Endocrine (2012) 42:577–583
DOI 10.1007/s12020-012-9656-5

ORIGINAL ARTICLE

Possible role of microsomal epoxide hydrolase gene polymorphism as a risk factor for developing insulin resistance and type 2 diabetes mellitus

Maivel H. Ghattas · Mahmoud A. Amer

Received: 27 January 2012 / Accepted: 6 March 2012 / Published online: 4 May 2012

Abstract The purpose of this study was to investigate the effects of mEPHX1 polymorphisms on risk of type 2 diabetes mellitus (T2DM) and insulin resistance (IR). One hundred and twelve patients with the diagnosis of T2DM and 150 control subjects were enrolled in the study. We investigated the two polymorphisms of the mEPHX1 gene (exon 3 Tyr113His and exon 4 His139Arg) using PCR–RFLP. Among diabetics, the frequencies obtained for the exon 3 mEPHX1 Tyr113 and His113 alleles were 46.9 and 53.1 %, respectively. In the control group, the frequencies were 70.7 and 29.3 %, respectively ($P = 0.0001$, OR = 2.73, 95 % CI = 1.9–3.91). The prevalence of mEPHX1 exon 3 Tyr/His and His/His was statistically significant ($P = 0.004$; 0.0001, respectively) when compared with the mEPHX1 exon 3 Tyr/Tyr homozygous carriers in both T2DM patients and in controls. We found that the His113 allele carriers had higher fasting insulin level, HOMA-IR, β cell activity, and lower insulin sensitivity compared to the wild type ($P = 0.0001$, 0.029, 0.0001, and 0.001, respectively). In contrast, there was no significant difference in exon 4 polymorphisms between patients and controls. However, our data revealed that the His139/His139 genotype carriers had higher fasting insulin level, and lower insulin sensitivity compared to Arg139 allele carriers ($P = 0.02$, and 0.001, respectively). Our study has shown for the first time that minor Tyr113 allele of mEPHX1 polymorphism had a higher risk of T2DM and IR occurrence with lower insulin sensitivity, while mEPHX1 exon 4 polymorphism had no significant association with T2DM and IR.

Keywords Type 2 diabetes · Insulin resistance · mEPHX1 · Polymorphism · PCR–RFLP

M. H. Ghattas (✉)
Department of Medical Biochemistry, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
e-mail: m_gattas@hotmail.com

M. A. Amer
Department of Pharmacology, Faculty of Pharmacy, Sinai University, El-Arish, Egypt

Introduction

The prevalence of diabetes is high and it is associated with high rates of morbidity and mortality. By the year 2030, an estimated 350 million individuals worldwide will suffer from diabetes [1]. Type 2 diabetes mellitus (T2DM), which accounts for more than 90 % of diabetes worldwide, is a common, complex, chronic disease with rapidly growing global importance [2]. Identifying high-risk groups will likely allow for effective prevention and treatment and is a topic of great interest in diabetic research [3]. There are strong evidences that genetic and environmental factors jointly determine its susceptibility [4].

T2DM is characterized by insulin resistance and pancreatic B-cell dysfunction [5]. The homeostasis model assessment of insulin resistance (HOMA-IR), which is developed for application in large epidemiologic investigations [6], is an alternative to the glucose clamp and the most commonly used surrogate measure of insulin resistance in vivo. Using HOMA-IR makes it possible to study a large number of subjects and with a single glucose and insulin measurement in the fasting state [7]. HOMA can be used to assess changes in B-cell function and IR in patients with diabetes to examine the natural history of diabetes and to assess the effects of treatment [8]. HOMA can be used to track changes in insulin sensitivity and B-cell function in

individuals to indicate whether reduced insulin sensitivity or B-cell failure predominates. The computer model can be used to determine insulin sensitivity (%S) and B-cell function (%B) from paired fasting plasma glucose and insulin data [9].

Microsomal epoxide hydrolase (mEPHX, EC 3.3.2.3), is an enzyme involved in the first-pass metabolism of epoxide intermediates, has received particular attention as two functional variants of the gene, which confer slow and fast metabolic activity, have been identified [9]. The human mEPHX gene is localized to the long arm of chromosome 1p11 [10], and two common aberrant alleles can be detected, which confer slow and fast enzyme activity [9]. An exon-3 thymine (T) to cytosine (C) polymorphism changes tyrosine residue 113 to histidine, and enzyme activity is reduced by ≥50 % (slow allele). The second mutation, an adenine (A) to guanine (G) transition in exon 4 of the gene, changes histidine residue 139 to arginine, and produces an enzyme with an activity increased by ≥25 % (fast allele) [11, 12].

While recent evidence suggests that genetic variability in xenobiotic-metabolizing enzymes, such as glutathione S transferases (GSTs), CYP1A1, and CYP2J2, play a role in T2DM development [13–16], no study has examined the effect of the mEPHX1 exon 3 (Tyr113His) and exon 4 (His139Arg) polymorphisms on risk of T2DM. The purpose of this study was to investigate the effects of mEPHX1 polymorphisms on risk of T2DM based on case–control study and to determine whether mEPHX1 gene polymorphisms synergistically affect insulin resistance.

Materials and methods

Subjects

Total of 112 T2DM (42 men, 70 women) and control group of 150 healthy subjects (58 men, 92 women) were examined in this study. Diagnosis of T2DM was based on American diabetes association definition of diabetes [17] plus not taking exogenous insulin. Healthy subjects do not meet the criteria for the diagnosis of diabetes mellitus. The present study was conducted according to the principles of the Dec-laration of Helsinki, and all patients provided written informed consent, following a protocol approved by the Sinai University Research Ethics Committee. Table 1 summarizes the clinical features of patients and controls.

Laboratory measurements

Venous blood samples were drawn after an overnight fasting. Hypertension was defined as a systolic blood pressure (BP) ≥ 140 mmHg, a diastolic BP ≥ 90 mmHg, or both. Current smoker was defined as a subject who continued to smoke cigarettes regularly. The HOMA-IR was calculated as described by Matthews et al. [6]: HOMA-IR = [FPI (μIU/ml) × FPG (mg/dl)]/405. Insulin sensitivity (%S) and β-cell function (%B) values were calculated using the computer model as described by Wallace et al. [8].

Table 1 Main characteristics of the study population

	Control (n = 150)	Case (n = 112)	P value
Age (y)	48.03 ± 7.35	48.74 ± 6.96	0.429
Sex (M/F)	58/92	42/70	0.847
FPG (mg/dl)	96.12 ± 9.84	202.96 ± 86.31	0.0001**
HbA_{1c} (%)	4.98 ± 0.73	9.64 ± 1.79	0.0001**
BMI	24.56 ± 2.27	29.85 ± 3.8	0.0001**
Fasting insulin level (mIU/l)	6.47 ± 2.24	9.38 ± 3.58	0.0001**
Total cholesterol (mg/dl)	150.58 ± 25.89	217.21 ± 44.38	0.0001**
Triglyceride (mg/dl)	125.16 ± 28.11	176.27 ± 91.24	0.0001**
HDL-C (mg/dl)	47.19 ± 10.66	45.63 ± 10.21	0.234
VLDL-C (mg/dl)	26.26 ± 5.99	35.65 ± 18.37	0.0001**
LDL-C (mg/dl)	98.19 ± 25.13	135.93 ± 47.23	0.0001**
Current smoking (±)	20/130	30/82	0.006*
Systolic BP (mmHg)	121.5 ± 14.97	137.18 ± 29.42	0.0001**
Diastolic BP (mmHg)	75.4 ± 7.83	82.63 ± 12.2	0.0001**
HOMA-IR	1.54 ± 0.57	4.61 ± 2.37	0.0001**
B-cell activity (%B)	75.16 ± 21.53	34.22 ± 23.48	0.0001**
Insulin sensitivity (%S)	132.5 ± 47.43	74.78 ± 27.8	0.0001**

Values are expressed as means ± SD

* P value <0.05, ** P value <0.001; all P values are noted for the comparison with the control group; comparisons were performed by Student t test and Chi-square test

Statistical analysis

Statistical analyses were performed with the SPSS ver. 17 software package. The relationship between mEPHX exon 3 (Tyr113 and His113 alleles) and exon 4 (His139 and Arg139 alleles) genotypes and risk of T2DM was assessed by the means of the odds ratio (OR) with 95 % confidence limits. Data were expressed as mean ± SD. Frequencies of categorical variables were compared by the χ^2 and Fishers' exact tests. Departure in the distribution of genotypes from Hardy–Weinberg equilibrium was assessed by means of the Chi-square test. Associations of genotypes with biochemical findings were evaluated by the two-way Student *t* test. Multiple regression analysis was used to adjust for confounding factors. A value of $P < 0.05$ was taken as indicative of significant differences.

Genotyping

Genomic DNA was isolated from 100 ml whole blood collected in EDTA anti-co-agulated tubes using the Wizard genomic DNA purification kit (Promega, USA). Genetic polymorphism in mEPHX exon 3 (Tyr113 and His113 alleles) and exon 4 (His139 and Arg139 alleles) were determined by PCR–RFLP analysis after amplification of the exons. For amplification of exon 3, we used primers described by Smith and Harrison [18] (5′-GATCGATAAG TTCCGTTTCACC-3′ and 5′-ATCTTAGTCTTGAAGT GAGGAT-3′). A mismatch in the reverse primer incorporated an *Eco*RV restriction site in the amplicon of the Tyr113 allele resulting in digestion products of 20 and 140 bp. The His113 allele remained undigested (162 bp). Amplification of exon 4 was done using primers described by Hassett et al. [9] (5′-GGGGTACCAGAGCCTGACC GT-3′ and 5′-AACACCGGGCCCACCCTTGGC-3′), followed by restriction analysis with RsaI. The His139 allele (357 bp) was digested into two fragments (295 and 62 bp), and the Arg139 allele (295 and 62 bp) was digested into fragments of 174, 121, and 62 bp in size. The amplified products were visualized under UV light after DNA electrophoresis on an ethidium bromide-stained agarose gel (Fig. 1). Individuals with combinations of alleles in their exon 3 and exon 4 genotypes have been classified according to their expected mEH activity as described by Smith and Harrison [18].

Results

A total of 262 subjects (112 patients with T2DM and 150 control subjects) were enrolled in the study. The descriptive data concerning the patient and control groups are presented in Table 1. No significant differences were observed in age, sex, HDL-C ($P > 0.05$) between patients and the control group. The proportion of FPG, HbA_{1c}, BMI, total cholesterol TG, VLDL-C, LDL-C smoking status, blood pressure, fasting insulin, and HOMA-IR was higher in T2DM patients than in controls ($P < 0.05$). Hardy–Weinberg equilibrium was tested for the two polymorphisms and no obvious deviation was found.

On multivariate analysis of the two genes mEPHX1 His139Arg polymorphism (rs2234922) genotype was not associated with risk of T2DM ($P > 0.05$). In control subjects the His139/His139 and His139/Arg139 genotypes were 54.67 and 45.33 %, respectively. Corresponding frequencies among cases were 59.8 and 40.2 %. The Arg139/Arg139 genotype was not detected in the studied groups. Compared to individuals with the His139/His139 genotype, the OR (95 % CI) for T2DM was 0.81 (0.49–1.33) for those with the His139/Arg139 genotype and 0.85 (0.56–1.31) for those with the polymorphic G allele when compared to individuals with wild type A allele (Table 2).

Among diabetics, the frequencies obtained for the exon 3 mEPHX1 wild type T allele and polymorphic C allele were 46.9 and 53.1 %, respectively. In the control group, the frequencies were 70.7 and 29.3 % for T and C alleles, respectively ($P = 0.0001$, OR = 2.73, 95 % CI = 1.9–3.91). The distribution of the exon 3 genotypes in controls and T2DM

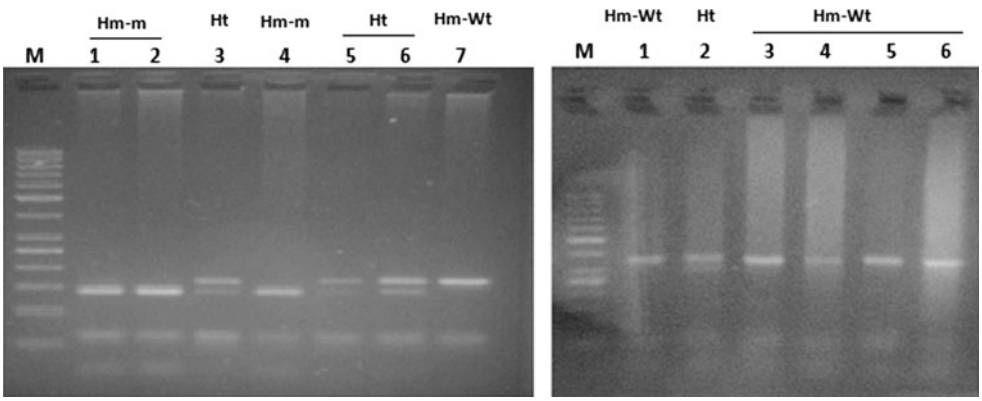

Fig. 1 Polymerase chain reaction (PCR) restriction fragment length polymorphism analysis of microsomal epoxide hydrolase (mEPHX) **a** exon 3, **b** exon 4; *M* 50-bp ladder, *Ht* heterozygote, *Hm* homozygote, *m* mutant; *Wt* wild type

Table 2 Genotype frequencies of microsomal epoxide hydrolase exon 3 and exon 4 polymorphisms relative to their association with insulin resistance groups

Genotype	Control ($n = 150$)	Case ($n = 112$)	P	OR	95 % CI
mEPHX1 exon 3					
Tyr113/Tyr113	75 (50)	27 (24.1)			
Tyr113/His113	62 (41.33)	51 (45.5)	0.004**	2.28	1.28–4.06
His113/His113	13 (8.67)	34 (30.4)	0.0001**	7.26	3.34–15.78
Tyr113/His113 + His113/His113	75 (50)	85 (75.9)	0.0001**	3.15	1.84–5.39
T allele	212 (70.7)	105 (46.9)			
C allele	88 (29.3)	119 (53.1)	0.0001**	2.73	1.9–3.92
mEPHX1 exon 4					
His139/His139	82 (54.67)	67 (59.8)			
His139/Arg139	68 (45.33)	45 (40.2)	0.405	0.81	0.49–1.33
Arg139/Arg139	0	0			
His139/Arg139 + Arg139/Arg139	68 (45.33)	45 (40.2)	0.405	0.81	0.49–1.33
A allele	232 (77.3)	179 (79.9)			
G allele	68 (22.7)	45 (20.1)	0.478	0.85	0.56–1.31
Combined mEPHX1 genotypes					
Tyr113Tyr and His139His	52 (34.7)	25 (22.3)	0.03*	1.85	1.06–3.22
Tyr113Tyr and His139Arg	23 (15.3)	2 (1.8)	0.0002**	9.96	2.29–43.2
Tyr113His and His139His	30 (20)	38 (33.9)	0.011*	0.49	0.28–0.85
Tyr113His and His139Arg	32 (21.3)	13 (11.6)	0.039*	2.06	1.03–4.15
His113His and His139His	0	4 (3.6)	0.032*		
His113His and His139Arg	13 (8.7)	30 (26.8)	0.0001**	0.26	0.13–0.53
Predicted mEH activity					
Very low	13 (8.7)	34 (30.4)	0.0001**	0.21	0.11–0.44
Low	30 (20)	38 (33.9)	0.011*	0.48	0.28–0.85
Intermediate	84 (56)	38 (33.9)	0.0004**	2.48	1.49–4.11
High	23 (15.3)	2 (1.8)	0.0002**	9.96	2.29–43.2

Values are expressed as n (%)

OR odds ratio computed between selected genotype/predicted mEH activity versus all other genotypes/predicted mEH activities in corresponding group, *CI* confidence interval; classification of predicted mEH activity based on Smith and Harrison [yy] as follows: very low: His113/His113 and either His 139/His139 or His139/Arg or Arg139/Arg139; low: Tyr113/His113 and either His139/His139 or Arg139/Arg139; intermediate: Tyr113/Tyr113 and His139/His139, Tyr113/His113 and His139/Arg139; high: Tyr113/Tyr113 and either His139/Arg139 or Arg139/Arg139; *mEPHX* microsomal epoxide hydrolase, *Tyr* tyrosine, *His* histidine, *Arg* arginine. Comparisons were performed by Chi-square test and Fisher's exact test

* P value <0.05, ** P value <0.001

patients was found to be highly significantly different. The prevalence of mEPHX1 exon 3 Tyr113/His113 heterozygosity was 45.5 % (51/112) in T2DM patients and 41.3 % (62/150) in controls. This difference was statistically significant ($P = 0.004$, OR = 2.28, 95 % CI = 1.28–4.06) (Table 2) when compared with the mEPHX1 exon 3 Tyr113/Tyr113 homozygous carriers in both T2DM patients and in controls (24.1 and 50 %, respectively). The prevalence of mEPHX1 exon 3 His113/His113 homozygosity was 30.4 % in T2DM patients and 8.67 % in controls. This difference was statistically significant ($P = 0.0001$, OR = 7.26, 95 % CI = 3.34–15.78) (Table 2).

We have investigated whether a combination of the Tyr113His and His139Arg genotypes was associated with T2DM. Our results showed that the Tyr113/Tyr113 and His139/His139 genotype combination was the most common genotype in controls (34.7 %). In addition, we found that the Tyr113/Tyr113 and His139/His139 genotype combination (with intermediate predicted mEH activity) was associated with a decreased risk for T2DM compared to all other combinations ($P = 0.03$, OR = 1.85, 95 % CI = 1.06–3.22). The Tyr113/His113 and His139/Arg139 genotypes combination (with low predicted mEH activity) was more common in the T2DM group ($P = 0.011$, OR = 0.49, 95 % CI = 0.28–0.85) compared to all other combinations. His113/His113 and either His139/His139 or His139/Arg139 and His113/His113 and Arg139/Arg 139 genotype combinations (very low predicted mEH activity)

were found more frequently in the T2DM group compared to all other combinations ($P = 0.0001$, OR = 0.21, 95 % CI = 0.11–0.44). A significant negative correlation between genotype combinations that predicted high mEH activity (Tyr113/Tyr113 and either His139/Arg139 or Arg139/Arg139) and T2DM risk was found compared to all other combinations ($P = 0.0002$, OR = 9.96, 95 % CI = 2.29–43.2) (Table 2).

We found that carriers of the rare allele (His113/His113 and Tyr113/His113) and (His139/Arg139 and Arg139/Arg139) had no effect on the clinical parameters in T2DM patients except in fasting insulin and insulin sensitivity when compared with Tyr113/Ty113 and His139/His139, respectively (Table 3). The smoking status showed a statistically different (His113/His113 and Tyr113/His113) genotypes combination when compared to Tyr113/Tyr113 genotype group ($P = 0.001$) (Table 3).

In the current report, we demonstrated that a polymorphism of mEPHX1 exon 3 His113/His113 homozygosity and the carriers of the polymorphic C allele occur at higher frequency in T2DM than in control subjects is associated with a higher risk of T2DM (OR = 7.26 and 2.73, respectively). Insulin sensitivity in type 2 diabetic patients in the carriers of (Tyr113/His113 + His113/His113) were significantly lower than those with the (Tyr113/Tyr113) carriers ($P = 0.001$). These results indicate that the presence of the His113 allele in the mEPHX1 exon 3 gene is associated with the insulin resistance status found in type 2 diabetic patients ($P = 0.029$). Meanwhile, we found that mEPHX1 exon 4 polymorphism had no significant association with T2DM and IR.

Discussion

IR has been implicated in the pathogenesis of T2DM [19]. Many studies have examined the involvement of gene polymorphisms with insulin resistance [20, 21]. Since insulin resistance is associated with an increase in oxygen free radicals [22], mEPHX may affect insulin resistance and T2DM.

Epoxide hydrolases are classified into two xenobiotic-metabolizing forms, microsomal (mEH), localized predominantly in the endoplasmic reticulum, is encoded by the EPHX1 gene and soluble (sEH) which is confined mainly to cytoplasm; this enzyme, encoded by the EPHX2

Table 3 The baseline characteristics of diabetic subjects according to microsomal epoxide hydrolase genotypes

	Tyr113His			His139Arg		
	Carriers of Tyr113/tyr113 ($n = 27$)	Carriers of tyr113/His113 + His113/His113 ($n = 85$)	P value	Carriers of His139/His139 ($n = 67$)	Carriers of His139/Arg139 + Arg139/Arg139 ($n = 45$)	P value
Age (y)	48.26 ± 8.79	48.89 ± 6.34	0.684	48.61 ± 7.19	48.93 ± 6.68	0.714
Sex (M/F)	11/16	31/54	0.689	27/40	15/30	0.455
FPG (mg/dl)	220.44 ± 104.11	197.41 ± 79.75	0.229	203.35 ± 84.09	202.37 ± 90.46	0.953
HbA1c (%)	9.22 ± 2.05	9.77 ± 1.69	0.165	9.58 ± 1.79	9.74 ± 1.79	0.644
Fasting insulin level (mIU/l)	6.99 ± 2.05	10.14 ± 3.63	0.0001**	10.02 ± 3.35	8.43 ± 3.73	0.02*
BMI	29.12 ± 3.42	30.07 ± 3.91	0.26	29.88 ± 3.74	29.78 ± 3.94	0.922
Total cholesterol (mg/dl)	206.55 ± 35.35	220.6 ± 46.56	0.153	214.46 ± 43.47	221.31 ± 45.88	0.423
Triglyceride (mg/dl)	163.62 ± 89.45	180.28 ± 91.96	0.411	170.49 ± 94.7	184.86 ± 86.15	0.416
HDL-C (mg/dl)	47.48 ± 9.6	45.05 ± 10.38	0.283	45.75 ± 10.29	45.47 ± 10.2	0.888
VLDL-C (mg/dl)	33.03 ± 17.92	36.48 ± 18.54	0.398	34.49 ± 19.08	37.36 ± 17.33	0.42
LDL-C (mg/dl)	126.03 ± 28.98	139.07 ± 51.44	0.213	134.22 ± 42.67	138.48 ± 53.71	0.642
Current smoking (±)	1/26	29/56	0.001**	15/52	15/30	0.199
Systolic BP (mmHg)	136.85 ± 24.73	137.29 ± 30.89	0.946	138.13 ± 24.46	135.78 ± 35.82	0.68
Diastolic BP (mmHg)	80.18 ± 11.14	83.41 ± 12.49	0.233	82.23 ± 11.46	83.22 ± 13.36	0.676
HOMA-IR	3.74 ± 1.86	4.88 ± 2.46	0.029*	4.92 ± 2.33	4.14 ± 2.37	0.087
B-cell activity (%B)	24.3 ± 14.23	37.33 ± 24.97	0.0001**	35.04 ± 23.87	32.96 ± 23.1	0.647
Insulin sensitivity (%S)	89.95 ± 26.46	70.03 ± 26.62	0.001**	68 ± 25.56	85.18 ± 28.16	0.001**

Values are expressed as means ± SD

* P value <0.05, ** P value <0.001; all P values are noted for the comparison with the control group; comparisons were performed by Student t test, Chi-square test and Fisher's exact test

gene [23]. The microsomal (EPHX1) and soluble (EPHX2) epoxide hydrolases function to regulate the oxidation status of a wide range of xenobiotic- and lipid-derived substrates. Previous report by Ohtoshi et al. [24] showed no relation between the genotype distribution of sEH (EPHX2) Arg287Gln gene polymorphism and T2DM. This is the first report showing the association of mEH (EPHX1) polymorphisms on risk of T2DM and insulin resistance.

Our results on Egyptian population suggest the hypothesis that mEPHX1 exon 3 polymorphism (rs1051740) is related to T2DM risk. The frequency of exon 3 Tyr113/Tyr113 genotype was the most significant genotype among the controls on contrast to the diabetics ($P = 0.0001$, OR = 3.15, 95 % CI = 1.84–5.39) suggesting that individuals with this homozygous wild genotype had 3.15 folds decreased risk of developing T2DM. We found that the polymorphic His113 allele carriers had higher fasting insulin levels, HOMA-IR, β-cell activity, and lower insulin sensitivity compared to the Tyr113/Tyr113 ($P = 0.0001$, 0.029, 0.0001 and 0.001, respectively).

On the other hand, our results showed that mEPHX1 His139Arg polymorphism in exon 4 (rs2234922) had no significant association with T2DM. However, our data revealed that the His139/His139 genotype carriers had higher fasting insulin levels, and lower insulin sensitivity compared to Arg139 allele carriers ($P = 002$, and 0.0001, respectively) and had no significant effect on HOMA-IR and β-cell activity. These observations were made on lung cancer risk, COPD, emphysema [25, 26], and [18], and aflatoxin-associated hepatocellular carcinoma [27] but our study has recorded for the first time this observation on T2DM risk and its relation to insulin resistance.

In our study we have also investigated whether a combination of the Tyr113His and His139Arg genotypes is associated with T2DM risk. We found that Tyr113/Tyr113 and His139/His139 (with intermediate predicted mEH activity) genotype combination was the most common genotype in our controls (34.67 %) in accordance with Erkisi et al. [25]. While the combination of Tyr113/His113 and His 139/His139 (with low predicted mEH activity) was the most common genotype in diabetics (33.93 %), therefore, we assumed the association between the predicted mEH low-activity and susceptibility to T2DM. Our determination of an association between genetically defined Tyr113His polymorphism in mEH activity and T2DM suggests that highly reactive epoxide intermediates may have a role in the pathogenesis of the disease.

Neither the exon 3 Tyr113His nor the exon 4 His139Arg genotypes were found to be associated with the clinical parameters in the diabetics except the relation between the exon 3 Tyr113His with smoking, this result was in consistence with Smith and Harrison [18]. The association between this polymorphism and cigarette smoking could be explained by the fact that cigarette contains high amount of free radicals per puff and an array of other highly toxic electrophiles [28]. Many of these compounds generate reactive epoxides which are detoxified by mEPHX [18].

Conclusion

The results indicated that minor Tyr113 allele of mEPHX1 exon 3 polymorphism had a higher risk of T2DM and IR occurrence with lower insulin sensitivity, which might be taken as a marker in clinical diagnosis and prevention of T2DM or as a therapeutic target in the treatment of T2DM patients. In our study, however, we found that mEPHX1 exon 4 polymorphism had no significant association with T2DM and IR. Low predicted mEPHX activity was the most common genotype combination in the diabetics. Although some of our data were statistically significant, we acknowledge that the study requires confirmation in a separate, large cohort.

References

1. S. Wild, G. Roglic, A. Green, R. Sicree, H. King, Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care **27**, 1047–1053 (2004)
2. M. Stumvoll, B.J. Goldstein, T.W. Haeften, Type 2 diabetes: principles of pathogenesis and therapy. Lancet **365**(9467), 1333–13461 (2005)
3. Y.Y. Zheng, X. Xie, Y.T. Ma, Y.N. Yang, Z.Y. Fu, X.M. Li, X. Ma, B.D. Chen, F. Liu, Relationship between type 2 diabetes mellitus and a novel polymorphism C698T in C5L2 in the Chinese Han population. Endocrine (2011). doi:10.1007/s12020-011-9574-y
4. W. Du, Q. Li, Y. Lu, X. Yu, X. Ye, Y. Gao, J. Ma, J. Cheng, Y. Cao, J. Du, H. Shi, L. Zhou, Genetic variants in ADIPOQ gene and the risk of type 2 diabetes: a case–control study of Chinese Han population. Endocrine **40**, 413–422 (2011)
5. K. Das, Y. Lin, E. Widen, Y. Zhang, P.E. Scherer, Chromosomal localization, expression pattern and promoter analysis of the mouse gene encoding adipocyte-specific secretory protein Acrp30. Biochem. Biophys. Res. Commun. **280**(4), 1120–1129 (2001)
6. D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia **28**, 412–419 (1985)
7. E. Bonora, G. Targher, M. Alberiche, R.C. Bonadonna, F. Saggiani, M.B. Zenere, T. Monauni, M. Muggeo, Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care **23**(1), 57–63 (2000)
8. T.M. Wallace, J.C. Levy, D.R. Matthews, Use and abuse of HOMA modeling. Diabetes Care **27**(6), 1487–1495 (2004)
9. C. Hassett, L. Aicher, J.S. Sidhu, C.J. Omiecinski, Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. Hum. Mol. Genet. **3**(3), 421–428 (1994)

10. R.C. Skoda, A. Demierre, O.W. McBride, F.J. Gonzalez, U.A. Meyer, Human microsomal xenobiotic epoxide hydrolase complementary DNA sequence, complementary DNA directed expression in COS-1 cells, and chromosomal localization. J. Biol. Chem. **263**(3), 1549–1554 (1988)
11. C. Hassett, K.B. Robinson, N.B. Beck, C.J. Omiecinski, The human microsomal epoxide hydrolase gene (EPHX1): complete nucleotide sequence and structural characterization. Genomics **23**(2), 433–442 (1994)
12. C. Kiyohara, K. Yoshimasu, K. Takayama, Y. Nakanishi, EPHX1 polymorphisms and the risk of lung cancer: a HuGE review. Epidemiology **17**(1), 89–99 (2006)
13. M.A. Amer, M.H. Ghattas, D.M. Abo-Elmatty, S.H. Abou-El-Ela, Influence of glutathione *S*-transferase polymorphisms on type-2 diabetes mellitus risk. Genet. Mol. Res. (2011). doi: 10.4238/2011.October.31.14
14. G. Wang, L. Zhang, Q. Li, Genetic polymorphisms of GSTT1, GSTM1, and NQO1 genes and diabetes mellitus risk in Chinese population. Biochem. Biophys. Res. Commun. **341**, 310–313 (2006)
15. X.L. Wang, M. Greco, A.S. Sim, N. Duarte, J. Wang, D.E. Wilcken, Effect of CYP1A1 MspI polymorphism on cigarette smoking related coronary artery disease and diabetes. Atherosclerosis **162**(2), 391–397 (2002)
16. C.P. Wang, W.C. Hung, Y. TH, C.A. Chiu, L. LF, F.M. Chung, C.H. Hung, S.J. Shin, H.J. Chen, Y.J. Lee, Genetic variation in the G-50T polymorphism of the cytochrome P450 epoxygenase CYP2J2 gene and the risk of younger onset type 2 diabetes among Chinese population: potential interaction with body mass index and family history. Exp. Clin. Endocrinol. Diabetes **118**(6), 346–352 (2010)
17. American Diabetes Association, Diagnosis and classification of diabetes mellitus. Diabetes Care **30**, 42–47 (2007)
18. C.A.D. Smith, D.J. Harrison, Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. Lancet **350**, 630–633 (1997)
19. Z. Shi, J. Liu, Q. Guo, X. Ma, L. Shen, S. Xu, H. Gao, X. Yuan, J. Zhang, Association of TRB3 gene Q84R polymorphism with type 2 diabetes mellitus in Chinese population. Endocrine **35**(3), 414–419 (2009)
20. H. Akasaka, T. Katsuya, S. Saitoh, K. Sugimoto, Y. Fu, S. Takagi, H. Ohnishi, H. Rakugi, N. Ura, K. Shimamoto, T. Ogihara, Effects of angiotensin II type 1 receptor gene polymorphisms on insulin resistance in a Japanese general population: the Tanno–Sobetsu study. Hypertens. Res. **29**(12), 961–967 (2006)
21. O.Y. Kim, H.H. Lim, L.I. Yang, J.S. Chae, J.H. Lee, Fatty acid desaturase (FADS) gene polymorphisms and insulin resistance in association with serum phospholipid polyunsaturated fatty acid composition in healthy Korean men: cross-sectional study. Nutr. Metab. (Lond) **8**(1), 24 (2011)
22. K. Shinozaki, K. Ayajiki, Y. Nishio, T. Sugaya, A. Kashiwagi, T. Okamura, Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. Hypertension **43**(2), 255–262 (2004)
23. S. Saito, A. Iida, A. Sekine, C. Eguchi, Y. Miura, Y. Nakamura, Seventy genetic variations in human microsomal and soluble epoxide hydrolase genes (EPHX1 and EPHX2) in the Japanese population. J. Hum. Genet. **46**(6), 325–329 (2001)
24. K. Ohtoshi, H. Kaneto, K. Node, Y. Nakamura, T. Shiraiwa, M. Matsuhisa, Y. Yamasaki, Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients. Biochem. Biophys. Res. Commun. **331**(1), 347–350 (2005)
25. Z. Erkisi, I. Yaylim-Eraltan, A. Turna, U. Görmüs, H. Camlica, T. Isbir, Polymorphisms in the microsomal epoxide hydrolase gene: role in lung cancer susceptibility and prognosis. Tumori **96**(5), 756–763 (2010)
26. J. Zidzik, E. Slabá, P. Joppa, Z. Kluchová, Z. Dorková, P. Skyba, V. Habalová, J. Salagovic, R. Tkácová, Glutathione *S*-transferase and microsomal epoxide hydrolase gene polymorphisms and risk of chronic obstructive pulmonary disease in Slovak population. Croat. Med. J. **49**(2), 182–191 (2008)
27. E.W. Tiemersma, R.E. Omer, A. Bunschoten, P. van't Veer, F.J. Kok, M.O. Idris, A.M. Kadaru, S.S. Fedail, E. Kampman, Role of genetic polymorphism of glutathione-*S*-transferase T1 and microsomal epoxide hydrolase in aflatoxin-associated hepatocellular carcinoma. Cancer Epidemiol. Biomarkers Prev. **10**(7), 785–791 (2001)
28. J.L. Farber, Mechanism of cell injury by activated oxygen species. Environ. Health Perspect. **102**, 17–24 (1994)