Polymorphisms in the gene encoding hepatocyte nuclear factor-4α and susceptibility to type 2 diabetes in a Polish population

K Wanic1,2, MT Malecki1, PP Wolkow2, T Klupa1, J Skupien1, J Bobrek1, E Kozek1, AS Krolewski2, J Sieradzki1

SUMMARY
Recently, several association studies of type 2 diabetes mellitus (T2DM) and the hepatocyte nuclear factor (HNF)-4α gene were reported with conflicting results. Our aim was to search for association between two polymorphisms of HNF-4α and T2DM in Polish Caucasians. The study groups comprised of 461 T2DM cases and 366 controls. Genotype-quantitative trait analyses were based on the oral glucose tolerance test (OGTT), glucose and insulin results, and comprised 310 glucose-tolerant subjects. All individuals were genotyped for two HNF-4α polymorphisms. The frequencies of the minor alleles were as follows: 19.2% in T2DM vs. 17.6% in controls for rs2144908; and 20.6% vs. 20.1% for rs4810424, respectively. The distributions of alleles, genotypes, and haplotypes of the HNF-4α polymorphisms did not differ between the study groups (lowest P = 0.41). None of the examined SNPs showed an association in control subjects with quantitative traits of fasting plasma glucose, fasting insulin, as well as plasma glucose and insulin 2 hours after glucose load in OGTT. We conclude that both examined polymorphisms in HNF-4α are not associated with T2DM and prediabetic phenotypes in Polish Caucasian study groups of this size.

Key-words: Diabetes · Gene · Polymorphism · HNF-4α.

Résumé
Plusieurs études d’association du diabète de type 2 (T2DM) au gène du facteur nucléaire hépatocytaire (HNF)-4α ont été récemment publiées, avec des résultats contradictoires. Notre objectif était d’étudier l’association de deux polymorphismes d’HNF-4α au T2DM dans une population caucasienne polonaise. Deux groupes de sujets ont été étudiés : 461 patients atteints de T2DM et 366 sujets sains (témoins). Des corrélations entre le génotype et des traits quantitatifs ont été recherchées en analysant les résultats de glycémie et d’insulinémie au cours d’une hyperglycémie provoquée par voie orale (HGPO) réalisée chez 310 témoins. Tous les individus ont été génotypés pour les polymorphismes du gène HNF-4α. La fréquence des allèles mineurs était la suivante : 19,2 % pour les patients T2DM contre 17,6 % dans le groupe témoin pour le polymorphisme rs2144908 ; et 20,6 % contre 20,1 % pour le polymorphisme rs4810424. Les distributions des allèles, génotypes et haplotypes des polymorphismes du gène HNF-4α n’étaient pas significativement différentes entre les deux groupes (valeur P la plus basse = 0,41). Chez les sujets non diabétiques, aucun des marqueurs étudiés n’était significativement associé à la glycémie ou à l’insulinémie mesurées à jeun et à deux heures de l’HGPO. En conclusion, les deux polymorphismes du gène HNF-4α ne sont pas associés au T2DM ni au phénotype pré-diabétique dans la population étudiée.

Mots-clés : Diabète · Gène · Polymorphisme · HNF-4α.
Introduction

Type 2 diabetes mellitus (T2DM) is a complex disorder with inherited and environmental factors influencing its occurrence. Genetic background of complex T2DM remains mostly unknown. Linkage between a region on chromosome 20q13 and susceptibility to T2DM has been established in several family studies [1-3]. Recently, association between T2DM and polymorphisms in the hepatocyte nuclear factor (HNF)-4α, a gene within the linkage region, was reported in some, but not all, examined populations and this association possibly accounts for linkage of T2DM with chromosome 20q [4-8]. Whether this association can be replicated in other ethnic groups remains to be investigated. HNF-4α is an orphan nuclear receptor that plays a key role in the development and function of the β cell. Rare mutations in the HNF-4α gene were linked to the monogenic form of diabetes (MODY1) [9].

The aim of our study was to search for the association between HNF-4α and T2DM in Polish Caucasians. In previously published studies, a number of polymorphisms were used. For this project, we selected two SNPs of the HNF-4α gene: rs2144908 and rs4810424, both adjacent to its P2 promoter [10]. The first one was analysed in the majority of recently published studies, the second produced positive results in the UK population [6].

Materials and methods

We included 827 unrelated individuals in this study: 461 T2DM patients and 366 non-diabetic controls. All the study individuals were Caucasians and residents of Southeastern Poland. The ascertainment process, through which the current WHO definitions and criteria were used, was as previously described in detail [11]. Briefly, only patients with clinical diagnosis of T2DM and with no insulin therapy for at least two years after diagnosis were recruited. The control group contained only individuals with normal fasting glucose, mainly the spouses of T2DM patients and volunteers from the medical personnel. The clinical characteristics of the study groups are shown in table I. The OGTT glucose and insulin results were available for 310 glucose-tolerant subjects from the control group and genotype-quantitative trait analyses were based on these results. This subgroup derived from our controls included 198 women and 112 men, they were on average 50 years old at the time of examination, and the mean BMI was 30.3. This study was performed according to the Helsinki Declaration and it was accepted by the Ethical Committee of the Jagiellonian University, Medical College.

All individuals were genotyped for two HNF-4α polymorphisms using a fluorescence polarization method. Alleles and genotypes were determined by single base extension/fluorescence polarization (AcycloPrime-FP SNP Detection System) method [12,13] using a Wallace Victor Multilabel Plate Reader (Perkin Elmer). Primers and probes designed in accordance with public databases were used. Using blinded duplicate samples in each 96-well assay, we checked the quality of genotyping. We evaluated differences between groups in allele, genotype, and haplotype frequencies by chi-square analysis. Mathematical framework of generalized linear models (PROC GLM in SAS software) was used to test whether the genotyped polymorphisms had influence on four quantitative traits: fasting plasma glucose, fasting insulin, as well as, glucose and insulin 2 hours after glucose load in OGTT. For these analyses of quantitative traits, we used outcome variables of plasma glucose concentrations as measured while insulin concentrations were log transformed to fulfil the requirement of the normal distribution. We tested crude associations of each polymorphism with each trait under assumptions of dominant, additive and recessive modes of inheritance and finally we tested whether these associations change when covariates such as age of examination, gender and BMI were included in the model. Linkage disequilibrium between the SNPs was also tested using SAS Software Release 8.2 (SAS Institute Inc., Cary, NC, USA). HAPLO.STAT software was used to infer and compare haplotype distributions. Estimation of power to determine association was calculated as described previously [14].

Results and discussion

Both HNF-4α polymorphisms were in Hardy-Weinberg equilibrium. There was evidence of almost complete linkage disequilibrium between the analysed SNPs (D’ = 0.902). The frequencies of the alleles and genotypes of both examined polymorphisms did not differ between T2DM patients and controls (lowest P = 0.41) (table II).

Table I

Characteristics of the study groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>T2DM</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Women</td>
<td>54.4</td>
<td>64.6</td>
</tr>
<tr>
<td>Age at examination (years)a</td>
<td>58.6</td>
<td>54.6</td>
</tr>
<tr>
<td>Age of diagnosis (years)a</td>
<td>49.2</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of disease (years)a</td>
<td>9.2</td>
<td>NA</td>
</tr>
<tr>
<td>BMI (kg/m²)a</td>
<td>31.1</td>
<td>30.1</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)a</td>
<td>7.6</td>
<td>4.7</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.7</td>
<td>NA</td>
</tr>
<tr>
<td>% on insulin treatment</td>
<td>54.7</td>
<td>NA</td>
</tr>
<tr>
<td>% Positive family history of T2DM in first or second degree relatives</td>
<td>51.7</td>
<td>23.0</td>
</tr>
</tbody>
</table>

NOTE - The high proportion of insulin-treated (either alone or in combination with oral medications) T2DM patients is related to the relatively long mean duration of disease. Fasting glucose was measured in T2DM patients prior to the administration of the morning dose of oral hypoglycaemic agents or insulin. a Data shown as mean.
The alleles of rs4810424 and rs2144908 formed two frequent haplotypes: G-G and C-A. Their frequencies were as follow: 78% in T2DM vs. 78.2% in controls for G-G; and 18% vs. 16% for C-A, respectively (global statistic $P = 0.24$). None of the examined polymorphisms showed an association with quantitative traits such as glucose and insulin levels, both fasting and 2hrs. Lowest $P = 0.06$ was found for SNP rs 4810424 and quantitative trait of plasma glucose concentration at 2 hours after glucose load using an additive model; but adjustment for age, BMI and sex removed the borderline significance.

We were not able to confirm the positive results of association studies in Finns, Ashkenazi Jews and in the UK population [4-6]. There are several possible explanations for this fact. This study had > 80% power to detect ORs from one of the initial HNF-4α studies [4]. Our analysis, however, may be lacking power to detect weaker association such as in the Finnish and British studies [5,6]. The alternative explanation is genetic heterogeneity among examined ethnic groups and the true absence of the association in the Polish population. It should be noted that two large, recently published association studies failed to confirm the previously reported association [7,8]. In the first one, none of the previously associated SNPs conferred an increased risk for diabetes in French Caucasians. The second study of more than 7000 individuals included a subgroup of samples from Poland provided by a biotechnological company. This group was examined for the association with HNF-4α gene SNPs with negative results [7].

In summary, both examined polymorphisms in HNF-4α were not associated with T2DM and prediabetic phenotypes in Polish Caucasian study groups of this size. It seems feasible that a future large meta-analysis could eventually help to define the role of the HNF-4α gene in complex T2DM.

Acknowledgements – This research was supported by the FIRCA 1 R03 TW01351-01 and DK47475 NIH Grants.

References

4. Love-Gregory LD, Wesson J, Ma J, et al. A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 alpha gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an ashkenazi jews-