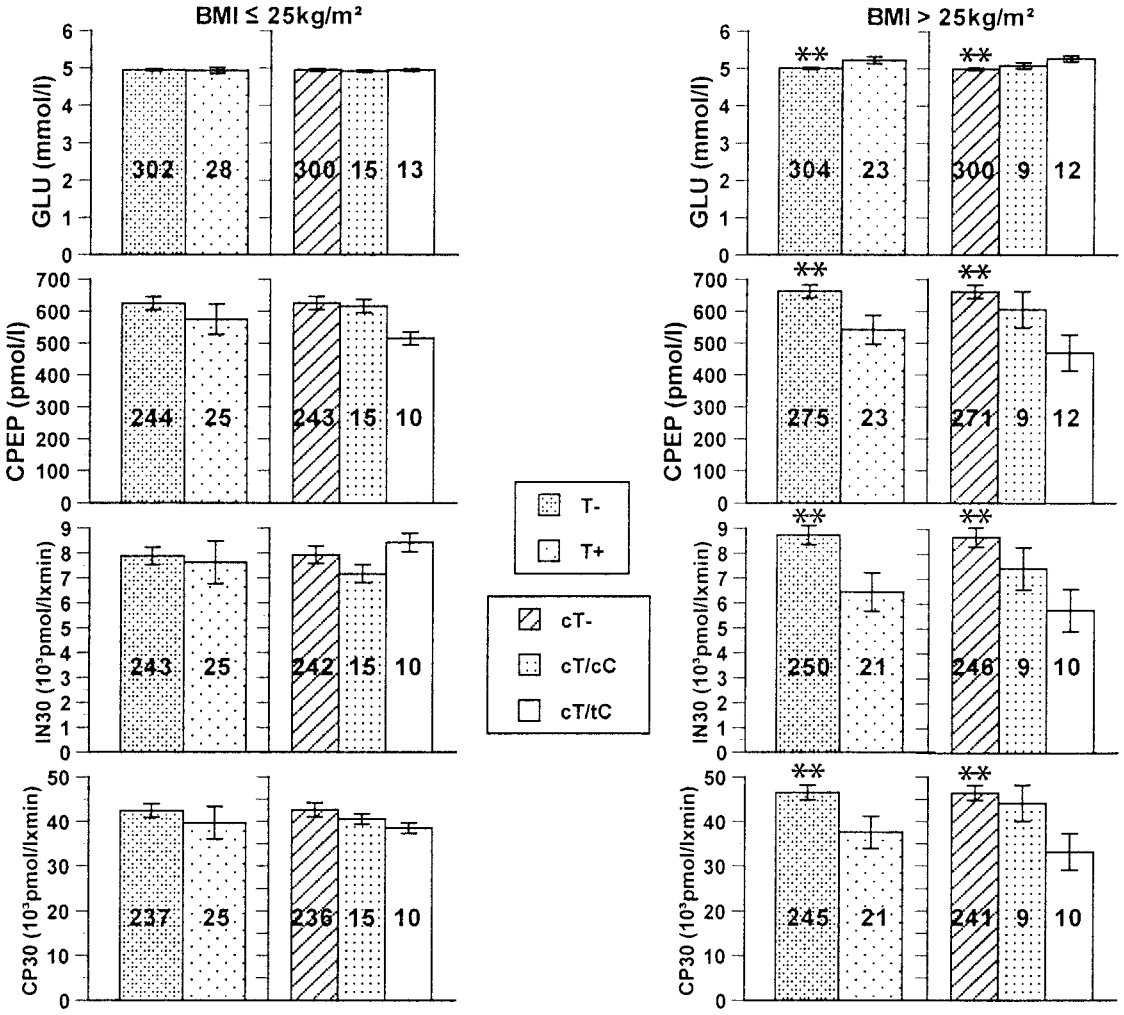

FIG. 2. Fasting plasma glucose and C-peptide and acute OGTT insulin and C-peptide responses in relation to exon 18 Thr759(ACC→ACT) genotype and IVS15-3c→t/exon 18 Thr759(ACC→ACT) haplotype in ND QFS subjects by BMI subgroup. Results given are back-transformed least-square means + SE from the general linear model multiple regression procedure, adjusting for age, age^2, age^3, WC, and SF6; **0.05 > *P* > 0.01 for comparisons with exon 18 Thr759(ACC→ACT) T allele carriers or the cT/tC haplotype. Numbers given are those for each subgroup.

of greater insulin sensitivity with lower insulin requirements. Still, we cannot rule out a primary or compensatory increase in insulin sensitivity. Interestingly, in the Hansen et al. (8) study, the subjects displaying decreased insulin and C-peptide responses also showed a 30% increase in glucose effectiveness. Also, in a recent K_{ATP} channel–knockout mouse model (15), not only was insulin secretion decreased, but insulin action was enhanced, as measured by an insulin tolerance test, due either to a direct peripheral effect of the K_{ATP} channel deficiency or to an unknown compensatory mechanism.

The main genotype differences were found in C-peptide measures, although OGTT insulin responses also tended to be lower overall and were significantly lower in overweight subjects. This apparent discrepancy could be explained by the insulin assay used here, which crossreacts with proinsulin (PI). It has been shown that in deficient insulin secretion states, such as maturity-onset diabetes of the young, OGTT responses for C-peptide are more suppressed than those for immunoreactive insulin (IRI) (16). Moreover, in type 2 diabetes, PI is proportionately increased with respect to IRI and specific insulin (17). Hence, our results would suggest that the SUR1 variant carriers are also characterized by a deficient insulin secretion state. However, measures of PI and specific insulin would be required to clarify this point.

Significant associations were only seen in younger subjects. It is possible that in older individuals, age and accumulated environmental influences might predominantly modulate insulin secretion, overshadowing any underlying genetic factor and clouding differences between genotypes. Also, with increasing BMI, differences between T^+ and T^- and between cT/tC and non-cT genotype groups became more pronounced for GLU, CPEP, IN30, and CP30. This pattern of higher GLU and lower OGTT secretory responses suggests that BMI directly influences these relationships. A possible explanation is the association between obesity and increased insulin resistance that leads to greater demand on β-cells, allowing differences in metabolic parameters to become more evident in SUR1 variant carriers, who would respond insufficiently because of limited secretion.

The exon 18 Thr759(ACC→ACT) polymorphism is silent; thus, it cannot alone explain our results. The IVS15-3c→t polymorphism is located by the intron-exon 16 splice junction, and thus an effect on mRNA splicing is possible

but unproven. The most likely explanation is that these variants are in linkage disequilibrium with a nearby unidentified functional mutation, either in the SUR1 gene or in a gene close by. In the latter case, the Kir6.2 gene located only 4.5-kb distant on 11p15.1 is a good candidate.

In conclusion, the present study has shown for the first time that the SUR1 gene exon 18 Thr759(ACC→ACT) T allele and the cT haplotype, resulting from the combination of this polymorphism and the IVS15-3c→t polymorphism, are significantly associated with lower CPEP and OGTT C-peptide responses and possibly higher GLU and lower OGTT insulin responses in a large Caucasian population of French descent. Sex, age, and particularly BMI status modulated these associations. Because differences were greater with the cT haplotype, using two SUR1 variants may more clearly define the allele associated with altered glucose metabolism. However, these variants have not been shown to have direct functional consequences. Additional studies are needed to define the role of SUR1 or some nearby gene in insulin secretion and to identify the mutations that fully explain the results reported here.

RESEARCH DESIGN AND METHODS

Subjects. The QFS cohort is composed of Caucasian nuclear families of French descent from the Québec City area, representing a mixture of random sampling and ascertainment through obese (BMI >32 kg/m^2) probands (18). In this study, 731 adults from 200 families were measured during phase two (1989–1997) and phase three (1998 to the present) of the QFS. Mean family size was 4.0 (range 1–13); 69% of families had two parents, 24% had 0–1 parent, and the remainder were extended families with more than two members from the parental generation. Written consent was obtained from all participating subjects, and the Medical Ethics Committee of Laval University approved the protocol.

Glucose tolerance status and metabolic parameters. A 75-g OGTT was performed after an overnight fast. Fasting and OGTT plasma glucose, insulin, and C-peptide were assayed as previously described (19). OGTT areas under the curve were calculated using the trapezoidal method. The area over the first 30 min defined the acute response, whereas the complete area (0–180 min) was the total response. 1997 American Diabetes Association diagnostic criteria determined the glucose tolerance status. Because OGTTs were initiated in QFS in 1993, 161 subjects had only GLU values and 3 had missing values. To avoid misclassification based on GLU, glucose tolerance was limited to diabetic and ND categories. From OGTT data on 567 subjects, the possibility of misclassifying a diabetic subject as ND using a GLU value <6 mmol/l was very low (0.77%), and in the group of 161 subjects, only 1 or 2 diabetic subjects would possibly be missed using this GLU cutoff, representing a very low number in the overall cohort. Only the 685 ND subjects were used in the metabolic study, because glucose homeostasis is perturbed in the diabetic state, making fasting and OGTT-derived measures more difficult to interpret and less well correlated with insulin sensitivity and secretory parameters obtained from more sophisticated measurements.

Adiposity phenotypes. BMI was derived from body weight divided by height squared (kg/m^2). WC and skinfold thicknesses (evaluated at six sites: biceps, triceps, medial calf, abdominal, suprailiac, and subscapular areas) were measured by a single observer as previously described (19).

Genotype and haplotype determination. Genomic DNA was obtained from cultured lymphoblastoid cell lines by proteinase K and phenol/chloroform extraction procedure followed by dialysis. Polymerase chain reaction (PCR) amplifications of the DNA segments encompassing the SUR1 IVS15-3c→t and exon 18 Thr759(ACC→ACT) variants were carried out using primers previously reported (8,9), and specific information is available in the online appendix at www.diabetes.org/diabetes/appendix.asp. Using both polymorphisms of the SUR1 gene, haplotypes were obtained. Because the family structures are known in QFS and genotypes of parents and offspring were both available, Mendelian analysis determined that the double heterozygote was cT/tC and not tT/cC (see online appendix at www.diabetes.org/diabetes/appendix.asp). No subjects were found with the genotypes tT/tT, tT/cT, or tT/tC, and the single individual carrying the cT/cT genotype was not included in the analysis. The exon 18 Thr759(ACC→ACT) T^+ group was thus comprised of the cT/tC double heterozygote and the cT/cC subgroups.

Statistical Analysis. Nonnormally distributed variables were log-transformed before analysis. Metabolic parameters were adjusted for age, age^2, age^3, and sex. Moreover, data were adjusted for WC and SF6, but not BMI, because BMI never achieved significance when WC and SF6 were present. Analysis of variance through the General Linear Model procedure in SAS (version 6.12; Cary, NC) was used to test for differences in metabolic parameters between genotypes. A χ^2 test was applied to evaluate whether genotype and allele frequencies were in Hardy-Weinberg equilibrium and to test for genotype and allele frequency differences. Linkage disequilibrium between the polymorphisms was assessed as described by Terwilliger and Ott (20).

All subjects were used in association analyses, despite the relatedness of the subjects. A recent simulation study compared three methods of accounting for nonindependence in family sampling designs to a method that ignored the within-family dependencies (Michael A. Province, Treva Rice, D.C. Rao, unpublished data). Results showed that failure to take into account dependencies among subjects of the same family in statistical analyses did not induce any bias, and ignoring these dependencies resulted in a small reduction in power without affecting type I error, except in cases of extreme within-family correlation, which is rare in family studies. We are primarily concerned with failure to detect significant associations, especially if the significance level is borderline; therefore, we believe that it is more appropriate to use all subjects in these association studies. Data are presented as least square-means ± SE. For log-transformed variables, the results are the back-transformed least-square means ± SE derived from the 95% confidence intervals. A P value <0.05 was considered significant.

ACKNOWLEDGMENTS

This study was supported by grants from the Medical Research Council of Canada (PG-11811, MT-13960, and GR-15187). We thank Claude Leblanc, François Michaud, and Christian Couture for their assistance with the computer database and information systems. Thanks are also expressed to Monique Chagnon and Chantal Paré for their technical assistance; Guy Fournier, Lucie Allard, and Anne-Marie Bricault for their dedicated work in QFS; and Diane Drolet for manuscript and secretarial support.

C.B. was previously supported by the Donald B. Brown Chair on Obesity funded by the Medical Research Council of Canada and Roche Canada, and he is currently funded by the George A. Bray Chair in Nutrition.

Preliminary results from this study were presented at the 59th Scientific Sessions of the American Diabetes Association Meeting, San Diego, California, in June 1999.

REFERENCES

1. Gottlieb MS: Diabetes in offspring and siblings of juvenile- and maturity-onset-type diabetics. *J Chronic Dis* 33:331–339, 1980
2. Newman B, Selby JV, King MC, Slemenda C, Fabsitz R, Friedman GD: Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. *Diabetologia* 30:763–768, 1987
3. Kahn CR, Vicent D, Doria A: Genetics of non-insulin dependent (type-II) diabetes mellitus. *Annu Rev Med* 47:509–531, 1996
4. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennet PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of NIDDM. *N Engl J Med* 329:1988–1992, 1993
5. Ashcroft FM, Rorsman P: Electrophysiology of the pancreatic β-cell. *Prog Biophys Mol Biol* 54:87–143, 1989
6. Aguilar-Bryan L, Clement JP 4th, Gonzalez G, Kunjilwar K, Babenko A, Bryan J: Toward understanding the assembly and structure of K_{ATP} channels. *Physiol Rev* 78:227–245, 1998
7. Thomas PM: Genetic mutations as a cause of hyperinsulinemic hypoglycemia in children. *Endocrinol Metab Clin North Am* 28:647–656, 1999
8. Hansen T, Echwald SM, Hansen L, Moller AM, Almind K, Clausen JO, Urhammer SA, Inoue H, Ferrer J, Bryan J, Aguilar-Bryan L, Permutt MA, Pedersen O: Decreased tolbutamide-stimulated insulin secretion in healthy subjects with sequence variants in the high-affinity sulfonylurea receptor gene. *Diabetes* 47:598–605, 1998
9. Inoue H, Ferrer J, Welling CM, Elbein SC, Hoffman M, Mayorga R, Warren-Perry M, Zhang Y, Millns H, Turner R, Province M, Bryan J, Permutt MA, Aguilar-Bryan L: Sequence variants in the sulfonylurea

receptor (SUR) gene are associated with NIDDM in Caucasians. *Diabetes* 45:825–831, 1996
10. Hani EH, Clement K, Velho G, Vionnet N, Hager J, Philippi A, Dina C, Inoue H, Permutt MA, Basdevant A, North M, Demenais F, Guy-Grand B, Froguel P: Genetic studies of the sulfonylurea receptor gene locus in NIDDM and in morbid obesity among French Caucasians. *Diabetes* 46:688–694, 1997
11. 't Hart LM, de Knijff P, Dekker JM, Stolk RP, Nijpels G, van der Does FE, Ruige JB, Grobbee DE, Heine RJ, Maassen JA: Variants in the sulphonylurea receptor gene: association of the exon 16–3t variant with type II diabetes mellitus in Dutch Caucasians. *Diabetologia* 42:617–620, 1999
12. Ohta Y, Tanizawa Y, Inoue H, Hosaka T, Ueda K, Matsutani A, Repunte VP, Yamada M, Kurachi Y, Bryan J, Aguilar-Bryan L, Permutt MA, Oka Y: Identification and functional analysis of sulfonylurea receptor 1 variants in Japanese patients with NIDDM. *Diabetes* 47:476–481, 1998
13. 't Hart LM, Dekker JM, van Haeften TW, Ruige JB, Stehouwer CD, Erkelens DW, Heine RJ, Maassen JA: Reduced second phase insulin secretion in carriers of a sulphonylurea receptor gene variant associating with type II diabetes mellitus. *Diabetologia* 43:515–519, 2000
14. Hovorka R, Jones RH: How to measure insulin secretion. *Diabetes Metab Rev* 10:91–117, 1994
15. Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gonoi T, Iwanaga T, Miyazaki J, Seino S: Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. *Proc Natl Acad Sci U S A* 95:10402–10406, 1998
16. Mohan V, Snehalatha C, Ramachandran A, Jayashree R, Viswanathan M: C-peptide responses to glucose load in maturity-onset diabetes of the young (MODY). *Diabetes Care* 8:69–72, 1985
17. Kahn SE, Leonetti DL, Prigeon RL, Boyko EJ, Bergstrom RW, Fujimoto WY: Relationship of proinsulin and insulin with noninsulin-dependent diabetes mellitus and coronary heart disease in Japanese-American men: impact of obesity–clinical research center study. *J Clin Endocrinol Metab* 80:1399–406, 1995
18. Bouchard C: Genetic epidemiology, association and sib-pair linkage: results from the Québec Family Study. In *Molecular and Genetic Aspects of Obesity*. Bray GA, Ryan DH, Eds. Baton Rouge, Louisiana, Louisiana State University Press, 1996, p. 470–481
19. Rice T, Nadeau A, Pérusse L, Bouchard C, Rao DC: Familial correlations in the Quebec family study: cross-trait familial resemblance for body fat with plasma glucose and insulin. *Diabetologia* 39:1357–1364, 1996
20. Terwilliger J, Ott J: *Handbook for Human Genetic Linkage*. John Hopkins University Press, Baltimore, 1994