**INTRODUCTION**

Endothelial dysfunction participated in type 2 diabetes (T2DM) from the onset to its outcomes (Schalkwijk & Stehouwer, 2005), and it may be the key factor during the development of insulin resistance or cardiovascular disease (Reaven, 2005; Steinberg, Brechtel, Johnson, Fineberg, & Baron, 1994; Vincent et al., 2004). The endothelial dysfunctions and development of atherosclerosis should impute the decreased bioavailability of nitric oxide (NO). Nitric oxide synthesis disruption induced by asymmetric dimethylarginine (ADMA) has been implicated as an important contributing factor for endothelial dysfunction (Cooke, 2004).
synthase (NOS) isozymes could be inhibited by ADMA via competing with l-arginine as the substrate (Kakimoto & Akazawa, 1970; Tsikas, Boger, Sandmann, Bode-Boger, & Frolich, 2000; Vallance, Leone, Calver, Collier, & Moncada, 1992).

Plasma ADMA levels have been elevated in patients with several risk factors for atherosclerosis, including insulin resistance and type 1 and type 2 diabetes (Abassi et al., 2001; Altinova et al., 2007; Stuhlinger et al., 2002), and have been reported to be associated with diabetic micro/macroversel complications (Abhary et al., 2009; Krzyzanowska et al., 2006). Moreover, elevated plasma ADMA level could be a predictor for the adverse cardiovascular events in type 2 diabetic patients in some prospective studies (Kanazawa et al., 2011; Krzyzanowska, Mittermayer, Woltz, & Schernthaner, 2007). Taken together, it seemed that ADMA may be a newly contributing factor for diabetes.

Most of ADMA would be degraded to citrulline by dimethylarginine dimethylaminohydrolase (DDAH) or excreted by the kidneys in vivo (Achan et al., 2003). There are two isoforms of DDAH, DDAH1 (OMIM No. 604743) and DDAH2 (OMIM No. 604744). DDAH1, but not DDAH2, is essential for metabolizing endogenous ADMA in vivo (Hu et al., 2009, 2011). Mice overexpressing DDAH1 exhibited enhanced insulin sensitivity and lower plasma ADMA levels (Sydow, Mondon, Schrader, Konishi, & Cooke, 2008). Our previous work discovered that a loss-of-function DDAH1 promoter polymorphism led to reduced expression of DDAH1 gene, and this -396 4N deletion–insertion polymorphism was associated with increased risk in both thrombosis stroke and coronary heart disease (CHD). The -396 4N insertion variant led to disruption of metal regulatory transcription factor 1 (MTF1) binding to this promoter region, resulting in inhibited transcriptional activity of DDAH1 gene, which in turn elevated the ADMA level in plasma (Ding et al., 2010).

The elevation of plasma or tissue ADMA and inhibition of NOS may partly explain the coexistence of insulin resistance and the endothelial dysfunction. We hypothesized that this DDAH1: -396_-395insGGGT polymorphism may be also associated with the risk of T2DM. To test this hypothesis, we conducted a case–control study of T2DM in Chinese Han population, with enrollment of 2,417 cases and 2,990 controls in total. The results demonstrated the association of DDAH1: -396 4N insertion variant with the risk of T2DM. The DDAH1: -396_-395insGGGT Ins allele increased the risk of T2DM in males, but not in females. Thus, we found a new gender-related risk factor for T2DM.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This study was approved by the Ethics Committee of Tongji Medical College. Experiments were conducted in accordance with the principles expressed in the Declaration of Helsinki. Each patient provided written informed consent at the enrollment.

2.2 | Recruitment for the discovery and replication population

In total, two sets of independent “discovery” and “replication” populations comprising T2DM patients and nondiabetic control subjects were included in our case–control study. Detailed inclusion criteria of the study population have been described in our previous study (Liu et al., 2012). Briefly, T2DM cases were confirmed by OGTT or FPG results according to the American Diabetes Association criteria (American Diabetes Association, 2008) or by reports of the use of antihyperglycemia medication or by reviews of medical records. Controls were recruited from geographically matched local communities from Central China (Wuhan, Hubei) by excluding those with a current diagnosis of diabetes or with a history of diabetes in their first-degree relatives. In addition, all the cases and controls were self-reported unrelated individuals at enrollment.

2.3 | Power estimation and the sample size

The sample size was considered with a power estimation using QUANTO program (Version 1.2.4). Assuming a minor allele frequency of 0.1 (according to our previous publication) (Ding et al., 2010) and disease prevalence of 0.5%–1%, 95% power would be achieved to detect genetic effects at an odds ratio (OR) between 1.45 and 1.50, with a sample size of approximate 1,200 cases and 1,200 controls.

2.4 | Data collection and definition of risk factors

Demographic data and other risk factors, including history of hypertension, diabetes, hyperlipidemia, smoking, and physical exercise were collected by structured questionnaire. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or current treatment with an antihypertensive drug. Diabetes was diagnosed by a fasting glucose level of >7.8 mmol/L and/or a glucose level of >11.1 mmol/L at 2 hr after oral glucose challenge. Hyperlipidemia was defined as total plasma cholesterol level of >5.72 mmol/L or plasma triglyceride (TG) >1.70 mmol/L. Smoking was defined as a history of smoking >2 pack-years and/or smoking within the preceding 1 year. Body mass index (BMI) (kg/m²) was calculated from measurements of height and weight. Biochemical measurements including levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C),
low-density lipoprotein cholesterol (LDL-C), TG, and insulin were performed using standard laboratory assays. The homeostasis model assessment of insulin resistance (HOMA-IR) and the homeostasis model assessment of pancreatic β-cell function (HOMA-β) were calculated from fasting insulin and glucose levels with the following equations: HOMA-IR = fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5 and HOMA-β = [20 × fasting insulin (IU/mL)]/[fasting glucose (mmol/L)-3.5].

2.5 | DNA isolation

Genomic DNA was extracted from peripheral leukocytes isolated from blood collected in K3-EDTA tubes, using a commercially available DNA isolation kit (DP305-03; TIANGEN; Life Science Products Division), according to the protocol provided by the manufacturer.

2.6 | Genotyping

All participants were genotyped for DDAH1 gene (GenBank reference sequence: NC_000001.11) polymorphism in promoter region (DDAH1: -396_–395insGCGT) according to standard TaqMan allelic discrimination assay (Applied Biosystems). Allelic discrimination was measured automatically using the Sequence Detection Systems 2.1 software (autocaller confidence level 95%). A total of 10% of all genotypes were repeated to check for consistency and to ensure intraplate and interplate genotype quality control. No genotyping discrepancies were detected between the repeated samples. DNA samples for cases and controls were run in the same batches. Probe sequences for this TaqMan 5′-nuclease assay were synthesized by Applied Biosystems, Foster City, CA, USA. Primer sequences for single nucleotide polymorphisms (SNPs) were designed and synthesized by TSINGKE, Beijing, as described in our previous study (Ding et al., 2010).

2.7 | Statistical analysis

Continuous variables were compared between groups by univariate analysis of variance. Categorical values were compared by the chi-squared test or Fisher’s test when appropriate. The distributions of genotype of variants were analyzed for deviation from Hardy–Weinberg Equilibrium (HWE) using chi-squared test. Disease association was assessed by unconditional logistic regression model after adjusting for covariates (sex, age, BMI, hypertension, hyperlipidemia, and smoking status). All analyses were performed using SPSS 22.0 (SPSS Inc.). All tests were two-sided, and p values less than .05 were considered statistically significant.

3 | RESULTS

3.1 | Characteristics of study population

The general characteristics of the T2DM case–control study populations were shown in Table 1 (the discovery population) and Table S1 (the replication population). The discovery population included 1,227 OGTT-confirmed T2DM patients and 1,339 ethnically and geographically matched controls. The replication population included 1,190 T2DM patients and 1,651 controls. As for the discovery population, the cases generally had higher TG, LDL-C, and lower HDL-C than that in controls. The frequencies of classical risk factors, such as hypertension, smoking status, and BMI were significantly higher than in the controls. Similar for the replication population, the frequencies of classical risk factors including hypertension, smoking status, and BMI were also significantly higher than in the controls. The subjects were all of self-reported unrelated Chinese Han population.

3.2 | Association of DDAH1: -396_–395insGCGT polymorphism with type 2 diabetes

We tested the association of the DDAH1: -396_–395insGCGT polymorphism with T2DM in the discovery populations (1,227 cases and 1,339 controls). No deviations from Hardy-Weinberg equilibrium were observed in cases or controls from any of our populations (Table S2). The DDAH1: -396_–395insGCGT Ins allele was significantly associated with increased risk of T2DM, both with or without adjustment for conventional risk

| TABLE 1 Clinical characteristics of the discovery population |
|-----------------|-----------------|
|                | Control (n = 1,339) | Case (n = 1,227) |
| Male (%)       | 45.56%           | 57.38%*          |
| Age (years)    | 57.77 ± 10.31    | 56.23 ± 13.61    |
| BMI (kg/m²)    | 23.93 ± 6.92     | 24.70 ± 3.79*    |
| SBP (mmHg)     | 130.74 ± 29.60   | 140.87 ± 26.07*  |
| DBP (mmHg)     | 80.08 ± 16.57    | 105.78 ± 15.67*  |
| Hypertension (%) | 32.86%          | 45.64%*          |
| Hyperlipidemia (%) | 28.08%         | 30.24%           |
| Total cholesterol (mmol/l) | 4.80 ± 1.58 | 4.75 ± 1.80      |
| Triglyceride (mmol/l)  | 1.48 ± 0.77     | 2.27 ± 0.91*     |
| HDL-C (mmol/l)     | 1.40 ± 0.11     | 1.06 ± 0.47*     |
| LDL-C (mmol/l)     | 2.30 ± 0.43     | 2.46 ± 0.53*     |
| Smoking (%)       | 30.25%           | 49.96%*          |

Note: Data are means ± SD, or n (%). Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; LDL-C, low-density lipoprotein cholesterol.

*p < .05.
factors including sex, age, hypertension, hyperlipidemia, smoking status, and BMI (unadjusted OR = 1.255, 95% CI = 1.081–1.456, p = .003; adjusted OR = 1.380, 95% CI = 1.128–1.687, p = .002). Similar association was found in the replication population (1,190 cases and 1,651 controls) (unadjusted OR = 1.231, 95% CI = 1.007–1.504, p = .043) (Table 2). Then we calculated the HOMA-IR and HOMA-β in age- and gender-matched groups of three genotypes of this polymorphism (n = 30 for each group). The HOMA-IR was significantly increased in the Ins/Ins group than in the Del/Del group (p < .05). For HOMA-β, no difference was observed between all three groups (Figure 1).

### 3.3  DDAH1: -396_-395insGCGT Ins Allele increases the risk of type 2 diabetes in males but not in females

We next analyzed the association of the DDAH1: -396_-395insGCGT deletion–insertion polymorphism in the populations grouped by the gender. In male subjects of the discovery population (704 cases and 610 controls), the Ins allele was significantly associated with increased risk of T2DM, both with or without adjustment for conventional risk factors (unadjusted OR = 1.455, 95% CI = 1.170–1.811, p = .001; adjusted OR = 1.528, 95% CI = 1.141–2.047, p = .004). The analysis of association in the replication population (695 cases and 828 controls) found similar results in male subjects (unadjusted OR = 1.308, 95% CI = 1.068–1.602, p = .009; adjusted OR = 1.439, 95% CI = 1.083–1.911, p = .012) (Table 3).

However, the analysis of association of the DDAH1: -396_-395insGCGT polymorphism in female subjects displayed unexpected results. In the discovery population (523 cases and 729 controls), the Ins allele was not associated with increased risk of T2DM in females, both with or without adjustment for conventional risk factors (unadjusted OR = 1.098, 95% CI = 0.888–1.358, p = .387; adjusted OR = 1.218, 95% CI = 0.913–1.626, p = .18). The analysis of female subjects in the replication population (495 cases and 823 controls) also showed no association (unadjusted OR = 1.144, 95% CI = 0.930–1.408, p = .115; adjusted OR = 1.161, 95% CI = 0.871–1.548, p = .308) (Table 4).

| TABLE 2  | Association of the DDAH1: -396_-395insGCGT polymorphism with T2DM between two independent populations |
|----------|--------------------------------------------------------------------------------------------------|
| Genotype, n (%) | Del/Del | Del/Ins | Ins/Ins | 4N Del/Ins + Ins/Ins Frequency | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
| Samples       |         |         |         |                                  |                      |                        |
| Discovery     |         |         |         |                                  |                      |                        |
| Control (n = 1,339) | 1,085 (81.03%) | 236 (17.63%) | 18 (1.34%) | 20.31% | 1.255 (1.081–1.456) | 1.380 (1.128–1.687) |
| Case (n = 1,227) | 935 (76.20%) | 272 (22.17%) | 20 (1.63%) | 25.43% | p = .003** | p = .002** |
| Replication   |         |         |         |                                  |                      |                        |
| Control (n = 1,651) | 1,338 (81.04%) | 286 (17.31%) | 27 (1.65%) | 20.61% | 1.182 (1.021–1.368) | 1.231 (1.007–1.504) |
| Case (n = 1,190) | 916 (76.97%) | 250 (21.01%) | 24 (2.02%) | 25.05% | p = .026* | p = .043* |

Note: GenBank reference sequence of DDAH1 gene is NC_000001.11. Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by logistic regression, with and without adjustment for sex, age, body mass index, hypertension, hyperlipidemia, and smoking status.

*p < .05.

**p < .01.

**FIGURE 1  DDAH1: -396_-395insGCGT Ins/Ins genotype was associated with impaired insulin sensitivity. (a) The homeostasis model assessment of insulin resistance (HOMA-IR) and (b) the homeostasis model assessment of pancreatic β-cell function (HOMA-β) were calculated in age- and gender-matched subject groups of three genotypes (DDAH1: -396_-395insGCGT Del/Del, Del/Ins and Ins/Ins), n = 30 for each group.**

*p < .05**
In this study, we identified that *DDAH1*: -396_-395insGCGT Ins allele was associated with increased risk of T2DM, but only in males, while no similar association was observed in females. To our knowledge, this study first investigated the association between *DDAH1*: -396_-395insGCGT polymorphism and the risk of T2DM. It was recognized that genetic variation of *DDAH1* was additively associated with circulating ADMA levels in participants with T2DM (Abhary et al., 2010). They tested 26 tag SNPs in *DDAH1* and showed that, among the 26 tag SNPs, rs1498373 was significantly associated with serum ADMA level, while rs1241321 and rs587843 were not. However, as all these SNPs are included in the intron regions of *DDAH1*, the altered protein function and how *DDAH1* polymorphisms affect the plasma ADMA levels remain unclear. Previous work in our laboratory showed that the transcriptional activity of the *DDAH1* gene is reduced in Ins allele due to the disruption of MTF1 transcription factor binding to the promoter region, and this Ins allele increased the risk of coronary heart disease and stroke (Ding et al., 2010). In this study, we demonstrated that the Ins allele also increased the risk of T2DM in both discovery and replication populations, with or without the adjustment of conventional risk factors (i.e. age, gender, hypertension, hyperlipidemia, smoking status, and BMI), suggesting that the contribution of this polymorphism to the risk of T2DM is independent of conventional risk factors (Tables 2). Then, the calculation of HOMA-IR and HOMA-β showed that subjects with Ins/Ins genotype had higher HOMA-IR than Del/Del genotype, but no difference of HOMA-β was observed among all genotypes (Figure 1). Our previous work showed that individuals with the *DDAH1*: -396_-395insGCGT Ins allele had significantly higher ADMA level than those with two copies of the Del allele (Ding et al., 2010), and other researcher demonstrated a positive correlation between the plasma

| TABLE 3 | Association of the *DDAH1*: -396_-395insGCGT polymorphism with T2DM in male subjects between two independent populations |
| Samples | Genotype, n (%) | 4N Del/Ins + Ins/Ins |
| --- | --- | --- | --- |
| | Del/Del Del/Ins Ins/Ins Frequency | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
| Discovery |  |
| Control (n = 610) | 507 (83.11%) 96 (15.74%) 7 (1.15%) | 18.04% |  | 1.455 (1.170–1.811) 1.528 (1.141–2.047) |
| Case (n = 704) | 531 (75.43%) 164 (23.30%) 9 (1.28%) | 25.86% | p = .001** | 1.528 (1.141–2.047) p = .004** |
| Replication |  |
| Control (n = 828) | 685 (82.73%) 130 (15.70%) 13 (1.57%) | 18.84% |  | 1.308 (1.068–1.602) 1.439 (1.083–1.911) |
| Case (n = 695) | 538 (77.41%) 144 (20.72%) 24 (3.57%) | 24.46% | p = .009** | 1.439 (1.083–1.911) p = .012* |

Note: GenBank reference sequence of *DDAH1* gene is NC_000001.11. Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by logistic regression, with and without adjustment for sex, age, body mass index, hypertension, hyperlipidemia, and smoking status.

* p < .05.
** p < .01.

| TABLE 4 | Association of the *DDAH1*: -396_-395insGCGT polymorphism with T2DM in female subjects between two independent populations |
| Samples | Genotype, n (%) | 4N Del/Ins + Ins/Ins |
| --- | --- | --- | --- |
| | Del/Del Del/Ins Ins/Ins Frequency | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
| Discovery |  |
| Control (n = 729) | 578 (79.29%) 140 (19.20%) 11 (1.51%) | 22.22% |  | 1.098 (0.888–1.358) 1.218 (0.913–1.626) |
| Case (n = 523) | 404 (77.25%) 108 (20.65%) 11 (2.10%) | 24.85% | p = .387 | 1.218 (0.913–1.626) p = .18 |
| Replication |  |
| Control (n = 823) | 654 (79.47%) 155 (18.83%) 14 (1.70%) | 22.23% |  | 1.144 (0.930–1.408) 1.161 (0.871–1.548) |
| Case (n = 495) | 378 (76.36%) 106 (21.41%) 11 (2.23%) | 25.87% | p = .204 | 1.161 (0.871–1.548) p = .308 |

Note: GenBank reference sequence of *DDAH1* gene is NC_000001.11. Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by logistic regression, with and without adjustment for sex, age, body mass index, hypertension, hyperlipidemia, and smoking status.
ADMA level and HOMA-IR (Chou et al., 2019). So, these findings support a hypothesis that genetic variants affecting DDAH1 gene expression modify T2DM susceptibility due to impaired insulin sensitivity but not insulin secretion, through the ADMA/NOS pathway.

It is interesting that gender difference appeared in the association of this polymorphism and the risk of T2DM. The DDAH1: -396_-395insGCGT Ins allele increased the risk in male subjects, but not in females, both in discovery and replication populations (Tables 3 and 4). It is not surprising that adult men have higher risk for T2DM, as well as for other cardiovascular disease. Two large national study of prevalence of T2DM in Chinese adults demonstrated higher incidence of T2DM in males than in females, in different age groups and residence groups (Wang et al., 2017; S. H. Yang, Dou, & Song, 2010). Studies also showed that men could develop diabetes at lower average BMI than women, and men were more susceptible to insulin resistance due to ectopic distribution of fat and lower level of lipid-regulating hormones like adiponectin and leptin (Sattar, 2013). A prospective follow-up study of 14,786 middle-aged men and women showed that the overall cardiovascular risk factor level was more favorable in women. The difference in the HDL/total cholesterol ratio was the major determinant of the sex difference in CHD risk. In addition, differences in smoking rate contributed markedly to the excess CHD risk of men (Jousilahti, Vartiainen, Tuomilehto, & Puska, 1999). Here we showed that the DDAH1: -396_-395insGCGT polymorphism might also be involved in the gender difference for diabetes susceptibility.

The differences in vascular endothelial functions between genders have been discussed in previous studies. Young Finns Study showed that men were more easily to have impaired vascular endothelium functioning under chronic stress and at increased risk of atherosclerotic progression (Chumaeva, Hintsanen, Juonala, Raitakari, & Keltikangas-Jarvinen, 2010). The female advantage in vasculature is mainly attributed to an enhanced vasodilative capacity of the endothelium, with the fact that basal and agonist-induced NO release from endothelium is elevated in vasculature from females compared to males (Kauser & Rubanyi, 1994; Orshal & Khalil, 2004; Yang, Bae, & Zhang, 2000). The mechanisms of hormone effects involved in the differences between the males and females on NO production and endothelial functions are mainly rely on the roles of estrogen, which have been shown to stimulate NO production in cultured human endothelial cells (CaulinGlaser, GarciaCardena, Sarrel, Sessa, & Bender, 1997). The binding of estrogen (predominantly 17β-estradiol) to estrogen receptors or specific subtypes of G protein coupled receptors (GPCRs) can trigger multiple signaling pathways including activation of ERK, PI3K, Akt, and c-Src (Filardo, Quinn, Bland, & Frackelton, 2000; Revankar, Cimino, Sklar, Arterburn, & Prossnitz, 2005; Sharma & Prossnitz, 2011), which in turn promote the activation of eNOS via the phosphorylation of residue Ser1177 (Haynes et al., 2003, 2000; Li et al., 2007; Simoncini et al., 2000). Studies in murine aortae have also verified the involvement of these pathways in the estrogen-induced endothelium-dependent NO-mediated vasodilation (Florian, Lu, Angle, & Magder, 2004; Guo, Razandi, Pedram, Kassab, & Levin, 2005; Li et al., 2007). Moreover, the ET-1 concentrations are usually higher in men versus women (Polderman et al., 1993). It was also reported that the ADMA level is lower in females than that in males (Schulze et al., 2005). A study in rats under restraint stress observed enhanced levels of ADMA and reductions in levels of NO metabolites in the brain, and the effects being greater in intensity in males as compared to females (Chakraborti, Gulati, & Ray, 2014).

Since NO synthase-derived free radical production may be one of the resources of oxidative stress in diabettes (Rochette et al., 2013; Vanhoutte, Zhao, Xu, & Leung, 2016), the prooxidant environment is also found to be less pronounced in women as reflected by different activity and expression of vascular NADPH oxidases (Chen et al., 2015; Wong, Randall, & Roberts, 2015). In vascular smooth muscle cells (VSMCs) harvested from rat aortae, male VSMCs have higher levels of superoxide, one of the main ROS, but lower levels of SOD-1, compared to females (Morales et al., 2015). So the gender difference in risk of T2DM which is related to DDAH1 promoter polymorphism might be explained by differences in NO production and oxidative status between males and females. Males seem to be more susceptible to NO reduction-induced vascular endothelium malfunction.

5 | LIMITATION

This is a retrospective case–control study, thus the information bias cannot be excluded. To limit this bias, all subjects were examined in a standardized manner, with well-defined diagnostic criteria, and genotyping was performed blind. Although we calculated adjusted ORs with conventional variables like sex, age, BMI, hypertension, hyperlipidemia, and smoking status, there might be unknown confounding variables which would affect the genetic variation and the risk of T2DM. Next, this is a single-center study, though we employed two independent sets of case–control populations to verify the association, the further external implementation is still important, especially in population with different ethnic backgrounds. Then, we could just conclude in an association between the genetic variation and the risk of T2DM, but the evidences here were inadequate to support a causal association. Finally, the biological mechanism underlying the association between this polymorphism and risk of T2DM remains unclear. In summary, additional studies are needed to investigate the impact of this polymorphism on
specific T2DM related pathways, and on disease susceptibility in a multicentered design.

6  |  CONCLUSION

In this study, our findings suggested that the \textit{DDAHI} -396_ -395insGGGT insertion allele is associated with increased risk of T2DM in a gender-dependent manner, affects males but not females. These findings indicate that DDAH1 promoter polymorphism represents an important locus for predicting inherited susceptibility to T2DM. Gender might be also an important factor in evaluating risk of T2DM in combination of gene polymorphism.

ACKNOWLEDGMENTS

We especially acknowledge all the participants in this study. This work was supported from the Natural Science Foundation of China (NSFC) (No. 81600314) and the Scientific Research Program of Wuhan (No. 2016060101010063).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Z.W. and D. W. W. designed this research. F. Z. and C. Z. performed all the experiments. F. Z. and Z. W. made the statistical analysis of all data, and drafted the manuscript. Z. W and D. W. W. edited and revised manuscript, and approved final version of manuscript.

ORCID

Zheng Wen https://orcid.org/0000-0003-4931-6615

REFERENCES

Abbasi, F., Asagmi, T., Cooke, J. P., Lamendola, C., McLaughlin, T., Reaven, G. M., … Tsao, P. S. (2001). Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. \textit{American Journal of Cardiology}, 88(10), 1201–1203. https://doi.org/10.1016/S0002-9149(01)02063-X

Abhay, S., Burdon, K. P., Kuot, A., Javadiyan, S., Whiting, M. J., Kasmeridis, N., … Craig, J. E. (2010). Sequence variation in DDAH1 and DDAH2 genes is strongly and additively associated with serum ADMA concentrations in individuals with type 2 diabetes. \textit{PLoS One}, 5(3), e9462. https://doi.org/10.1371/journal.pone.0009462

Abhay, S., Kasmeridis, N., Burdon, K. P., Kuot, A., Whiting, M. J., Yew, W. P., … Craig, J. E. (2009). Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines. \textit{Diabetes Care}, 32(11), 2084–2086. https://doi.org/10.2337/dc09-0816

Achan, V., Broadhead, M., Malaki, M., Whitley, G., Leiper, J., MacAllister, R., & Vainance, P. (2003). Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. \textit{Arteriosclerosis, Thrombosis, and Vascular Biology}, 23(8), 1445–1459. https://doi.org/10.1161/01.ATV.0000081742.92006.59

Altinova, A. E., Arslan, M., Sepci-Dincel, A., Akturk, M., Altan, N., & Toruner, F. B. (2007). Uncomplicated type 1 diabetes is associated with increased asymmetric dimethylarginine concentrations. \textit{Journal of Clinical Endocrinology and Metabolism}, 92(5), 1881–1885. https://doi.org/10.1210/jc.2006-2643

American Diabetes Association (2008). Standards of medical care in diabetes—2008. \textit{Diabetes Care}, 31(Suppl 1), S12–54. https://doi.org/10.2337/dc08-S012

CaulinGlaser, T., GarciaCardena, G., Sarrel, P., Sessa, W. C., & Bender, J. R. (1997). 17 beta-estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca2+ mobilization. \textit{Circulation Research}, 81(5), 885–892.

Chakraborti, A., Gulati, K., & Ray, A. (2014). Possible role of nitric oxide (NO) in the regulation of gender related differences in stress induced anoxigenesis in rats. \textit{Nitric Oxide}, 43, 74–80. https://doi.org/10.1016/j.niox.2014.08.005

Chen, C., Wang, Y., Yang, S., Li, H., Zhao, G., Wang, F., … Wang, D. W. (2015). MiR-320a contributes to atherogenesis by augmenting multiple risk factors and down-regulating SRF. \textit{Journal of Cellular and Molecular Medicine}, 19(5), 970–985. https://doi.org/10.1111/jcmm.12483

Chou, C.-Y., Shu, K.-H., Chen, H.-C., Wang, M.-C., Chang, C.-C., Hsu, B.-G., … Huang, C.-C. (2019). Development and validation of a nomogram of urothelial cancer in patients with chronic kidney disease. \textit{Scientific Reports}, 9, 3473. https://doi.org/10.1038/s41598-019-40276-4

Chumaeva, N., Hintsanen, M., Juonala, M., Raitakari, O. T., & Keltikangas-Jarvinen, L. (2010). Sex differences in the combined effect of chronic stress with impaired vascular endothelium functioning and the development of early atherosclerosis: The Cardiovascular Risk in Young Finns study. \textit{BMC Cardiovascular Disorders}, 10, 34. https://doi.org/10.1186/1471-2261-10-34

Cooke, J. P. (2004). Asymmetrical dimethylarginine: The Uber marker? \textit{Circulation}, 109(15), 1813–1818. https://doi.org/10.1161/01. CIR.0000126837.07732.D5

Ding, H., Wu, B., Wang, H., Lu, Z., Yan, J., Wang, X., … Wang, D. W. (2010). A novel loss-of-function DDAH1 promoter polymorphism is associated with increased susceptibility to thrombosis stroke and coronary heart disease. \textit{Circulation Research}, 106(6), 1145–1152. https://doi.org/10.1161/CIRCRESAHA.109.215616

Filardo, E. J., Quinn, J. A., Bland, K. I., & Frackelton, A. R. (2000). Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. \textit{Molecular Endocrinology}, 14(10), 1649–1660. https://doi.org/10.1210/me.14.10.1649

Florian, M., Lu, Y., Angle, M., & Magder, S. (2004). Estrogen induced changes in Akt-dependent activation of endothelial nitric oxide synthase and vasodilation. \textit{Steroids}, 69(10), 637–645. https://doi.org/10.1016/j.steroids.2004.05.016

Guo, X. M., Razandi, M., Pedram, A., Kassab, G., & Levin, E. R. (2005). Estrogen induces vascular wall dilation—Mediation through kinase signaling to nitric oxide and estrogen receptors alpha and
Haynes, M. P., Li, L., Sinha, D., Russell, K. S., Hisamoto, K., Baron, R., … Bender, J. R. (2003). Src kinase mediates phosphatidylinositol-3-kinase/Akt-dependent rapid endothelial nitric-oxide synthase activation by estrogen. Journal of Biological Chemistry, 278(4), 2118–2123. https://doi.org/10.1074/jbc.M210828200

Haynes, M. P., Sinha, D., Russell, K. S., Collinge, M., Fulton, D., Morales-Ruiz, M., … Bender, J. R. (2000). Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-Akt pathway in human endothelial cells. Circulation Research, 87(8), 677–682. https://doi.org/10.1161/01.RES.87.8.677

Hu, X. L., Atzler, D., Xu, X., Zhang, P., Guo, H. P., Lu, Z. B., & Chen, Y. J. (2011). Dimethylarginine Dimethylaminohydrolase-1 Is the Critical Enzyme for Degrading the Cardiovascular Risk Factor Asymmetrical Dimethylarginine. Arteriosclerosis Thrombosis and Vascular Biology, 31(7), 1540–1546. https://doi.org/10.1161/Arvba ha.110.222638

Hu, X. L., Xu, X., Zhu, G. S., Atzler, D., Kimoto, M., Chen, J., & Chen, Y. J. (2009). Vascular Endothelial-Specific Dimethylarginine Dimethylaminohydrolase-1-Deficient Mice Reveal That Vascular Endothelium Plays an Important Role in Removing Asymmetric Dimethylarginine. Circulation, 120(22), 2222–2229. https://doi.org/10.1161/Circulationaha.108.819912

Jousilahti, P., Vartiainen, E., Tuomilehto, J., & Puska, P. (1999). Sex, age, cardiovascular risk factors, and coronary heart disease—A prospective follow-up study of 14 786 middle-aged men and women in Finland. Circulation, 99(9), 1165–1172. https://doi.org/10.1161/01.CIR.99.9.1165

Kakimoto, Y., & Akazawa, S. (1970). Isolation and identification of N-G, N-G'-dimethyl-arginine, N-epsilon-monoo-, di-, and trimethyllysine, and glucosylgalactosyl- and galactosyl-delta-hydroxylysine from human urine. Journal of Biological Chemistry, 245(21), 5751–5758.

Kanazawa, I., Yano, S., Notsu, Y., Yamaguchi, T., Nabika, T., & Sugimoto, T. (2011). Asymmetric dimethylarginine as a risk factor for cardiovascular disease in Japanese patients with type 2 diabetes mellitus. Clinical Endocrinology, 74(4), 467–472. https://doi.org/10.1111/j.1365-2265.2010.03946.x

Kaiser, K., & Rubanyi, G. M. (1994). Gender difference in bioassayable endothelium-derived nitric oxide from isolated rat aortae. American Journal of Physiology, 267(6 Pt 2), H2311–H2317. https://doi.org/10.1152/ajphenh.1994.267.6.H2311

Krzynowowska, K., Mittermayer, F., Krugluger, W., Schnack, C., Hofer, M., Wolzt, M., & Schernthaner, G. (2006). Asymmetric dimethylarginine is associated with macrovascular disease and total homocysteine in patients with type 2 diabetes. Atherosclerosis, 189(1), 236–240. https://doi.org/10.1016/j.atherosclerosis.2005.12.007

Krzynowowska, K., Mittermayer, F., Wolzt, M., & Schernthaner, G. (2007). Asymmetric dimethylarginine predicts cardiovascular events in patients with type 2 diabetes. Diabetes Care, 30(7), 1834–1839. https://doi.org/10.23736/sdc07-0190

Li, L., Hisamoto, K., Kim, K. H., Haynes, M. P., Bauer, P. M., Sanjay, A., … Bender, J. R. (2007). Variant estrogen receptor-c-Src molecular interdependence and c-Src structural requirements for endothelial NO synthase activation. Proceedings of the National Academy of Sciences of the United States of America, 104(42), 16468–16473. https://doi.org/10.1073/pnas.0704315104

Liu, L., Ding, H., Wang, H. R., Xu, Y. J., Cui, G. L., Wang, P. H., … Wang, D. W. (2012). Polymorphism of HMGA1 is associated with increased risk of type 2 diabetes among Chinese individuals. Diabetologia, 55(6), 1685–1688. https://doi.org/10.1007/s00125-012-2518-0

Moraes, R. C., Bahnsen, E. S., Havelka, G. E., Cantu-Medellin, N., Kelley, E. E., & Kibbe, M. R. (2015). Sex-based differential regulation of oxidative stress in the vasculature by nitric oxide. Redox Biology, 4, 226–233. https://doi.org/10.1016/j.redox.2015.01.007

Orshol, J. M., & Khalil, R. A. (2004). Gender, sex hormones, and vascular tone. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 286(2), R233–R249. https://doi.org/10.1152/jpregu.00338.2003

Polderman, K. H., Stehouwer, C. D., van Kamp, G. J., Dekker, G. A., Verheugt, F. W., & Gooren, L. J. (1993). Influence of sex hormones on plasma endothelin levels. Annals of Internal Medicine, 118(6), 429–432. https://doi.org/10.7326/0003-4819-118-6-19930.3150-00006

Reaven, G. (2005). Insulin resistance, type 2 diabetes mellitus, and cardiovascular disease: The end of the beginning. Circulation, 112(20), 3030–3032. https://doi.org/10.1161/CIRCULATIONHA.104.504670

Revankar, C. M., Cimino, D. F., Sklar, L. A., Artberburn, J. B., & Prossnitz, E. R. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science, 307(5715), 1625–1630. https://doi.org/10.1126/science.1106943

Rochette, L., Lorin, J., Zeller, M., Guillard, J. C., Lorgis, L., Cottin, Y., & Vergely, C. (2013). Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: Possible therapeutic targets? Pharmacology & Therapeutics, 140(3), 239–257. https://doi.org/10.1016/j.pharmthera.2013.07.004

Sattar, N. (2013). Gender aspects in type 2 diabetes mellitus and cardiometabolic risk. Best Practice & Research Clinical Endocrinology & Metabolism, 27(4), 501–507. https://doi.org/10.1016/j.beem.2013.05.006

Schalkwijk, C. G., & Stehouwer, C. D. (2005). Vascular complications in diabetes mellitus: The role of endothelial dysfunction. Clinical Science, 109(2), 143–159. https://doi.org/10.1042/CS20050025

Schulze, F., Maas, R., Freese, R., Schwedhelm, E., Silberhorn, E., & Boger, R. H. (2005). Determination of a reference value for N(G), N(G)-dimethyl-L-arginine in 500 subjects. European Journal of Clinical Investigation, 35(10), 622–626. https://doi.org/10.1111/j.1365-2362.2005.01561.x

Sharma, G., & Prossnitz, E. R. (2011). Mechanisms of estradiol-induced insulin secretion by the G protein-coupled estrogen receptor GPR30/ GPER in pancreatic beta-cells. Endocrinology, 152(8), 3030–3039. https://doi.org/10.1210/en.2011-0091

Simoncini, T., Hafez-Moghadam, A., Brazil, D. P., Ley, K., Chin, W. W., & Liao, J. K. (2000). Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature, 407(6803), 538–541. https://doi.org/10.1038/35035131

Steinberg, H. O., Brechtel, G., Johnson, A., Fineberg, N., & Baron, A. D. (1994). Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. Journal of Clinical Investigation, 94(3), 1172–1179. https://doi.org/10.1172/JCI117433

Stuhliger, M. C., Abbasi, F., Chu, J. W., Lemandola, C., McLaughlin, T. L., Cooke, J. P., … Tsao, P. S. (2002). Relationship between insulin resistance and an endogenous nitric oxide synthase
inhibitor. *JAMA*, 287(11), 1420–1426. https://doi.org/10.1001/jama.287.11.1420

Sydow, K., Mondon, C. E., Schrader, J., Konishi, H., & Cooke, J. P. (2008). Dimethylarginine dimethylaminohydrolase overexpression enhances insulin sensitivity. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 28(4), 692–697. https://doi.org/10.1161/ATVBAHA.108.162073

Tsikas, D., Boger, R. H., Sandmann, J., Bode-Boger, S. M., & Frolich, J. C. (2000). Endogenous nitric oxide synthase inhibitors are responsible for the L-arginine paradox. *FEBS Letters*, 478(1–2), 1–3. https://doi.org/10.1016/S0014-5793(00)01686-0

Vallance, P., Leone, A., Calver, A., Collier, J., & Moncada, S. (1992). Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *The Lancet*, 339(8793), 572–575. https://doi.org/10.1016/0140-6736(92)90865-z

Vanhoutte, P. M., Zhao, Y., Xu, A., & Leung, S. W. (2016). Thirty years of saying NO: Sources, fate, actions, and misfortunes of the endothelium-derived vasodilator mediator. *Circulation Research*, 119(2), 375–396. https://doi.org/10.1161/CIRCRESAHA.116.306531

Vincent, M. A., Clerk, L. H., Lindner, J. R., Klibanov, A. L., Clark, M. G., Rattigan, S., & Barrett, E. J. (2004). Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. *Diabetes*, 53(6), 1418–1423. https://doi.org/10.2337/diabetes.53.6.1418

Wang, L., Gao, P., Zhang, M., Huang, Z., Zhang, D., Deng, Q., … Wang, L. (2017). Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. *JAMA*, 317(24), 2515–2523. https://doi.org/10.1001/jama.2017.7596

Wong, P. S., Randall, M. D., & Roberts, R. E. (2015). Sex differences in the role of NADPH oxidases in endothelium-dependent vasorelaxation in porcine isolated coronary arteries. *Vascular Pharmacology*, 72, 83–92. https://doi.org/10.1016/j.vph.2015.04.001

Yang, S., Bae, L., & Zhang, L. (2000). Estrogen increases eNOS and NOx release in human coronary artery endothelium. *Journal of Cardiovascular Pharmacology*, 36(2), 242–247. https://doi.org/10.1097/00005344-200008000-00015

Yang, S. H., Dou, K. F., & Song, W. J. (2010). Prevalence of Diabetes among Men and Women in China. *New England Journal of Medicine*, 362(25), 2425–2426.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Zhu F, Zhou C, Wen Z, Wang DW. *DDAH1* promoter -396 4N insertion variant is associated with increased risk of type 2 diabetes in a gender-dependent manner. *Mol Genet Genomic Med.* 2020;8:e1011. https://doi.org/10.1002/mgg3.1011