ALELLIC VARIATION IN EXON 18 OF THE SULFONYLUREA RECEPTOR 1 (SUR1) GENE, INSULIN SECRETION AND INSULIN SENSITIVITY IN NONDIABETIC RELATIVES OF TYPE 2 DIABETIC SUBJECTS


SUMMARY - Background: We have previously observed associations of the T-allele of the exon 18 variant (ACC → ACT; Thr759Thr) of the sulfonylurea receptor 1 (SUR1) gene with type 2 diabetes mellitus (T2DM). Here we assess β-cell function and insulin sensitivity in carriers of different genotypes at this locus.

Methods: Pre-hepatic insulin secretion rates (ISR) derived by deconvolution of circulating C-peptide levels, and glucose clearance were assessed during graded infusions of intravenous glucose in CC-homozygous (n = 6) and CT-heterozygous (n = 6) nondiabetic relatives of CT-heterozygous type 2 diabetic subjects.

Results: Average ISR over the duration of the study, adjusted for sex, age, BMI and prevailing glucose levels, were lower in CT-heterozygous subjects as compared with CC-homozygous subjects (3.91 ± 0.40 vs. 4.84 ± 0.28 pmol/kg.min⁻¹; p = 0.048). The correlation curves relating ISR to glucose levels were significantly different in the two groups (analyses of covariance p = 0.029). Glucose clearance was similar in both groups.

Conclusions: Insulin secretion rates, but not insulin sensitivity, assessed during graded infusion of glucose were mildly decreased in nondiabetic relatives of type 2 diabetic subjects, who carry the at risk T-allele of exon 18 variant of the SUR1 gene. These results suggest that the at-risk allele might have a small effect on pancreatic β-cell function and contribute to the development of T2DM in these families.

Key-words: sulfonylurea receptor, insulin secretion, insulin sensitivity, glucose infusion, glucose clearance.

REZUMÉ - Variation allélique de l’exon 18 du gène du récepteur 1 des sulfamides (SUR1), insulinosécrétion et sensibilité à l’insuline chez des apparentés non diabétiques de patients diabétiques de type 2. Contexte : Nous avons précédemment observé une association de l’allèle T du variant de l’exon 18 (ACC → ACT; Thr759Thr) du gène codant le récepteur 1 des sulfamides (SUR1) avec le diabète de type 2 (DT2). Ici, nous évaluons la fonction β-pancréatique et la sensibilité à l’insuline chez des porteurs des différents génotypes de ce variant.

Méthodes : Douze sujets non diabétiques, hétérozygotes CT (n = 6) ou homozygotes CC (n = 6), apparentés de patients diabétiques de type 2 hétérozygotes CT, ont été étudiés. Les taux de sécrétion d’insuline pré-hépatiques (ISR), calculés par déconvolution du peptide-C, et la clairance du glucose ont été évalués au cours de perfusions intraveineuses de glucose à multiples paliers.

Résultats : ISR, ajusté pour le sexe, l’âge, le BMI et la glycémie concomitante, était inférieur chez les sujets hétérozygotes CT, par rapport aux taux chez les sujets homozygotes CC (moyenne sur la durée de l’étude ± SEM : 3,91 ± 0,40 vs. 4,84 ± 0,28 pmoI/kg.min⁻¹; p = 0,048). Les courbes de corrélation entre ISR et glycémie étaient significativement différentes dans les deux groupes de sujets (analyse de covariance p = 0,028). La clairance du glucose était semblable dans les deux groupes.

Conclusions : La sécrétion d’insuline en réponse au glucose, mais pas la sensibilité à l’insuline, était modérément diminuée chez les porteurs de l’allèle T à risque du variant de l’exon18 du gène SUR1, apparentés non diabétiques de sujets diabétiques de type 2. Ces résultats suggèrent que cet allèle est associé dans ces familles à un petit effet sur la fonction β-pancréatique qui pourrait contribuer au développement du DT2.

Mots-clés : récepteur des sulfamides, sécrétion d’insuline, sensibilité à l’insuline, perfusion de glucose, clairance du glucose.
he sulphonylurea receptor 1 (SUR1) is a subunit of the pancreatic β-cell ATP-sensitive potassium channel (K\textsubscript{ATP} channel), which plays a central role in glucose-induced insulin secretion by linking signals derived from glucose metabolism to cell membrane depolarisation and insulin exocytosis [1]. Studies in various populations provided evidence for associations of single nucleotide polymorphisms (SNPs) in intron 15 splice acceptor site (IVS15-3c\textrightarrow;t; cagGCC \rightarrow tagGCC), exon 18 (ACC \rightarrow ACT; Thr759Thr) and exon 31 (AGG \rightarrow AGA; Arg1273Arg) of the SUR1 gene with type 2 diabetes mellitus (T2DM) [2-8]. We have observed associations of the rare T allele of the exon 18 SNP with T2DM and with morbid obesity (BMI = 40 kg/m\textsuperscript{2}) in two cohorts of French Caucasian subjects characterised by a strong family history either of diabetes or obesity [3]. T2DM and obesity are strongly associated and share many metabolic abnormalities such as hyperinsulinaemia. We have observed no clear-cut phenotypic differences in carriers of the different alleles of this SNP [3]. However, no detailed measurements of insulin secretion and insulin sensitivity were made in these subjects.

Functional studies performed in euglycaemic individuals, positive for a genetic marker associated with diabetes, provide a unique opportunity to assess alterations in β-cell function and insulin sensitivity independent of the presence of hyperglycaemia, a well known confounding factor. Thus, the present study was undertaken to address the potential pathophysiological implications of the allelic variation in exon 18 SNP of the SUR1 gene. We have assessed β-cell function and insulin sensitivity in CC-homozygous and CT-heterozygous nondiabetic relatives of CT-heterozygous type 2 diabetic subjects, members of the original cohort where this SNP was found to be associated with T2DM [3].

### METHODS

#### Detection of the exon 18 variant (ACC → ACT; Thr759Thr)

The SNP in exon 18 was examined by a PCR-RFLP based method as described previously [3]. This variant corresponds to the variant of exon 22 of previous publications, in which the exons were mistakenly numbered starting from the 3' end instead of the 5' end of the SUR1 gene [2, 3].

#### Subjects

Subjects participating in the present study belong to a cohort of French Caucasian families with multiple cases of T2DM [3]. An association of diabetes with the SNP in exon 18 of the SUR1 gene, but not with the SNP in intron 15, was previously observed in this cohort [3]. The frequency of the T-allele of exon 18 SNP was 0.038 in the probands from these families and 0.009 in unrelated nondiabetic control subjects (Fisher’s exact test p = 0.033). Genotype frequencies were 0.923 vs 0.983 CC and 0.077 vs 0.017 CT (p = 0.031) for probands and controls, respectively. No TT homozygosity was observed in the probands, their relatives or in the controls. In the present investigation, metabolic studies were performed in 12 subjects (7 women, 5 men) from 9 kindred. They had normal glucose tolerance as assessed by a 75-g oral glucose tolerance test. Four subjects were overweight (BMI > 26 kg/m\textsuperscript{2}) and another four were obese (BMI > 30 kg/m\textsuperscript{2}), but otherwise, all were in good general health. None were taking medication known to interfere with glucose homeostasis. All subjects had at least one first-degree relative with T2DM who carried the rare T allele at the SUR1 exon 18 SNP, including a sib in all but one case and a parent in the remaining case. For the purpose of these investigations, subjects were divided in two groups according to the genotype of the exon 18 SNP: CC-homozygous (n = 6) and CT-heterozygous (n = 6) carriers. Basic clinical profile of participants are shown in Table I.

#### Study Design

Investigations were performed at the clinical research centre (Centre d’Investigation Clinique) of Hôpital Necker-Enfants Malades, Paris, France. Insulin secretion and insulin sensitivity were evaluated during the administration of graded infusions of glucose. Subjects were instructed to eat a diet in which carbohydrates comprised at least 50% of total calories for at least five days preceding the test. Studies were started at 8:00-9:00 AM with subjects in the recumbent position after a 12h overnight fast. The experimental protocol was detailed elsewhere [9]. Briefly, intravenous catheters were placed in the right and in the left forearms for glucose administration and blood sampling. Following a 30 minute baseline sampling period, a graded intravenous infusion of 30% glucose was then started at a rate of 1 mg/kg body weight.min\textsuperscript{-1}, followed by infusions at 2, 3, 4, 6 and 8 mg/kg body weight.min\textsuperscript{-1} (Infusomat, Secura Braun, Melssungen, Germany). Each infusion rate was maintained for a period of 40 minutes, except the first one that lasted 60 minutes. Blood samples were drawn every 10 minutes throughout the experiment for measurement of glucose, insulin and C-peptide concentrations.
Assays

Plasma glucose was measured by the glucose oxidase technique (Yellow Springs Instruments Co., Yellow Springs, OH). Serum insulin and C-peptide were measured by radioimmunoassays (CIS-Bio, Gif-sur-Yvette, France). Cross-reactivity with intact pro-insulin was at least 20% for insulin and 15% for C-peptide assays.

Data Analysis

Relationships between glucose levels and prehepatic insulin secretion rates (ISR) during graded intravenous glucose infusions were explored. ISR and glucose levels used in the analysis represented the average of the values for each individual at each glucose infusion rate. ISR was then plotted against the corresponding glucose level to define a dose-response relationship. ISR were derived by deconvolution of circulating C-peptide concentrations [10] using version 3.4a of the ISEC software [11]. Individual kinetic parameters of C-peptide clearance are computed by ISEC from standard kinetic parameters, taking into account the age, sex, body surface area, and glucose tolerance status of the subject [12]. Glucose clearance was used as an index of insulin sensitivity. It was computed for each step of the graded intravenous glucose infusion by dividing the glucose infusion rate (mmol.kg body weight$^{-1}$.min$^{-1}$) by the corresponding average plasma glucose concentration (mmol/l) [13].

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistics were performed with the JMP software (SAS Institute Inc, Cary, NC). The Shapiro-Wilk W test was used to test the gaussian distribution of clinical and biological parameters, and data was log-transformed for the statistical analyses when appropriate. Comparisons between groups were made with Student’s t-test. In order to adjust by sex, age and BMI the metabolic parameters under comparison, statistical differences were also assessed by analysis of variance through the multiple regression procedure of JMP. Qualitative traits were analysed by contingency table chi-square tests. Repeated measures analyses of variance (ANOVA) were employed to compare glucose, insulin and C-peptide values across a period of time during the graded intravenous infusions of glucose. Analyses of covariance (ANCOVA) were employed to compare correlation of parameters between groups.

RESULTS

Insulin secretion response to graded intravenous glucose infusion

Baseline glucose, insulin and C-peptide levels were similar in CT-heterozygous and CC-homozygous subjects: $5.42 ± 0.3$ vs $5.8 ± 0.1$ nmol/l (p = 0.25), $82 ± 17$ vs $90 ± 14$ pmol/l (p = 0.59) and $0.73 ± 0.18$ vs $0.87 ± 0.15$ nmol/l (p = 0.43), respectively. Glucose, insulin and C-peptide responses to the glucose infusion are shown in figure 1. Average concentrations of these parameters over the duration of the study were $8.0 ± 0.4$ vs $8.5 ± 0.3$ mM glucose (p = 0.26), $179 ± 23$ vs $196 ± 18$ pmol/l insulin (p = 0.17) and $1.62 ± 0.18$ vs $1.74 ± 0.12$ nM C-peptide (p = 0.11) for CT-heterozygous and CC-homozygous subjects, respectively. These comparisons were also performed with data adjusted for sex, age, BMI and prevailing glucose levels (for insulin and C-peptide comparisons only), with results expressed as back-transformed least-square means from the general model multiple regression analysis. Average values over the duration of the study were $7.4 ± 1.0$ vs $8.0 ± 1.0$ mM glucose (p = 0.19), $140 ± 15$ vs $156 ± 14$ pmol/l insulin (p = 0.24) and $1.18 ± 0.11$ vs $1.49 ± 0.12$ nM C-peptide (p = 0.06) for CT-heterozygous and CC-homozygous subjects, respectively. Average values for each glucose infusion step were similar in both groups.
(repeated measures ANOVA \( p = 0.12 \) for glucose, 
\( p = 0.29 \) for insulin and \( p = 0.12 \) for C-peptide ; data not shown).

Baseline ISR were \( 2.07 \pm 0.47 \) pmol/kg.min\(^{-1} \) for CT-heterozygous subjects and \( 2.50 \pm 0.36 \) pmol/kg.min\(^{-1} \) for CC-homozygous subjects (\( p = 0.35 \)). Average ISR over the duration of the study presented a trend towards lower values in CT-heterozygous subjects as compared with CC-homozygous subjects (\( 5.04 \pm 0.56 \) vs \( 5.41 \pm 0.36 \) pmol/kg.min\(^{-1} \), respectively ; \( p = 0.09 \)). When adjusted for sex, age, BMI and prevailing glucose levels, this difference became
statistically significant: $3.91 \pm 0.40$ vs $4.84 \pm 0.28 \text{ pmol/kg.min}^{-1}$, respectively ($p = 0.048$). In order to allow the ISR to be compared in the two groups at the same plasma glucose levels, the average ISR for each individual at each glucose infusion step was plotted against the corresponding glucose level (Fig. 2). ISR were well correlated with glucose levels both in CT-heterozygous ($R^2 = 0.28 \ p < 0.0001$) and in CC-homozygous ($R^2 = 0.58 \ p < 0.0001$) subjects. ANCOVA was performed to compare between groups these correlations. The curves relating ISR to glucose levels were significantly different in CT-heterozygous and in CC-homozygous subjects ($p = 0.029$) when sex, age, BMI were included in the model as covariate factors. This suggests that insulin secretion rates in response to graded infusions of glucose were lower in CT-heterozygous subjects as compared to their CC-homozygous relatives.

**Glucose clearance**

Glucose clearance significantly increased throughout the experiment in both groups (repeated measures ANOVA $p < 0.0001$). However, average values over the seven glucose infusion steps were similar in CT-heterozygous and in CC-homozygous subjects ($2.71 \pm 0.17$ vs $2.58 \pm 0.18 \text{ ml/kg bw.min}^{-1}$, respectively; $p = 0.54$). Figure 3 shows average glucose clearance at each glucose infusion step plotted against the corresponding insulin levels. The relationship between glucose clearance and insulin concentration was not significantly different between groups when tested by ANCOVA in a model including sex, age and BMI as co-variates.

**DISCUSSION**

In the present investigation, we have used a sensitive method to explore insulin secretion in vivo, consisting of graded infusion of intravenous glucose coupled with a mathematical model to compute pre-hepatic insulin secretion rates (ISR). We have observed that ISR in response to glucose were mildly decreased in a group of nondiabetic subjects heterozygous for the C- and T-alleles of exon 18 SNP of the SUR1 gene as compared to values in first-degree relatives homozygous for the C-allele. These subjects belong to families for which an association of the
T-allele with T2DM was observed [3]. Glucose clearance assessed as an index of insulin sensitivity was similar in CC-homozygous and CT-heterozygous relatives.

It is important to point out that our study had limited power to detect a direct effect of the genotype on ISR. Power, that is, the probability of getting a significant result at p = 0.05 level was only 0.27 (95% confidence interval 0.05-0.95). Power computation also showed that observations from three additional subjects per group might bring the difference between groups of average ISR to a p < 0.05 significance level. Unfortunately, no additional family member was available for these investigations. However, in the context of the small sample size of our study, the mild differences in insulin secretion between groups could be detected by analysing dose-response curves relating ISR to glucose levels and by taking into account covariates such as sex, age and the BMI. This might suggest that the at-risk allele for diabetes in these families has only a small effect on pancreatic β-cell function, or even only a modulator effect in the presence of a diabetic environment.

Nevertheless, our results agree with those from a much larger group of nondiabetic French-Canadians from the Quebec Family Study. In that study, after adjustment for sex, age and the BMI, C-peptide levels following an OGTT were mildly decreased (~13%) in CT-heterozygous as compared to CC-homozygous subjects [14]. Interestingly, an additive effect of the tagGCC allele of intron 15 and the T-allele of exon 18 SNPs was observed, with a ~20% reduction of C-peptide levels in carriers of both at risk genotypes [14].

A few other studies involving measurement of insulin secretion in vivo were performed in subjects with different SUR1 genotypes at SNPs in intron 15 and exons 18 and 31. These studies in various populations and with variable sample size used different methods for the assessment of β-cell function and yielded contrasting results. Insulin secretion and insulin sensitivity were assessed by the hyperglycaemic clamp in two groups of Dutch subjects, either with normal or with impaired glucose tolerance [15]. A 25% reduction in the second-phase insulin secretion was observed in carriers of the tagGCC allele of intron 15 SNP in both groups of subjects, while no differences in insulin sensitivity were noted. The exon 18 SNP and the combined presence of at risk genotypes of intron 15 and exon 18 SNPs were not associated with differences in insulin secretion or insulin sensitivity in that study. In a recent study, American subjects with normal or with impaired glucose tolerance, and selected for having at least two siblings with T2DM undergone a tolbutamide-modified intravenous glucose tolerance test [16]. Heterozygous carriers of the tagGCC allele of intron 15 SNP presented reduced insulin secretion in response to glucose or tolbutamide, as compared to homozygous carriers of either of the two alleles. Moreover, the tagGCC allele was associated with reduction in the β-cell compensation to decreased insulin sensitivity [16]. Here again, no effect was observed for the exon 18 SNP. In contrast with those studies,
insulin secretion in response to an intravenous bolus injection of glucose or of tolbutamide was similar in young nondiabetic Danish subjects, carriers of the different genotypes of intron 15 or exon 18 SNPs [4]. However, subjects who were either homozygous or heterozygous for the tagGCC allele of intron 15 SNP and also carried the T allele of the exon 18 SNP showed a 50% decrease in insulin secretion after tolbutamide injection, but a normal β-cell response upon glucose injection. Insulin sensitivity assessed by the minimal model was similar in both groups.

The A allele of the exon 31 SNP was associated with hyperinsulinemia both in the fasting state and following an oral glucose load in a cohort of nondiabetic Mexican-Americans subjects [17], a population with a high prevalence of T2DM. In that study, subjects who were homozygous for the A allele had insulin levels two hours after the glucose load twice as higher as the carriers of other genotypes. Finally, contrasting with all the previous studies, insulin secretion in response to oral or intravenous glucose tolerance tests was similar in nondiabetic Finnish subjects, carriers of the different genotypes in these three SNPs [7].

The genetic mechanism behind the association of these silent or intronic SNPs with β-cell defects and diabetes remains unexplained. Linkage disequilibrium with a functional variant in the SUR1 gene or nearby may be a possible explanation. The variable allelic distribution of the three SNPs in diabetic and nondiabetic subjects in different Caucasian populations [18], as well as the heterogeneous results of studies of β-cell function in these populations suggests that different risk alleles for diabetes may exist. This might be related to variable degrees of linkage disequilibrium of the SNPs with the putative functional variant. It is now clear that SUR1 is not a major diabetogenic locus [3, 19, 20], but plays only a minor role in a polygenic context. The identification of the functional variants in the SUR1 gene or nearby will allow a better assessment of the impact of this locus on the mechanisms of hyperglycaemia of T2DM.

Acknowledgements – We thank P. Gallina for managing the contact with the subjects who participated in the study. We are indebted to the nursing (A. Antoine, R. Nyckees, I. Ratet), technical (V. Leceru, A. Papot) and secretarial (H. Moretti-Larrieu, N. Paris) staff at the CIC-Necker for their expert assistance. This work was supported by grants from INSERM for research in Clinical Investigation Centres (INSERM CIC 9303 NEM), the Ministry for Research and Technology (France), and the European Union (Biomed 2). A.F. Reis was supported by a doctoral grant from CAPES, Brasilia, Brazil. The ISEC software was kindly provided by Dr. R. Hovorka (Centre for Measurement and Information in Medicine, Department of Systems Science, City University, London, UK).

REFERENCES

8 Reis AF, Ye WZ, Dubois D, Bellamé-Chantelot C, Timsit J, Velho G. Association of a variant in exon 31 of the sulfonylurea receptor 1 (SUR1) gene with type 2 diabetes mellitus in French Caucasians. Hum Genet, 2000, 107, 138-144.
11 Hovorka R. A computer program to reconstruct insulin secretion. Diabetologia, 1993, 36 (suppl.1), A68.