The authors aimed to determine whether 2 functional polymorphisms in the heme oxygenase-1 (HO-1) gene promoter are associated with type 2 diabetes mellitus (T2DM). A Chinese case-control study involving 1,103 newly diagnosed T2DM patients, 371 patients with impaired glucose regulation (IGR), and 1,615 controls was performed (December 2004–December 2007). A (GT)n microsatellite polymorphism and a single nucleotide polymorphism, T(-413)A, were genotyped, and their functional relevance was evaluated by examining the level of HO-1 protein expression. For the (GT)n microsatellite polymorphism, genotypes with the L (GT)n allele (≥25 GT repeats) were associated with increased odds of IGR or T2DM compared with the S/S genotype (<25 GT repeats) (S/L genotype: odds ratio (OR) = 1.35, \( P = 0.048 \); L/L genotype: OR = 1.65, \( P = 0.006 \)). Subsequent haplotype analysis showed that haplotype TL contributed to increased odds of IGR or T2DM compared with haplotype TS (OR = 1.56, \( P = 0.003 \)). In functional analyses, HO-1 expression level was significantly reduced in persons with IGR and T2DM carrying the L/L (GT)n genotype compared with persons with the S/S genotype. Further haplotype combination assay confirmed the functional dominance of the (GT)n microsatellite polymorphism over the T(-413)A single nucleotide polymorphism. These results support an association between the HO-1 (GT)n microsatellite polymorphism, HO-1 expression levels, and the odds of T2DM.

**Association Between Heme Oxygenase-1 Gene Promoter Polymorphisms and Type 2 Diabetes in a Chinese Population**

Fangfang Song, Xiangyang Li, Muxun Zhang, Ping Yao, Nianhong Yang, Xiufa Sun, Frank B. Hu, and Liegang Liu

*Initially submitted December 1, 2008; accepted for publication June 9, 2009.*

Although the underlying mechanisms for the pathogenesis of type 2 diabetes mellitus remain to be fully determined, oxidative stress has been shown to contribute, at least in part, to the progression of type 2 diabetes (1, 2). This hypothesis is supported by observations in diabetic patients of a striking increase in oxidative damage to lipids and DNA, as well as impairment of the antioxidative defense system (3). Antioxidants used for adjuvant therapy in the treatment of type 2 diabetes seem to be promising, but results have been inconsistent (4, 5), possibly because of large interindividual differences in responses to antioxidant supplementation. Among a panel of potential candidate genes related to oxidative stress, the heme oxygenase-1 (HO-1) gene has drawn much attention with its potent antiinflammatory, antioxidant, and antiproliferative effects (6).

Heme oxygenase is a microsomal rate-limiting enzyme responsible for the oxidation and degradation of heme into biologically active metabolites—biliverdin, which is rapidly reduced to bilirubin by biliverdin reductase, carbon monoxide, and iron (7). Three mammalian heme oxygenase isoforms have been identified. Among them, the inducible isof orm HO-1 is a stress-responsive protein ubiquitously distributed in mammalian tissues and can be induced by various oxidative agents (8, 9). The induction of HO-1 has been considered an adaptive cellular defense response protecting cells or tissues against injuries in pathophysiologic
states, attributed to the antioxidant properties of bilirubin and biliverdin, the extrusion and sequestration of cellular free iron by ferritin, and the antiapoptotic and antiinflammatory effects of carbon monoxide (6, 8–10). The beneficial role of HO-1 in diabetes has been reported in animal models and in vitro assays involving exposure to glucose (6, 11), but little information on humans is available.

Recently, Exner et al. (12) proposed that genetic variability may modulate the HO-1 gene’s response to exogenous stimuli. Two potentially functional polymorphisms in the promoter region of the human HO-1 gene have been identified: a (GT)$_n$ microsatellite polymorphism (also called rs3074372) and a single nucleotide polymorphism (SNP), T(-413)A (rs2071746) (12). The (GT)$_n$ repeat is the most frequent simple repeat scattered throughout human and animal genomes, and many of these are length-polymorphic (13). Previous studies have evaluated the associations of the HO-1 (GT)$_n$ microsatellite polymorphism with risks of multiple diseases (12), and several of these studies have suggested a positive association between longer (GT)$_n$ repeats and higher risks of cardiovascular disease and renal transplantation (14–18). However, to our knowledge, no investigators have examined the association between this polymorphism and risk of type 2 diabetes. Data on the potential role of the T(-413)A SNP in human diseases are more limited (16, 19–21), although there is some evidence that the A allele of this SNP is related to higher HO-1 gene promoter activity than the T allele (19–21).

Our aim in this study was to examine associations between these polymorphisms in the HO-1 gene and odds of type 2 diabetes in Chinese persons with different states of glucose metabolism. In addition, we evaluated the functional relevance of these 2 polymorphisms by measuring levels of intracellular HO-1 protein expression among persons with different genotypes.

**MATERIALS AND METHODS**

**Characteristics of subjects and clinical protocol**

The study population consisted of 371 patients with impaired glucose regulation (IGR; defined as impaired fasting glucose or impaired glucose tolerance) and 1,103 newly diagnosed type 2 diabetes patients consecutively recruited from persons visiting the outpatient clinics of the Department of Endocrinology at Tongji Medical College Hospital (Wuhan, People’s Republic of China) between December 2004 and December 2007. Concomitantly, we recruited 1,615 healthy controls who were frequency-matched to patients by age (±5 years) and sex from an unselected population undergoing routine health check-ups at the same hospital. The controls, patients with IGR, and patients with type 2 diabetes met the respective diagnostic criteria recommended by the World Health Organization in 1999 (22). The inclusion criteria for the study population were: age ≥30 years, body mass index (weight (kg)/height (m)$^2$) <40, no history of diagnosis of IGR or diabetes, and no history of receiving pharmacologic treatment for hyperlipidemia or hypertension. Persons with clinically systemic diseases, acute illness, and chronic inflammatory diseases were excluded. The response rates among eligible incident cases and controls were approximately 95% and 90%, respectively. All subjects enrolled were of Chinese Han ethnicity. The Ethics Committee of Tongji Medical College approved the study protocol, and written informed consent was obtained from all participants.

A standard questionnaire was used to collect information about age, sex, history of diseases (diabetes, hyperlipidemia, and hypertension), family history of diabetes in first-degree relatives, cigarette smoking, alcohol consumption, and physical activity. For smoking and alcohol status, the subjects were divided into 3 categories: never, past, and current use. The participants’ responses to the question regarding occupational physical activity were categorized into low activity (office work), moderate activity (driver), and high activity (farming or steelmaking). Recreational physical activity was also defined in 3 categories: Irregular participation in sports for less than 1 hour per week was defined as low activity; participation for more than 1 hour per week but less than 2 hours per week was defined as moderate activity; and participation for more than 2 hours per week was defined as high activity (23). Anthropometric measurements included height (m), weight (kg), and blood pressure (mm Hg), obtained using standardized techniques. Body mass index was calculated as described previously (24). Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg.

All subjects underwent a complete physical examination in the morning after an overnight fast; venous blood samples were drawn from an antecubital vein into heparinized tubes for plasma separation. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation (TBD Company, Tianjin, China). Plasma levels for biochemical parameters, including glycemic control (fasting plasma glucose, hemoglobin A1c), fasting plasma insulin, and lipid profile (triglyceride, total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol) were determined through previously described methods (24). Intra- and interassay coefficients of variation were less than 4% for all of these tests. In addition, the homeostasis model assessment of insulin resistance index and the homeostasis model assessment of beta cell function index were calculated (25).

**Determination of HO-1 protein expression in peripheral blood mononuclear cells**

Intracellular HO-1 protein expression in peripheral blood mononuclear cells was detected by flow cytometry as described in our previous paper (26) and was expressed as the mean fluorescence intensity (which has no units). We randomly selected for this analysis a subgroup of 606 healthy controls, 65 patients with IGR, and 217 patients with diabetes. The characteristics of this analysis population were similar to those found in the original study sample.

**Analysis of (GT)$_n$ length polymorphism in the HO-1 gene promoter region**

A moiety of the venous blood samples was retained individually for genomic DNA extraction using a conventional
The 5'-flanking region containing a poly (GT)n repeat of the HO-1 gene was amplified by polymerase chain reaction using a 5'-carboxyl-fluorescein amidite (FAM)-labeled sense primer (FAM-5'-AGAGCCTGCAGCTTCTCAGA-3') and an unlabeled antisense primer (5'-ACAAAGTCTGGCCATAGGAC-3'). The sizes of amplification products were determined by capillary electrophoresis on an automated DNA capillary sequencer (ABI Prism Genetic Analyzer 3100; Applied Biosystems, Foster City, California). For analysis, aliquots containing 0.5 μL of polymerase chain reaction product were mixed with 0.5 μL of GeneScan-500 LIZ Size Standard solution (size range, 50–500 base pairs) as an internal lane size standard and 9 μL of Hi-Di formamide (Applied Biosystems). Mixtures were then denatured at 95°C for 5 minutes and snap-cooled on ice for 5 minutes, followed by electrophoresis on a polyacrylamide gel POP-4 (Applied Biosystems) in a 36-cm capillary for genetic analysis. The size of the polymerase chain reaction fragment was analyzed and converted to dinucleotide repeat lengths using GeneMapper 3.5 analysis software (Applied Biosystems). The genotyping error rate was assessed by randomly re-genotyping 10% of the samples as blind duplicates; the concordance rate was 100%.

### Table 1. Anthropometric and Biochemical Characteristics of the Study Population by Glucose Metabolism State, Wuhan, People's Republic of China, December 2004–December 2007

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n = 1,615) Mean (SD) No. %</th>
<th>Patients With Impaired Glucose Regulation (n = 371) Mean (SD) No. %</th>
<th>Patients With Newly Diagnosed Type 2 Diabetes (n = 1,103) Mean (SD) No. %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>917 56.7</td>
<td>187 50.4</td>
<td>624 56.5</td>
<td>0.072</td>
</tr>
<tr>
<td>Age, years</td>
<td>48.41 (12.01)</td>
<td>50.37 (11.98)</td>
<td>50.47 (11.24)</td>
<td>0.596</td>
</tr>
<tr>
<td>Body mass indexa</td>
<td>22.76 (3.81)</td>
<td>25.59 (4.64)*</td>
<td>25.11 (4.47)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>302 18.7</td>
<td>164 44.1*</td>
<td>476 43.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current alcohol consumption</td>
<td>530 32.8</td>
<td>139 37.6*</td>
<td>501 45.4*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Recreational physical activityb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1,175 72.8</td>
<td>273 73.6</td>
<td>811 73.5</td>
<td>0.363</td>
</tr>
<tr>
<td>Medium</td>
<td>321 19.9</td>
<td>77 20.8</td>
<td>231 20.9</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>119 7.4</td>
<td>21 5.7</td>
<td>61 5.5</td>
<td></td>
</tr>
<tr>
<td>Occupational physical activityc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1,373 85.0</td>
<td>321 86.5</td>
<td>971 88.0</td>
<td>0.174</td>
</tr>
<tr>
<td>Medium</td>
<td>114 7.1</td>
<td>26 7.0</td>
<td>70 6.3</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>128 7.9</td>
<td>24 6.5</td>
<td>62 5.6</td>
<td></td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>203 12.6</td>
<td>82 22.2*</td>
<td>222 20.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>4.76 (0.66)</td>
<td>6.08 (0.61)*</td>
<td>9.67 (2.96)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/mL</td>
<td>9.60 (7.04)</td>
<td>11.16 (7.73)*</td>
<td>10.73 (6.73)*</td>
<td>0.021</td>
</tr>
<tr>
<td>Homeostasis model assessment of insulin resistance indexd</td>
<td>2.27 (1.70)</td>
<td>3.02 (2.12)*</td>
<td>4.39 (4.58)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homeostasis model assessment of beta cell function indexd</td>
<td>115.71 (64.44)</td>
<td>92.93 (69.34)*</td>
<td>47.08 (27.48)**</td>
<td>0.004</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>5.69 (0.69)</td>
<td>6.02 (0.80)</td>
<td>8.94 (2.47)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.48 (1.14)</td>
<td>1.92 (1.03)*</td>
<td>2.09 (1.58)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.46 (0.90)</td>
<td>4.65 (1.14)*</td>
<td>4.73 (1.36)*</td>
<td>0.034</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol, mmol/L</td>
<td>1.41 (0.44)</td>
<td>1.45 (0.69)</td>
<td>1.43 (0.73)</td>
<td>0.776</td>
</tr>
<tr>
<td>Low density lipoprotein cholesterol, mmol/L</td>
<td>2.62 (0.83)</td>
<td>2.49 (0.99)</td>
<td>2.53 (1.18)</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.

* P < 0.05 as compared with the control group; ** P < 0.05 as compared with the impaired glucose regulation group.

a Weight (kg)/height (m)2.

b Irregular participation in sports for less than 1 hour per week was defined as low activity; participation for more than 1 hour per week but less than 2 hours per week was defined as moderate activity; and participation for more than 2 hours per week was defined as high activity (23).

c Examples: office work (low), driving (medium), and farming or steelmaking (high).

d Calculated using fasting plasma glucose (mmol/L) and fasting plasma insulin (mU/L) levels.
were observed in diabetic patients (for genotype TS/TS vs. genotype AL/AL, AS/AS, TL/TL, TS/TS, n = 126) and subjects with type 2 diabetes mellitus (diplotypes: AL/AL, n = 65; AS/AS, n = 20; TL/TL, n = 13; TS/TS, n = 20) as detected by flow cytometry, Wuhan, People’s Republic of China, December 2004–December 2007. Within the A/A genotype class, carriers with haplotype pairs of AS/AS had a significantly higher HO-1 expression level than carriers with haplotype pairs of AL/AL in both the control group (P = 0.02) and the type 2 diabetes group (P < 0.001). When the T/T genotype was considered, similar effects were observed in diabetic patients (for genotype TS/TS vs. genotype TL/TL, P = 0.018). Bars, standard deviation.

Figure 1. Relation between the mean fluorescence intensity (MFI) of heme oxygenase-1 (HO-1) expression in peripheral blood mononuclear cells and estimated haplotype combinations in control subjects (diplotypes: AL/AL, n = 65; AS/AS, n = 20; TL/TL, n = 13; TS/TS, n = 20) and subjects with type 2 diabetes mellitus (diplotypes: AL/AL, n = 39; AS/AS, n = 5; TL/TL, n = 6; TS/TS, n = 25), as detected by flow cytometry, Wuhan, People’s Republic of China, December 2004–December 2007. Within the A/A genotype class, carriers with haplotype pairs of AS/AS had a significantly higher HO-1 expression level than carriers with haplotype pairs of AL/AL in both the control group (P = 0.02) and the type 2 diabetes group (P < 0.001). When the T/T genotype was considered, similar effects were observed in diabetic patients (for genotype TS/TS vs. genotype TL/TL, P = 0.018). Bars, standard deviation.

Statistical analysis

Differences in the clinical and laboratory characteristics of healthy controls and patients with IGR and type 2 diabetes were tested by 1-way analysis of variance, followed by the Student-Newman-Keuls multiple range test for continuous variables or the χ² test for categorical variables.

For analysis of the (GT)ₙ length polymorphism, allelic repeats were divided into subgroups according to their distributions. Differences in allelic and genotypic frequencies of the (GT)ₙ microsatellite polymorphism and T(-413)A SNP in healthy controls and patients with IGR and type 2 diabetes were compared by χ² test, which was also used to assess Hardy-Weinberg equilibrium for each individual locus. There was no evidence of deviation from Hardy-Weinberg equilibrium for the genotype distributions of these 2 polymorphisms among the 3 groups (all P’s > 0.05).

For analysis of linkage disequilibrium between these 2 polymorphisms, the linkage disequilibrium coefficient (D’) and the correlation coefficient (r²) were calculated by means of the LDA software program (27). Haplotypes were constructed using a Bayesian approach implemented in the PHASE 2.0 program (28). We used the multinomial logistic regression method to assess the association of IGR and type 2 diabetes events with the specific polymorphism or haplotypes. Odds ratios and 95% confidence intervals were unadjusted and adjusted for known risk factors for type 2 diabetes, including age, sex, body mass index, cigarette smoking, alcohol consumption, family history of diabetes, hypertension, and physical activity. We performed multivariate analysis of covariance to estimate differences in HO-1 expression level across genotypes, adjusted for the aforementioned risk factors and followed by Bonferroni correction for multiple comparisons. A 2-sided P value less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS software (Windows version 12.0; SPSS, Inc., Chicago, Illinois).

RESULTS

Compared with healthy controls, patients with IGR and type 2 diabetes had a higher body mass index, more hypertension, more alcohol consumption, a higher prevalence of family history of diabetes, and higher levels of triglyceride and total cholesterol. The homeostasis model assessment of insulin resistance index was higher in patients with IGR than in controls, and was further increased in diabetic patients. The homeostasis model assessment of beta cell function index was lower in patients with IGR and further decreased in patients with diabetes (Table 1).

Association between individual HO-1 protein expression levels and different glucose metabolism states

There was a significantly decreased HO-1 protein expression level in both the IGR and diabetic patients compared with healthy controls: Mean fluorescence intensity was 64.8 (standard deviation (SD), 13.09), 38.38 (SD, 20.7), and 37.0 (SD, 22.7) in healthy controls, patients with IGR, and patients with diabetes, respectively.

Association studies of (GT)ₙ microsatellite polymorphism and T(-413)A SNP

The number of (GT)ₙ repeats in the HO-1 gene promoter region in this study population ranged between 12 and 40. The distribution of allelic subgroups was comparable in groups with different glucose metabolism states. All were bimodal, with the 2 most common alleles having 22 and 30 repeats (see Web Figure 1, which is posted on the Journal’s Web site [http://aje.oxfordjournals.org/]). According to their distributions and previously reported results from HO-1 association and functional studies (14–18), we divided the allelic repeats into 2 subclasses: less than 25 (GT)ₙ repeats, defined as class S (short), and 25 or more (GT)ₙ repeats, defined as class L (long). Each participant was recoded for one of the 3 possible genotypes: S/S, S/L, or L/L. The frequency of the L allele was significantly higher in patients with IGR (53.2%) and patients with diabetes (51.1%) than in controls (47.3%) (P = 0.002). With regard to the genotypic distribution, patients with IGR and type 2 diabetes had higher proportions of class L/L than did control subjects (28.2% in patients with IGR and 24.6% in patients with diabetes vs. 21.9% in controls; P = 0.007) (Table 2). Table 2 shows the associations between the genotypes of the (GT)ₙ polymorphism and risks of type 2 diabetes; all odds ratios and 95% confidence intervals were calculated with the commonly homozygous genotype used as the reference group, adjusting for known risk factors for type 2 diabetes. The L/L genotype was associated with increased odds of IGR as compared with the S/S genotype (odds ratio = 1.74, 95% confidence interval (CI): 1.03, 2.95; P = 0.040); the presence of the L allele (S/L and L/L genotypes) was an independent risk factor for type 2 diabetes, and the adjusted
odds ratios were 1.45 (95% CI: 1.05, 1.99; \( P = 0.023 \)) and 1.62 (95% CI: 1.11, 2.36; \( P = 0.012 \)) for genotypes S/L and L/L, respectively, versus genotype S/S. When the IGR and type 2 diabetes groups were combined as an endpoint, similar results were obtained (Table 2).

Analysis of the \( T(-413)A \) SNP did not yield any significant differences among subjects with different glucose metabolism states for either allelic or genotypic frequencies (Table 3).

**Analysis of linkage disequilibrium and haplotypes of the \( (GT)_n \) microsatellite polymorphism and \( T(-413)A \) SNP**

Moderately strong linkage disequilibrium between the \( (GT)_n \) microsatellite polymorphism and the \( T(-413)A \) SNP was observed \( (D' = 0.72, r^2 = 0.53) \), and we subsequently conducted haplotype analysis to better understand the role of these 2 polymorphisms. Among the 4 estimated haplotypes constructed in our study population, the 2 most prevalent haplotypes were TS and AL (Table 4). Compared with the most frequent haplotype, TS (44.4% in controls, 41.6% in patients with IGR, and 41.4% in patients with type 2 diabetes), haplotype TL (10.6% in controls, 15.6% in patients with IGR, and 14.0% in patients with diabetes) was associated with increased odds of IGR (adjusted odds ratio = 1.82, 95% CI: 1.18, 2.80; \( P = 0.007 \)) and type 2 diabetes (adjusted odds ratio = 1.49, 95% CI: 1.09, 2.03; \( P = 0.012 \)). When IGR and type 2 diabetes endpoints were combined, the adjusted odds ratio was 1.56 (95% CI: 1.16, 2.09; \( P = 0.003 \)). Significant associations were not found for haplotypes AS and AL.

**Functional relevance of the \( (GT)_n \) microsatellite polymorphism and \( T(-413)A \) SNP**

To assess whether the \( (GT)_n \) microsatellite polymorphism and the \( T(-413)A \) SNP in the \( HO-1 \) gene promoter region could exert any functional effect, \( HO-1 \) protein expression levels for persons carrying different genotypes of each polymorphism were compared in different glucose metabolism states (Table 5). Interestingly, the \( HO-1 \) protein expression
level in peripheral blood mononuclear cells was significantly reduced in persons with IGR and type 2 diabetes carrying the L/L (GT)ₙ genotype as compared with the S/S genotype, but not among healthy controls. No significant differences in HO-1 expression level were observed between participants with different genotypes for the T(-413)A SNP.

On the basis of the haplotype analysis, the associations between HO-1 protein expression level and various haplotype pairs were subsequently explored. Overall, 9 haplotype pairs (diplotypes: AL/AL, AL/TL, AL/TS, AS/AL, AS/AS, AS/TS, TL/TL, TS/TL, TS/TS) were constructed in the control and type 2 diabetes groups, while only 7 haplotype pairs (except the AS/AS and TL/TL genotypes) were obtained for IGR patients because of the low frequencies of the TL and AS haplotypes and the relatively smaller sample size (see Tables 4 and 5). Thus, we compared differences in HO-1 protein expression level offered by distinct haplotype pairs in control subjects and diabetic patients (Figure 1). Within the A/A genotype class, S-allele carriers with haplotype pairs of AS/AS had a significantly higher HO-1 expression level than L-allele carriers (haplotype pairs: AL/AL) in both the control group (mean fluorescence intensity was 102.89 (SD, 16.71) and 59.13 (SD, 8.35) for the AS/AS and AL/AL genotypes, respectively (P = 0.02)) and the type 2 diabetes group (mean fluorescence intensity was 69.82 (SD, 10.11) and 28.59 (SD, 4.35) for the AS/AS and AL/AL genotypes, respectively (P < 0.001)). When the T/T genotype was considered, similar effects were observed in diabetic patients (mean fluorescence intensity was 52.74 (SD, 6.06) for the TS/TS genotype vs. 25.82 (SD, 4.22) for the TL/TL genotype (P = 0.018)).

**DISCUSSION**

Oxidative stress has been suggested to be an important mechanism in the development of type 2 diabetes (1, 2). In the antioxidative system, HO-1 acts as a key factor in mechanisms to mitigate oxidative stress because of its potent antiinflammatory, antioxidant, and antiproliferative actions (6). In addition, impaired HO-1 protein expression/activity may be related to the pathogenic process induced by oxidative stress. Although accumulating evidence from animal studies supports a beneficial role of HO-1 in the diabetic state (6, 11), it is still not clear whether HO-1 exerts a similar biologic effect in humans. There are 2 potentially functional polymorphisms in the promoter region of HO-1, the (GT)ₙ genotypes, respectively (P = 0.02) and the type 2 diabetes group (mean fluorescence intensity was 69.82 (SD, 10.11) and 28.59 (SD, 4.35) for the AS/AS and AL/AL genotypes, respectively (P < 0.001)). When the T/T genotype was considered, similar effects were observed in diabetic patients (mean fluorescence intensity was 52.74 (SD, 6.06) for the TS/TS genotype vs. 25.82 (SD, 9.22) for the TL/TL genotype (P = 0.018)).
length polymorphism and the T(-413)A SNP, raising the hypothesis that individual capacity to modulate the HO-1 gene’s response is genetically determined (12). In the present article, we describe for the first time the potential association between HO-1 expression level and different glucose metabolism states and elucidate the role of these 2 polymorphisms in susceptibility to type 2 diabetes, as well as their functional relevance.

As expected, compared with healthy controls, decreased levels of HO-1 protein expression were observed in prediabetic (IGR) and diabetic patients. These findings suggest that reduced HO-1 expression may contribute to the development of type 2 diabetes, consistent with the results of several small clinical studies which found decreased mRNA expression of HO-1 in peripheral blood mononuclear cells (29), skeletal muscle (30), and retinal pigment epithelium (31) among patients with type 2 diabetes. On the contrary, Avogaro et al. (32) demonstrated that monocyte HO-1 mRNA expression was increased in type 2 diabetes patients (32). The reason for the discrepant results may be the use of patients of different age groups, diabetes durations, or treatments, as well as small sample sizes. The sample used for our gene expression analysis was much larger (606 controls, 65 IGR patients, and 217 diabetes patients). Furthermore, our present study focused on the newly diagnosed diabetes patients, rather than on prevalent or treated cases, and thus the gene expression is unlikely to have been affected by drug treatment.

HO-1 gene promoter polymorphisms, especially the (GT)n microsatellite polymorphism, have been associated with several clinical outcomes, including cardiovascular disease and renal transplantation (12). These studies suggest that persons with a greater number of (GT)n repeats are at higher risk of developing these disorders (14–17). As far as type 2 diabetes is concerned, only 1 epidemiologic study conducted in a small population (99 diabetic patients and 90 controls) tested the associations among the length of (GT)n repeats, heme oxygenase enzymatic activity in peripheral blood mononuclear cells, and iron stores in diabetic patients (33). Those authors reported a correlation of increased S/M genotype frequency with increased HO-1 activity in diabetic patients as compared with controls (S defined as /C20–26 GT repeats, the middle components (M) defined as 27–32 GT repeats, and L defined as /C21–33 GT repeats) (33). In addition, diabetic patients less often have the S/S and M/M genotypes than control subjects. However, the contribution of this (GT)n polymorphism to type 2 diabetes has not been reflected in this paper. In the present study, a similar allelic...
distribution was observed as for previous studies conducted in Chinese populations (15, 34). We found that carriers of the L (GT)\textsubscript{n} alleles (S/L and L/L genotypes) were more susceptible to IGR and type 2 diabetes, whereas those carrying S (GT)\textsubscript{n} alleles had a decreased risk. Consistent with results from previous functional studies (14–18), IGR and diabetic patients with the L/L genotype had a lower HO-1 expression level than those with the S/S genotype, providing a plausible explanation for the observed adverse effect of the L (GT)\textsubscript{n} allele on the progression of type 2 diabetes. Although there is no universally accepted definition of short/long (GT)\textsubscript{n} repeats, most studies have used 25 or 27 repeats as the cutpoint (35). Given the allelic distribution in the populations studied previously and presently, the number of GT repeats ranging from 25 to 28 is rather small (Web Figure 1), and we also conducted additional statistical analysis according to the different division points within this range. As expected, our research indicated similar and comparable findings.

The potentially conformational changes (i.e., the purine-pyrimidine alternating sequence with Z-DNA conformation potential) may explain the transcriptional regulation of the HO-1 gene by the (GT)\textsubscript{n} length polymorphism (12), because no transcriptional factors or proteins have been found binding to this repeat region (12, 16). These conformational changes may bring structural interferences to other biologically important regulatory elements adjacent (i.e., binding sites for some transcription factors) in the HO-1 gene (12, 16) and then result in the modification of HO-1 promoter activity. Alternatively, the biologic function of the (GT)\textsubscript{n} polymorphism we observed could actually be ascribed to another genetic variant in linkage disequilibrium with the (GT)\textsubscript{n} polymorphism. To date, in only 2 genetic association studies have investigators performed linkage disequilibrium and haplotype analysis between the (GT)\textsubscript{n} microsatellite polymorphism and the T(-413)A SNP in the HO-1 gene promoter region; both studies, in the fields of liver transplantation (21) and rheumatoid arthritis (16), supported strong linkage disequilibrium between these 2 potentially functional polymorphisms. Furthermore, the 2 most frequently constructed haplotypes were “A(-413)(GT)\textsubscript{29} and (-413)T\textsubscript{22}(GT)\textsubscript{22}” (21) and “AL and TS” (16), respectively—similar to the results presented herein. We observed that the odds of type 2 diabetes, conferred by haplotype TL, were similar to those exerted by the L (GT)\textsubscript{n} allele, suggesting that the (GT)\textsubscript{n} microsatellite polymorphism may be the causal locus. Moreover, we observed no differences in HO-1 expression level between persons carrying different genotypes of the T(-413)A SNP. For each homozygous genotype of the T(-413)A SNP (A/A and T/T genotypes), haplotype combinations with the S allele (AS/AS and TS/TS genotypes) were both associated with higher levels of HO-1 expression than those with the L allele (AL/AL and TL/TL genotypes). Therefore, the (GT)\textsubscript{n} microsatellite polymorphism is likely to play a role in HO-1 expression and promoter activity.

To summarize, this is the first report describing the association between 2 potentially functional polymorphisms in the promoter region of the human HO-1 gene and odds of type 2 diabetes in a case-control study including subjects with different states of glucose metabolism. We found that the (GT)\textsubscript{n} microsatellite polymorphism was associated with increased odds of IGR or type 2 diabetes, ascribed to its regulatory effect on HO-1 protein expression. Carriers of long (GT)\textsubscript{n} repeats (≥25 GT repeats) showed decreased HO-1 protein expression and increased odds of type 2 diabetes. These data suggest that the HO-1 (GT)\textsubscript{n} microsatellite polymorphism is involved in type 2 diabetes genetics in
our study population, which may provide important implications for the pathophysiology and prevention of IGR and type 2 diabetes. Clearly, these promising data need further confirmation in larger studies, preferably with subjects of other ethnic backgrounds. In addition, future studies are needed to conduct comprehensive examination of other variants in the HO-1 locus.

ACKNOWLEDGMENTS

Author affiliations: Department of Nutrition and Food Hygiene and MOE Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People’s Republic of China (Fangfang Song, Xiangyang Li, Ping Yao, Nianhong Yang, Xiufa Sun, Liegang Liu); Department of Epidemiology and Biostatistics, Tianjin Medical University Cancer Hospital and Institute, Tianjin, People’s Republic of China (Fangfang Song); Department of Internal Medicine, The Institute of Endocrinology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People’s Republic of China (Muxun Zhang); and Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts (Frank B. Hu).

This work was supported by grants from the Program for New Century Excellent Talents at the University of China (grant NCET-04-0707) and the National Natural Science Foundation of China (grant NSFC-30471406).

Conflict of interest: none declared.

REFERENCES


