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Combined analysis of polymorphism variants in *hMTH1*, *hOGG1* and *MUTYH* genes on the risk of type 2 diabetes in the Chinese population

Lili Cao [a,c], Wei Zhou [a,c], Yanbei Zhu [a,b,c], Wenwen Guo [a,c], Zhenming Cai [a,c], Xuan He [a,c], Yuan Xie [a,c], Xinxiu Li [a,c], Dalong Zhu [a,b], Yaping Wang [a,c,d,*]

[a] Department of Medical Genetics, Nanjing University School of Medicine, Nanjing, Jiangsu, China
[b] Department of Endocrinology, Affiliated Drum Tower Hospital, Medical College of Nanjing University, Nanjing, Jiangsu, China
[c] Jiangsu Key Laboratory of Molecular Medicine, Nanjing University School of Medicine, Nanjing, Jiangsu, China
[d] The State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, Jiangsu, China

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ABSTRACT

Reactive oxygen species are considered to play a role in the development of type 2 diabetes mellitus (T2DM) and its complications. 8-Oxoguanine, which is one of the major oxidation base lesions produced by reactive oxygen species, may cause G:C to T:A transversion mutations because it can mispair with adenine. *hMTH1* (human mutT homolog 1), *hOGG1* (human 8-oxoguanine glycosylase 1) and *MUTYH* (human mutY homolog) genes constitute the 8-oxoG repair pathway. In this study, we screened for the polymorphism variants Val83Met (c.247G>A, rs4866) in *hMTH1*; c.-53G>C (rs56387615), c.-23A>G (rs1801129) and c.-18G>T (rs1801126) in the 5′-UTR of *hOGG1*; and AluYb8 insertion in *MUTYH* (*AluYb8MUTYH*, rs10527342) and investigated their synergistic effect on the risk of T2DM in the Chinese population. The genotypes were determined by electrophoresis, a high-resolution melting technique and sequencing of PCR products. Our results showed that the c.247G>A variant in the *hMTH1* gene increased the risk of T2DM in >55 years of age groups (OR = 1.579; 95%CI: 1.029–2.421). The set of c.-53G>C, c.-23A>G and c.-18G>T variants detected in the 5′-UTR of the *hOGG1* gene and the *AluYb8* insertion in the *MUTYH* gene were each associated with an increased risk of T2DM (OR = 1.507, 95%CI: 1.122–2.024; OR = 1.229, 95%CI: 1.030–1.466, respectively). Combined analysis of the variations among the three genes suggested that the c.247G>A variant in *hMTH1* combined with *AluYb8MUTYH* variant had a synergistic effect on increasing the risk of T2DM (OR = 1.635; 95%CI: 1.147–2.330). This synergy was also observed between the variants in the 5′-UTR of the *hOGG1* and the *AluYb8MUTYH* variant (OR = 1.804; 95%CI: 1.254–2.595). Our results suggest, for the first time, the combined effects of *AluYb8MUTYH* with either *hMTH1* c.247G>A or variants in the 5′-UTR of the *hOGG1* on the risk of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disease characterized by insulin resistance and/or abnormal insulin secretion (Zimmet et al., 2001). Reactive oxygen species (ROS) are involved in the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance and, ultimately, T2DM and its complications (Robertson, 2006). ROS are products of normal metabolism and xenobiotic exposures throughout the lifetime of an individual. Excessive ROS levels can cause structural and functional damage to proteins, lipids and DNA (McCord, 1985). 7,8-Dihydro-8-oxoguanine (8-oxoG) is the most important DNA lesion generated by ROS because of its abundance and mutagenicity. It can mispair with adenine in double-stranded DNA during DNA replication, resulting in G:C to T:A transversion mutations (Bruner et al., 2000). The base excision repair (BER) pathway plays a major role in the repair of mutations caused by ROS (David et al., 2007). *hMTH1* (human mutT homolog 1), *hOGG1* (human 8-oxoguanine glycosylase 1) and *MUTYH* (human mutY homolog) are the major components of the BER system. The *hMTH1* gene encodes an oxidized purine nucleoside triphosphatase that efficiently hydrolyzes oxidized dGTP such as 8-oxo-dGTP in nucleotide pools, thereby avoiding its incorporation into DNA (Nakabeppu et al., 2006). hOGG1 (human 8-oxoguanine glycosylase 1) is an 8-oxoG glycosylase enzyme that excises 8-oxoG from damaged DNA (Nishimura, 2002). Adenine-specific DNA glycosylase encoded by the *MUTYH* (human *mutY homolog*) gene removes post-replicative adenines misincorporated opposite 8-oxoG lesions (Lu et al., 2006; Slupska et al., 1999). Gene knockout in mouse or cell lines showed that these three enzymes play important

Abbreviations: 8-oxoG, 7,8-dihydro-8-oxoguanine; BER, base excision repair; hMTH1, human mutT homolog 1; MUTYH, human mutY homolog; hOGG1, human 8-oxoguanine glycosylase 1; HRM, high-resolution melting; ROS, reactive oxygen species; SNPs, single nucleotide polymorphisms; T2DM, type 2 diabetes mellitus.

* Corresponding author at: The Department of Medical genetics, Medical School, Nanjing University, Hankou Road 22, Nanjing 210093, China. Tel.: +86 25 83686495; fax: +86 25 83686451.
E-mail address: wangyap@nju.edu.cn (Y. Wang).

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roles in the suppression of spontaneous mutagenesis and tumorigenesis (Sakamoto et al., 2007; Tsuzuki et al., 2001; Xie, 2004).

Single nucleotide polymorphisms (SNPs) in DNA repair genes may alter the function of their encoded proteins and reduce DNA repair capacity, which may lead to genetic instability and increased susceptibility to age-related diseases (Goode et al., 2002; Hsieh and Yamane, 2008). The polymorphism c.247G>A, resulting in p.Val83Met, in exon 4 of the *hMTH1* gene has been well studied (Kohno et al., 2006; Miyako et al., 2004). It is suggested that the Met allele at codon 83 of the *hMTH1* gene might increase the risk of type 1 diabetes mellitus in the Japanese female population (Miyako et al., 2004). This variant reduces the protein activity in vitro (Yakushiji et al., 1997). It is unclear whether the *hMTH1* p.Val83Met polymorphism, which could affect the repair of oxidized nuclear and mitochondrial nucleotide pools, is associated with the risk of T2DM. We previously reported that the c.-23G>A variant in the 5′-UTR of the *hOGG1* gene might increase the risk of T2DM (Sun et al., 2010b). In addition, the variants c.-53G>C, c.-23A>G and c.-18G>T of the *hOGG1* gene could reduce the activity of its promoter (Chen et al., 2011b; Liu et al., 2011; Sun et al., 2010b). The association between the set of variants of the three SNPs in the *hOGG1* gene and the risk of T2DM is still unknown. Moreover, we previously observed a reverse insertion of the AluYb8 element in the *MUTYH* gene, which could be a novel genetic risk factor for T2DM (Chen et al., 2011a; Sun et al., 2010a).

In this article, we conducted a case–control study in a Chinese population, using a considerable sample size, to evaluate the role of the *hMTH1* gene variant Val83Met; the variants c.-53G>C, c.-23A>G and c.-18G>T in the 5′-UTR of the *hOGG1* gene; and the *AluYb8MUTYH* polymorphism in the risk for T2DM. Furthermore, we investigated whether there is a synergistic effect among the variants occurring in *hMTH1*, *hOGG1* and *MUTYH* on the risk of T2DM.

2. Materials and methods

2.1. Subjects

Type 2 diabetes patients, 20–85 years of age (n = 1197, mean age: 56.23 ± 10.97 years), were recruited from the Affiliated Drum Tower Hospital, Nanjing University School of Medicine, China. The type 2 diabetes patients were diagnosed according to the World Health Organization (WHO) 1999 criteria (Alberti and Zimmet, 1998). Sample collection was conducted from 2009 to 2011. Age/sex-matched healthy controls (n = 1197, mean age: 55.55 ± 11.46 years) were randomly screened from the healthy volunteers who accepted routine medical examinations at the same hospital. Controls that showed a normal fasting blood glucose level (<6.1 mmol/L) were included in this study, whereas those suffering from acute or chronic metabolic diseases or cancer were excluded.

2.2. DNA extraction

Blood samples were obtained from all subjects, and genomic DNA was extracted from peripheral blood samples using a TIANamp Blood DNA Kit according to the manufacturer's protocol (Tiangen BIOTECH CO., LTD., China).

2.3. Variant screening for the hMTH1 and hOGG1 genes by high-resolution melting (HRM) and DNA sequencing

Genotyping for *hMTH1* (NC 000007.13) and *hOGG1* (NG 012106.1) was performed using HRM analysis. The following primers were used for detecting variants in the *hMTH1* gene: F1, 5′-ATCGTGTTTGAG TTCGTGG-3′; and R1, 5′-GATGGGACCCGCATAGTG-3′, giving a PCR product of 139 bp. The following primers were used for the *hOGG1* gene: F2, 5′-ATGCAGGAGGTGGAGGAATT-3′; and R2, 5′-AGTGGAGGCTAGAGTAC GAT-3′, generating a PCR product of 221 bp.

PCR amplification was performed in a volume of 10 μL that contained 25 ng of genomic DNA, 0.2 pmol of each primer, 1 μL of 25 mM $MgCl_2$, 0.8 μL of 2.5 mM dNTPs, 1 μL of 10× Taq buffer with $(NH_4)_2SO_4$, 1 μL of 10× LC Green® PLUS (Idaho Technology, Inc.), 0.4 U of Taq DNA Polymerase (Fermentas), and 0.4 μL of dimethyl sulfoxide (DMSO). A thermal cycler (PTC-200, MJ research) was used for PCR, and the amplification protocol consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57.2/56 °C for 30 s, and extension at 72 °C for 30 s and with a final 10 min extension at 72 °C. After PCR was completed, genotyping of the polymorphisms was performed using the HRM assay (details previously described) (Sun et al., 2010b).

All heterozygotes identified by the HRM assay were confirmed by direct sequencing to determine the precise genotypes. Direct sequencing of the purified PCR products was performed using an ABI BigDye® Terminator v3.1 Cycle Sequencing Kit. Analyses were completed on a 3130 Genetic Analyzer (Applied Biosystems).

2.4. Variant screening for the MUTYH gene

The *AluYb8MUTYH* polymorphism was genotyped by electrophoresis using the primers F3: 5′-TCTTGACCTGGAGACCTTCC-3′ and R3: 5′-AGCTGCTTCCTCCAAACAGC-3′. The PCR products were separated on 1% agarose gels (Invitrogen, Carlsbad, CA) at 120 mA for 35 min to obtain the genotypes in the *MUTYH* (NG 008189.1) gene.

2.5. Statistical analyses

Statistical analyses were performed using the statistical program SPSS 16.0 software. Data were expressed as the mean ± SD (continuous variables) and as percent totals (categorical variables). Chi-squared tests with 2 × 2 contingency tables were used to compare the genotype frequencies in a case–control study, and the Student's t test was used to determine differences in means. The odds ratio (OR) is shown with a 95% confidence interval (CI). Differences were considered statistically significant for a 2-tailed $P<0.05$.

2.6. Ethics

Written informed consent was obtained from all the subjects who participated, and the study was approved by the Ethics Committee of Nanjing University School of Medicine.

Table 1
Comparison of genotypes of genetic polymorphisms in *hMTH1*, the 5′-UTR of the *hOGG1* and *MUTYH* between T2DM and healthy controls.

Polymorphisms	Numbers (%)		P value [a]	OR (95%CI)[b]
	T2DM (n = 1197)	Controls (n = 1197)		
hMTH1 c.247G>A				
G/G	1088(90.89%)	1110(92.73%)		
G/A	109(9.11%)	87(7.27%)	0.117	
hOGG1 (SNPs in 5′-UTR)				
Non-variants	1079(90.14%)	1116(93.23%)		
Variants[c]	118(9.86%)	81(6.77%)	**0.008**	**1.507(1.122–2.024)**
AluYb8MUTYH				
A/A	325(27.15%)	376(31.41%)		
A/P + P/P	872(72.85%)	821(68.59%)	**0.025**	**1.229(1.030–1.466)**

The bold values ($P<0.05$) were considered statically significant.
[a] P values were analyzed for the frequencies of genotypes between T2DM case and control groups, P values were determined by the χ^2 test.
[b] OR (95%CI) were calculated as the wild genotypes as reference.
[c] Variants including c.-53G>C, c.-23A>G and c.-18G>T in the 5′-UTR of *hOGG1*.