Diabetologia (1999) 42: 617–620

Diabetologia

Short communication

Variants in the sulphonylurea receptor gene: association of the exon 16–3*t* variant with Type II diabetes mellitus in Dutch Caucasians

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Abstract

Aims/hypothesis. We have analysed to what extent two previously reported single nucleotide polymorphisms in the sulphonylurea receptor gene (*SUR1*) are associated with Type II (non-insulin-dependent) diabetes mellitus in The Netherlands. Furthermore, we estimated haplotype frequencies in control and diabetic populations, including data extracted from three other studies.
Methods. Subjects with Type II diabetes ($n = 388$) and normoglycaemic subjects (n = 336) were randomly selected from two population-based studies, the Hoorn and Rotterdam studies. DNA was typed for variants in exon 16 (-3*c*→*t* variant in the splice acceptor site) and exon 18 (Thr^{759}Thr, AC**C**→AC**T**).
Results. The genotype frequencies in both populations were similar. We observed an association of the exon 16–3*t* variant with Type II diabetes (allele frequencies 0.41 % vs 0.48 % in NGT and Type II diabetes, respectively, $p = 0.01$). There was no association between Type II diabetes and the variant in exon 18 or the combination of both variants ($p > 0.5$). A strong linkage disequilibrium between the exon 16 and exon 18 variants was observed in the diabetic groups but not, or less pronounced, in the control groups from the different studies. Haplotype estimation shows that several different risk haplotypes exist in different Caucasian populations.
Conclusion/interpretation. The exon 16–3*t* allele of the *SUR1* gene is associated with Type II diabetes in the Netherlands. Based on estimated haplotype frequencies in different Caucasian populations we conclude that multiple haplotypes on the *SUR1* gene seem to confer a risk for developing Type II diabetes in Caucasians. [Diabetologia (1999) 42: 617–620]

Keywords Type II diabetes, diabetes, genetics, sulphonylurea receptor, prevalence.

Type II (non-insulin-dependent) diabetes mellitus is a phenotypic and genetic heterogeneous disorder. Both defects in insulin action and insulin secretion are involved [1]. Mutations in a wide variety of genes, such as the genes for glucokinase, insulin receptor, hepatocyte nuclear factors and the mitochondrial DNA, contribute to deregulation of glucose homeostasis. One study has provided evidence for an association between two single nucleotide polymorphisms (SNPs) in the *SUR1* gene and Type II diabetes in Caucasians [2]. Additional studies have corroborated this association with Type II diabetes in populations with different ethnic backgrounds [3–5].

It is known from other studies that even in study groups confined to Caucasians of north-western European descent, differences occur in the degree of as-

Received: 6 November 1998 and in final revised form: 14 January 1999

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Abbreviations: SNP, Single nucleotide polymorphism.

Table 1. Characteristics of the study groups

	Rotterdam Study		Hoorn Study	
	NGT	Type II	NGT	Type II
n	170	196	166	192
Sex (%, M/F)	56/44	53/47	60/40	54/46
Age (years)	64 ± 5.6	67 ± 5.0	61 ± 6.5	65 ± 7.8
BMI (kg/m^2)	25.9 ± 3.1	26.9 ± 3.8	26.3 ± 2.9	28.6 ± 4.9
W/H ratio	0.89 ± 0.09	0.94 ± 1.0	0.89 ± 0.08	0.94 ± 0.09
HbA_{1c}(%)	5.9 ± 0.4[a]	7.3 ± 1.3[a]	5.5 ± 0.5	7.2 ± 1.8
Fasting glucose (mmol/l)	5.7 ± 0.8[a]	9.1 ± 3.1[a]	5.4 ± 0.7	7.9 ± 2.7
Random glucose (mmol/l)	6.0 ± 1.0	12.0 ± 4.8	n. a.	n. a.
Fasting Insulin (pmol/l)	56 (44 – 82)	78 (52 – 114)	75 (60 – 96)	84 (59 – 107)

All values are expressed as means ± standard deviation or median (interquartile range).
[a] Only available for a part of the Rotterdam Study group (NGT, *n* = 116: Type II diabetes, *n* = 86).
n. a.: not available

sociation of particular gene variants and diabetes [6]. Our study was undertaken to analyse the association of the two SNPs, in exon 16 and exon 18 of the *SUR1* gene, with Type II diabetes in the Netherlands. We included subjects from two independent population studies, the Rotterdam and the Hoorn study, which allowed us to assess the homogeneity of the Dutch population for these SNPs. Furthermore we investigated whether the two SNPs showed linkage with each other in Type II diabetic and control groups from our study and from other published studies in Caucasian populations. Haplotype estimations were made to identify haplotype(s) at risk for developing Type II diabetes in these populations.

Subjects and methods

Study population. Subjects participating in this study were randomly selected from two population-based studies in the Netherlands, the Rotterdam and the Hoorn studies [7, 8]. All non-diabetic participants underwent an oral glucose tolerance test and were diagnosed based on World Health Organisation (WHO) criteria as described [7, 8]. Characteristics of both study populations are shown in Table 1. Participants gave informed consent and the studies were approved by the medical ethics committees. Furthermore, we included genotype data from three other published studies in Caucasian populations for haplotype estimations and linkage calculations [2–4].

Genetic studies. DNA was extracted from peripheral blood samples according to standard procedures. Two previously described SNPs in the sulphonylurea receptor gene in exon 16 (-3*c*→*t*) and exon 18 Thr^{759}-Thr (AC**C**→AC**T**) were examined by a PCR-RFLP based method as described by Hansen et al. [4]. With regard to the detection of the exon 16 (nt–3) SNP there is a discrepancy between this method and the method initially described by Inoue et al. [2] As described by both authors the presence of the variant allele results in the loss of a *Pst 1* site (recognition site ct**g**cag). This is, however, not the result of a t→c mutation as stated by Inoue et al. but from a change from the wildtype c allele to the variant t allele, as shown by Hansen et al. [4]. Therefore the description of the assignments of allelic variants given in reference 2 is wrong. In that publication, however, the correct allele frequencies are presented in the tables (A. Permutt, personal communication). In the publication by El Hani et al. [3] the method described by Inoue et al. was used for the assignment of the c and t alleles. As a result, the listed allele frequencies for the exon 16 SNP should be reversed (P. Froguel, personal communication).

Statistical analysis. Allele and genotype frequencies were compared between the Type II diabetic and control groups by Fisher exact test. Logistic regression analysis was used to adjust the association for age and BMI. We used *t* tests or Mann-Whitney U test to compare diabetes related variables between carriers and non-carriers using SPSS version 7.0. The algorithm described by Hill was used to detect linkage between the two variants and to estimate the prevalence of exon 16/exon 18 haplotypes in the different cohorts [9].

Results

Sulphonylurea receptor exon 16 (-3c→t) and exon 18 (T759T) SNPs. Genotype frequencies for both SNPs are given in Table 2. There were no significant differences in the distribution of the genotypes between the Rotterdam and the Hoorn study ($p > 0.2$). We observed no association of the exon 18 T allele with diabetes in the Rotterdam and Hoorn populations (allele frequency 5.3 % in NGT vs 5.2 % in Type II diabetes). The frequency distribution of the exon 16 genotypes in the NGT and Type II diabetic groups was different. When data from Rotterdam and Hoorn were combined, this difference reached statistical significance (NGT vs Type II diabetes, $p = 0.03$). Adjustment for age and BMI in a logistic regression analysis with glucose tolerance as a dependent variable did not change this association (OR 1.50, 95 % CI, 1.06–2.12, $p = 0.02$). Also, the allele frequency of the -3*t* allele in NGT and Type II diabetic subjects differed (-3*t* allele frequency 41 % vs 48 % in NGT and Type II diabetes, respectively, $p = 0.01$). The combined presence of exon 16 (-3*c*/*t* or -3*t*/*t*) and exon 18 (C/T or T/T) SNPs, which did associate with Type II diabetes in some other studies [2, 4], was not associated with it in our study. Clinical variables such as age, sex, BMI, HbA_{1c}, fasting and 2h-plasma glucose and

Table 2. Genotype frequencies of the exon 16 (nt –3) and exon 18 variants of the *SUR1* gene

Genotype	Rotterdam Study		Hoorn Study		Combined studies	
	NGT	Type II	NGT	Type II	NGT	Type II
Exon 16 (nt –3)						
–3 c/c	52 (0.31)	44 (0.22)	59 (0.35)	53 (0.28)	111 (0.33)	97 (0.25)
–3 c/t	90 (0.53)	111 (0.57)	81 (0.49)	98 (0.51)	171 (0.51)	209 (0.54)
–3 t/t	28 (0.16)	41 (0.21)	26 (0.16)	41 (0.21)	54 (0.16)	82 (0.21)
n	170	196	166	192	336	388
P value	0.18		0.18		0.03	
Exon 18						
C/C	153 (0.90)	178 (0.91)	127 (0.89)	172 (0.90)	280 (0.89)	350 (0.90)
C/T	17 (0.10)	17 (0.09)	16 (0.11)	19 (0.10)	33 (0.11)	36 (0.09)
T/T	0	1 (< 0.01)	0	1 (< 0.01)	0	2 (0.01)
n	170	196	143	192	313	388
P value	0.80		0.82		0.74	

Genotype frequencies in Type II diabetes and normal glucose tolerant (NGT) groups were compared using Fisher exact tests. Values in parenthesis represent fraction of total

Table 3. Estimated haplotype frequencies in four different Caucasian control and diabetic populations

	SUR1 Haplotype			
	Ex. 16–3*c*/Ex.18 C	Ex. 16 –3*t*/Ex. 18 C	Ex. 16–3*c*/Ex. 18T	Ex. 16–3*t*/Ex.18T
Controls				
Netherlands	0.532	0.415	0.053	0.000
Denmark	0.533	0.440	0.027	0.000
France	0.495	0.495	0.010	0.000
Utah + UK	0.453	0.525	0.007	0.016
Type II diabetic				
Netherlands	0.468 (–)	0.481 (+)	0.052 (–)	0.000
Denmark	0.499 (–)	0.451 (+)	0.051 (+)	0.000
France	0.488 (–)	0.474 (–)	0.038 (+)	0.000
Utah + UK	0.552 (+)	0.379 (–)	0.065 (+)	0.003 (–)

Haplotype estimates were calculated according the algorithm of Hill [9]. An increase (+) or decrease (–) in haplotype frequency relative to control groups is indicated for the ease of interpretation

insulin concentrations, lipid spectrum and age of onset of diabetes were not different in diabetic and normoglycaemic carriers of the SNPs compared with non-carriers.

SUR1 haplotype estimation and linkage disequilibrium. Since no genetic data on relatives of the study subjects are available, the data do not allow the reconstruction of the precise exon 16-exon 18 haplotype for each participant. We estimated the haplotype frequency in control and diabetic groups from the combined Rotterdam and Hoorn studies and from three additional studies in Caucasians [2–4] (Table 3). There was no consensus diabetes-risk haplotype present in all studies, but each study population showed differences in haplotype frequencies between control and diabetic groups. The exon 16-3*c*/exon 18T allele was raised in the diabetic group in most studies whereas the exon 16-3*t*/exon 18C haplotype was raised in two (Table 3). The exon 16-3*c*/exon 18C haplotype was raised only in the Utah and United Kingdom populations. Calculations of linkage between both SNPs in the control and diabetic group from the combined Rotterdam and Hoorn studies showed a stronger linkage between both SNPs in the diabetic group compared with the control group. This phenomenon was also present in the three other studies we examined (data not shown).

Discussion

Two SNPs in the *SUR1* gene have been associated with Type II diabetes in different ethnic populations [2–5, 10, 11]. In our study, involving populations from the cities of Rotterdam and Hoorn in the Netherlands, no association was seen between the exon 18 SNP and diabetes. We did detect an association of the exon 16–3*t* SNP with Type II diabetes. No evidence for regional differences in allele frequencies within the Netherlands was obtained. A similar association with the exon 16-3*t* SNP but not with the SNP in exon 18 was recently also reported for a Finnish population [11]. Remarkably, the associations

found in the Dutch and the Finnish study with regard to the exon 16 SNP are with the -3*t* allele whereas in the combined Utah and United Kingdom study the association was found with the -3*c* allele. Other studies in Caucasian populations showed association with the exon 18 SNP but not with the exon 16 SNP. This suggests that multiple risk alleles for the *SUR1* gene exist in different Caucasian populations.

We also estimated the degree of linkage disequilibrium between the two SNPs in the *SUR1* gene in the control and diabetic groups from different Caucasian populations. A strong linkage was calculated between the exon 16 and exon 18 variants in all diabetic groups tested, but not, or less pronounced, in the control groups. Since it is not possible to reconstruct the exact haplotype for each participant from the data, we were not able to determine precisely with which of the four possible haplotypes the diabetogenic locus associates. From the estimated haplotype frequencies for the whole diabetic and control groups we conclude that several different risk haplotypes exist in the different populations. The exon 16-3*c*/exon 18T was the most consistently found risk haplotype, found in three of four independent studies.

It has been reported that the exon 18T allele associates with marked obesity [3]. In the Rotterdam and Hoorn populations, however, we did not observe this association (data not shown). Recently normoglycaemic subjects with the exon 16-3*c*/*t*/exon 18C/T combination were shown to have decreased insulin and C-peptide secretion after tolbutamide stimulation [4]. We recently showed that in subjects with impaired glucose tolerance the exon 16-3*c*/*t* genotype is associated with impaired first phase insulin secretion during a hyperglycaemic clamp [12]. These findings suggest that these *SUR1* SNPs are associated with a functional change of the ATP-sensitive potassium channel in the beta cell, implying that these SNPs affect the function of the *SUR1* gene product. The nature of both variants is such, however, that they do not affect the coding region of the protein, although an effect on the stability or splicing of the mRNA product cannot be completely excluded. Alternatively the SNPs could be in linkage disequilibrium with other functional variants in the *SUR1* gene or the *Kir6.2* gene. The latter gene is located only 4.5 kb apart from the *SUR1* gene on chromosome 11. Together the SUR and the Kir6.2 protein form the beta cell specific ATP-sensitive potassium channel. Further analysis of the *SUR1*/*Kir6.2* locus on chromosome 11p15.1 will be necessary to precisely define the diabetogenic defect(s) in this chromosomal region.

Acknowledgements. The authors would like to thank the participants of the Rotterdam and Hoorn studies for their co-operation. We thank Dr H. El Hani for providing unpublished results, Dr T. Hansen is acknowledged for providing data prior to publication. This work was supported by grants from the Diabetes Fonds Nederland and the European Economic Committee grant No BMH4-CT98–3084.

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