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1 Title: ²Associations of *ACE* I/D and *AGTRI* rs5182 polymorphisms with diabetes and
2 their effects on lipids in an elderly Chinese population

3 Abstract

4 Background

5 Diabetes mellitus is generally accompanied by dyslipidaemia, but inconsistent
6 relationships between lipid profiles and diabetes are noted. Moreover, genetic variations
7 in ³insertion/deletion (I/D) polymorphisms at angiotensin-converting enzyme gene
8 (*ACE*) and T/C polymorphisms in the angiotensin type 1 receptor gene (*AGTRI*) are
9 related to diabetes and lipid levels, but the associations are controversial. Thus, the
10 current research aimed to explore the effects of *ACE* I/D, *AGTRI* rs5182 and diabetes
11 mellitus on serum lipid profiles in 385 Chinese participants with an average age of
12 75.01 years.

13 Methods

14 ⁷The *ACE* I/D variant was identified using the polymerase chain reaction (PCR) method,
15 whereas the *AGTRI* rs5182 polymorphism was identified using the PCR-based
16 restriction fragment length polymorphism (PCR-RFLP) method and verified with DNA
17 sequencing. ¹⁸Total cholesterol (TC), triglyceride (TG), apolipoprotein A (ApoA),
18 apolipoprotein B (ApoB), high-density lipoprotein cholesterol (HDL-C) and low-
19 density lipoprotein cholesterol (LDL-C) levels were measured using routine methods,
20 and the lipid ratios were calculated.

21 Results

22 *ACE* I/D, but not *AGTRI* rs5182, was a predictor ⁴⁷of TG/HDL-C for the whole study

population. Both *ACE I/D* and *AGTR1* rs5182 were predictors of HDL-C and LDL-C levels in females but not in males. Moreover, in females, diabetes mellitus and *ACE I/D* were identified as predictors of TG and TG/HDL-C, whereas *AGTR1* rs5182 and diabetes mellitus were predictors of TG/HDL-C. Moreover, diabetes mellitus and the combination of *ACE I/D* and *AGTR1* rs5182 variations were predictors of TG and TG/HDL-C exclusively in females.

Conclusions

The results demonstrated the potential for gender-dependent interactions of *ACE I/D*, *AGTR1* rs5182, and diabetes on lipid profiles. These findings may serve as an additional explanation for the inconsistent changes of blood lipids in individuals with diabetes mellitus, thereby offering a novel perspective for the clinical management of blood lipid levels in diabetic patients.

Keywords: diabetes mellitus, *ACE I/D* polymorphism, *AGTR1* rs5182 polymorphism, dyslipidaemia, combination analysis.

Background

Diabetes mellitus has become an expanding public health concern globally, affecting more than 37% of individuals aged 65 years and older [1]. Moreover, diabetes mellitus is generally accompanied by dyslipidaemia [2], the typical features of which include elevated triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels as well as decreased high-density lipoprotein cholesterol (HDL-C) levels [3]. Elevated TG levels and reduced HDL-C levels are often accompanied by diabetes mellitus [4]. In

45 addition, as a sensitive parameter reflecting blood lipid profiles, the TG-to-HDL-C ratio
 46 (TG/HDL-C) is significantly positively associated with diabetes mellitus [5]. However,
 47 inconsistent relationships between serum lipids and diabetes mellitus have also been
 48 reported. For example, TG² is associated with a reduced risk of diabetes mellitus in
 49 patients who exhibit genetic susceptibility to elevated TG levels [6]. Similarly, the
 50 protective effect of HDL for diabetes was found only in Iranian women but not in
 51 Iranian men [7]. Thus, the critical effects of genetic background should be considered
 52 in the context of the complex relationship between lipid fractions and diabetes mellitus.
 53 ⁴²The renin-angiotensin system (RAS), a complex hormonal regulatory system, is
 54 associated with not only blood pressure but also dyslipidaemia [8]. Interfering with the
 55 RAS using drugs has become a type of diabetes treatment [9]. In the RAS, ³¹angiotensin-
 56 converting enzyme (ACE) can convert angiotensin I to angiotensin II through the
 57 removal of carboxy-terminal dipeptides [8]. The significant effects of ACE on
 58 glycaemic disturbances suggest a correlation between ACE and diabetes mellitus [10],
 59 which was further confirmed by the utilization of ACE inhibitors to prevent diabetes
 60 mellitus [11]. The ACE¹⁵ gene (*ACE*), which is located on the long arm of chromosome
 61 17 (17q23), is 21 kb long and consists of 26 exons and 25 introns [12]. The ¹*ACE I/D*
 62 polymorphism is distinguished by a distinctive 287-base pair repetitive element located
 63 within intron 16, which gives rise to the variation of either an insertion or a deletion
 64 (I/D) within the genetic sequence [13]. This variation leads to 3 possible genotypes,
 65 including II, ID and DD [13], as well as a change in ACE concentration [14]. Previous
 66 ¹⁴studies have shown that *ACE I/D* is associated with diabetes mellitus [15], and II

67 homozygotes³ had higher HDL-C levels than did subjects with the D allele [13, 16]. On
 68 the other hand, other studies noted¹⁴ that the association between the *ACE I/D*
 69 polymorphism and dyslipidaemia was insignificant in Chinese diabetic patients [17, 18].
 70 Obviously, other factors should be considered to explain the conflicting findings
 71 regarding²² the influence of *ACE I/D* on lipid profiles in diabetic patients.

72 As the major biologically active hormone generated by the RAS system, angiotensin II
 73 regulates blood pressure via angiotensin type 1 receptor (AGTR1) [8, 19]. The
 74 human AGTR1 gene (*AGTR1*), which contains 5 exons and 4³⁰ introns, is located on the
 75 long arm of chromosome 3 (3q21-25) [20]. The rs5182 polymorphism (C573T) of
 76 *AGTR1* alters *AGTR1* expression [21] and is related to the presence of diabetes
 77 combined with hypertension in the Han population of Inner Mongolia [20]. The *AGTR1*
 78 rs5182 variant is related to nonalcoholic fatty liver disease, which is often characterized
 79 by dyslipidaemia [22, 23]. However, a study conducted in another Chinese population
 80 failed to observe a significant association between *AGTR1* rs5182 and dyslipidaemia
 81 [21]. Thus, the effects of *ACE I/D* and *AGTR1* rs5182 on lipid profiles, as well as their
 82 interaction with diabetes mellitus, should be studied to obtain a better understanding
 83 about the influence of diabetes and genetic polymorphisms on lipids.

84 This study seeks to explore possible explanations for the controversial findings
 85 concerning the relationship between diabetes and dyslipidaemia reported in previous
 86 studies and to evaluate¹ the cumulative effect of *ACE I/D* and *AGTR1* rs5182 on lipid
 87 levels, which has not yet been reported. It is hypothesized that interactions potentially
 88 occur among *ACE I/D* variation, the *AGTR1* rs5182 polymorphism and diabetes

mellitus to impact serum lipid profiles in the current study. Therefore, *ACE I/D* variation, the *AGTRI* rs5182 polymorphism and serum lipid levels were measured in 385 Chinese subjects with an average age of 75.01 years. The interactions between genetic backgrounds and diabetes mellitus, as well as their contributions to lipid profiles, were analysed. The investigation of the combined effects of *ACE I/D* and *AGTRI* rs5182 on lipid profiles, as well as their interactions with diabetes mellitus in the current study, may contribute to preventing and managing dyslipidaemia in individuals with diabetes mellitus.

2. Methods

Study population

One thousand one hundred nineteen volunteers were enrolled, and the inclusion criteria for the participants were as follows: (1) understood the procedures involved and provided written consent; (2) had a history of diabetes; (3) provided current medication use status for antidiabetic and/or lipid-lowering drugs; (4) provided complete serum lipid and glucose measurements; (5) provided blood samples; and (6) were aged ≥ 50 years. In total, 385 of the participants (average age = 75.01 ± 24.90 years) who met the above criteria were involved in the present study. The study was approved by the Human Research Ethics Committee of Chongqing Orthopedic Hospital of Traditional Chinese Medicine.

Biochemical measurements

Venous blood samples were collected from the participants in the morning after a 12-

hour fast. Serum was isolated via centrifugation (3000 rpm, 20 minutes) at 4 °C²⁰ and stored at -80 °C for further analyses. Glucose levels were measured using the glucose oxidase–peroxidase (GOD–POD) method [24]. Serum TG²⁷ levels were determined using the glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) method [25]. TC, HDL-C and LDL-C levels were measured using the cholesterol oxidase-peroxidase and 4-aminoantipyrine phenol (CHOD-PAP) method [25].⁴⁶ Apolipoprotein A (ApoA) and apolipoprotein B (ApoB) levels were determined via immunoturbidimetry [26]. Ratios of TG/HDL-C, TC/HDL-C and LDL-C/HDL-C were calculated.¹³

DNA extraction and genotyping

Genomic DNA¹⁷ was extracted using a DNA extraction kit per the manufacturer's instructions (Kuang Yuan, Suzhou, China). The *ACE I/D* genotype⁷ was detected via the polymerase chain reaction (PCR) method, and the *AGTR1* rs5182 variant was identified via the⁵ polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method followed by confirmation using DNA sequencing. Briefly, for *ACE I/D* genotype detection, the target DNA fragments were amplified with the primers 5'-CTGGAGACCACTCCCATCCTTTCT-3'⁶ (forward) and 5'-GATGTGGCCATCACATTCGTCAGAT-3' (reverse) [13]. The samples were denatured at 94 °C for 4 min, followed by 32 cycles, which consisted of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 90 s, with a final extension at 72 °C for 5 min. A 190-bp PCR fragment¹⁰ was produced in the absence of the insertion (D), and a 490-bp fragment was produced in the presence of the insertion (I). In the absence of the insertion (D), a PCR product of 190 bp was generated,

133 whereas¹⁰ the presence of the insertion (I) resulted in the amplification of a 490-bp
 134 fragment. Moreover, for *AGTR1* rs5182 genotype identification, two oligonucleotide
 135 primers, 5'-GGCTTTGCTTTGTCTTGTTG-3' (forward) and 5'-
 136 AATGCTTGTAGCCAAAGTCACCT-3' (reverse), were used for amplification [27].
 137 The PCR procedure consisted of⁸ 3 min at 94 °C for denaturation; 40 cycles of 30 sec at
 138 94 °C, 30 sec at 60 °C, and 90 sec at 72 °C; and a final elongation step of 5 min at 72 °C
 139 [27]. The PCR-amplified products were digested overnight with the restriction
 140 endonuclease *MnII*,³⁷ which cuts at position 580, when the C allele is present instead of
 141 the T allele at 573, and at positions 905, 1032, 1062, and 1147. The PCR-amplified
 142 products of *ACE I/D* and the restriction endonuclease *MnII*-digested products of
 143 *AGTR1* rs5182 were identified using³⁹ 1.5% agarose gel electrophoresis and verified with
 144 DNA sequencing.

145 Dummy variable coding

146 Owing to the limited number,⁵ the minor allele homozygotes were combined with their
 147 heterozygotes and defined as D allele carriers of *ACE I/D* and C allele carriers of
 148 *AGTR1* rs5182 for further analysis.¹ To further clarify the cumulative effects of *ACE I/D*
 149 and *AGTR1* rs5182 on lipid levels, the combined genotypes¹ of *ACE I/D* and *AGTR1*
 150 rs5182 were used as dummy variables:

151 Dummy variable 1 = *ACE* II + *AGTR1* rs5182 TT

152 Dummy variable 2 = *ACE* II + *AGTR1* rs5182 C allele

153 Dummy variable 3 = *ACE* D allele + *AGTR1* rs5182 TT

154 Dummy variable 4 = *ACE* D allele + *AGTR1* rs5182 C allele

155 ³² Statistical analyses

156 The data are expressed as the means $\pm$ standard deviations (SDs) unless otherwise
 157 specified. ²⁵ The sample size calculation was conducted using the G*Power software
 158 program (version 3.1.9.7, Germany) [28], and the current sample size was sufficient for
 159 a minimum power of 80%. The deviation from Hardy–Weinberg equilibrium [29],
 160 which can estimate the number of homozygous and heterozygous variant vectors in an
 161 unevolved population [30], was analysed with the χ^2 goodness-of-fit test. ²⁹ The chi-
 162 square test was used to determine the distribution of genotypes and alleles, the
 163 prevalence of diabetes and the percentage of drug use between subjects of different
 164 genders. The normal distribution of each variable was initially analysed using the
 165 Kolmogorov-Smirnov test. Because of the abnormal distribution, logarithmic
 166 transformations were applied to ⁹ TG and the TG/HDL-C ratio to reduce skewness before
 167 performing the statistical analyses. Independent sample t tests were conducted to
 168 compare the differences in blood lipid levels between the males and the females.
 169 Potential factors associated with blood lipid and blood glucose levels were analysed
 170 using the stepwise ³⁶ multiple linear regression analysis. Statistical significance was
 171 defined as $P < \sup{21} 0.05$.

172

173 **3. Results**

174 3.1. Characteristics of the study population

175 Table 1 shows the characteristics of the metabolic variables, prevalence of diabetes,
 176 and drug usage in the current study population. As displayed in Table 1, TG, ³ TC, HDL-

C, ApoA, LDL-C, and ApoB levels as well as the prevalence of diabetes were significantly greater in females than in males ($P = 0.017$, $p = 0.016$, $P = 0.049$, $P = 0.002$, $P = 0.021$, $P = 0.022$, and $P = 0.045$, respectively). However, no statistically significant differences in age or glucose were noted between the genders. When considering the use of lipid-lowering drugs and treatment with antidiabetic drugs, no significant difference was found between male and female subjects.

3.2. Genotypes and alleles of *ACE* I/D and *AGTR1* rs5182 in the participants

The identification of *ACE* I/D and *AGTR1* rs5182 using gel electrophoresis, followed by confirmation via DNA sequencing, is shown in Figure 1. The frequencies of genotypes and alleles are presented in Figure 2. The genotype frequencies of either *ACE* I/D or *AGTR1* rs5182 were in Hardy–Weinberg equilibrium in the current study ($P = 0.820$ and $P = 0.480$, respectively). No statistically significant differences in the genotype frequencies of the *ACE* I/D genotype and *AGTR1* rs5182 genotype were noted between male and female subjects. However, the D allele of *ACE* I/D and C allele of *AGTR1* rs5182 exhibited significantly greater frequencies in males compared with females. Owing to the limited numbers of samples, the minor allele homozygotes were combined with their heterozygotes and defined as D allele carriers of *ACE* I/D and C allele carriers of *AGTR1* rs5182, respectively, for further analysis.

3.3. Predictors of lipid levels in the subjects

To further explore the predictors of lipid profiles in the current study population, stepwise multiple linear regression analyses were performed. The *ACE* I/D genotype

(Model A) or *AGTRI* rs5182 genotype (Model B) was used as an independent variable separately or in combination with age, gender, diabetes mellitus, and the use of antidiabetic drugs or lipid-lowering drugs as other independent variables.

As shown in Table 2, gender was the only predictor of TG, TC, and ApoB levels, explaining 1.2%, 1.5%, and 1.4% of the total variance, respectively. Gender, antidiabetic drug use, and lipid-lowering drug use were predictors of HDL-C levels, accounting for 2.7%, 1.1% and 0.8% of the total variance, respectively. In addition, gender, the use of antidiabetic drugs, lipid-lowering drugs and diabetes mellitus were predictors of ApoA levels, accounting for 2.3%, 3.2%, 2.3%, and 0.9% of the total variance, respectively. Moreover, gender, the use of antidiabetic drugs, and diabetes mellitus were predictors of LDL-C levels, accounting for 0.9%, 1.3% and 1.2% of the total variance, respectively. The use of antidiabetic drugs, diabetes mellitus status and age were predictors of glucose levels, accounting for 12.3%, 2.8% and 2.3% of the total variance, respectively. Notably, *ACE* I/D and the use of antidiabetic drugs were predictors of the TG/HDL-C ratio, accounting for 1.0% and 1.6% of the total variance, respectively. However, *AGTRI* rs5182 was not a predictor of TG/HDL-C.

When gender was taken into consideration in the *ACE* I/D analysis, as displayed in Table 3, the use of antidiabetic drugs, the use of lipid-lowering drugs and diabetes mellitus were predictors of HDL-C (accounting for 2.4%, 2% and 2.8% of the total variance, respectively) and ApoA (accounting for 4.3%, 3.4%, and 3.6% of the total variance, respectively) levels in males. The use of antidiabetic drugs and diabetes mellitus were predictors of LDL-C levels, contributing 2.7% and 1.6% of the total

221 variance, respectively, whereas the use of antidiabetic drug was a predictor of ApoB
 222 levels in males, contributing 2.4% of the total variance. Furthermore, the use of
 223 antidiabetic drugs, diabetes mellitus, and age were predictors of glucose levels in males,
 224 contributing to 12.3%, 2.1%⁴ and 2.3% of the total variance, respectively. Although *ACE*
 225 *I/D* was not a predictor of any lipid profile in males, notably, in female subjects, *ACE*
 226 *I/D* and diabetes mellitus were predictors of TG (accounting for 3.7% and 3.5% of the
 227 total variance, respectively) and TG/HDL-C (accounting for 4.3% and 3.9% of the total
 228 variance, respectively). Moreover, age was the only predictor of ApoA levels, and *ACE*
 229 *I/D* was the only predictor of LDL-C levels in females, accounting for 3.6% and 4.4%
 230 ⁴⁴ of the total variance, respectively. In terms of HDL-C levels in females, *ACE I/D*, age
 231 and the use of antidiabetic drugs were predictors, accounting for 2.9%⁴, 2.7% and 2.3%
 232 of the total variance, respectively. The predictors of glucose in females were the same
 233 as those in identified males, including the use of antidiabetic drugs, diabetes mellitus,
 234 and age, contributing to 2.8%, 14% and 1.9% of the total variance, respectively.
 235 The results of the analysis of the effects of *AGTR1* rs5182 in males and females are
 236 ¹¹ shown in Table 4. The predictors of HDL-C, ApoA, LDL-C, ApoB, and glucose levels,
 237 as well as the contribution of each factor to the total variance in males, were the same
 238 as those identified in the *ACE I/D* analysis (Table 3). Similarly, the predictors of
 239 glucose levels and their contributions to the total variance in females were the same as
 240 those identified in the *ACE I/D* analysis (Table 3). Nevertheless, in female subjects,
 241 diabetes mellitus was the only predictor of TG levels, whereas age was the only
 242 predictor of ApoA levels, accounting for 3.5% and 3.6%, respectively, of the total

243 variance. Interestingly, *AGTR1* rs5182, age and the use of antidiabetic drugs were
 244 predictors of HDL-C levels in females, accounting for ⁴2.9%, 2.3%, and 2.2%, of the
 245 total variance, respectively. The *AGTR1* rs5182 was the only predictor of LDL-C levels
 246 in females, accounting for 2.0% of the total variance. Furthermore, *AGTR1* rs5182 and
 247 diabetes mellitus were predictors of TG/HDL-C, accounting for 2.4% and 3.9% of the
 248 total variance, respectively.

249 3.4.⁴⁹ *ACE* I/D and *AGTR1* rs5182 combination analysis in the subjects

250 To further investigate the combined influence ³⁸of the *ACE* I/D polymorphism and the
 251 *AGTR1* rs5182 variant on lipid profiles, stepwise multiple linear regression analyses
 252 were performed. Dummy variables 1 (*ACE* II + *AGTR1* rs5182 TT), 2 (*ACE* II + *AGTR1*
 253 rs5182 C allele), and 4 (*ACE* D allele + *AGTR1* rs5182 C allele) were incorporated as
 254 independent variables, whereas dummy variable 3 (*ACE* D allele + *AGTR1* rs5182 TT)
 255 served as the reference category. Additionally, age, gender, the prevalence of diabetes
 256 mellitus, and the use of antidiabetic drugs or lipid-lowering drugs were included as
 257 other independent variables to estimate their individual predictive value for lipid levels,
 258 lipid ratios and glucose levels.

259 In the whole study population, ¹¹as shown in Table 5, although the predictors of TG, TC,
 260 HDL-C, ApoA, ApoB, LDL-C, and glucose levels, as well as the contribution of each
 261 factor to the total variance, were the same as those in Table 2, it is interesting to note
 262 that dummy variable 1 and the use of antidiabetic drugs, accounting for 0.8% and 1.6%
 263 of the total variance, respectively, were predictors of TG/HDL-C.

When gender was taken into consideration, as displayed in Table 6, dummy variable 4 was the only predictor of TG levels in males, accounting for 3.8% of the total variance. Dummy variable 4 and diabetes mellitus were identified as predictors of TG levels in females, accounting for 3.8% and 3.7% of the total variance, respectively. None of the dummy variables were predictors of TC, HDL-C, ApoA, LDL-C, ApoB, TG/HDL-C, TC/HDL-C, LDL-C/HDL-C or glucose levels in males. In contrast, dummy variable 1 was the only predictor of TC (accounting for 5.8%), ApoB (accounting for 2.4%) and LDL-C (accounting for 7.7%) levels, whereas dummy variable 1 and age were predictors of ApoA (accounting for 3.3% and 3.6%, respectively) in females. Moreover, the predictors of HDL-C in females included dummy variable 1, age, and the use of antidiabetic drugs, accounting for 8.4%, 3.2%, and 2.4% of the total variance, respectively. Dummy variable 1, dummy variable 4, and diabetes mellitus were predictors of TG/HDL-C in female subjects and accounted for 5%, 1.9%, and 4% of the total variance, respectively.

278

279 4. Discussion

Diabetes mellitus is often accompanied by dyslipidaemia [5]. However, inconsistencies in the relationship between diabetes mellitus and dyslipidaemia have been reported [6, 31], and the mechanism of the observed discrepancy remains unclear. Moreover, the RAS has been reported as a critical system in controlling blood pressure. For example, ACE activity has been reported to be involved in changes in the retinoic acid receptor (RAR)/retinoid X receptor (RXR)-peroxisome proliferator-activated receptor (PPAR)

signalling pathway and the suppression of cellular retinol-binding protein 1 (CRBP1), ultimately affecting adipocyte homeostasis and blood lipids [32, 33]. Moreover, AGTR1 activation is related to lipid accumulation in both the livers of C57BL/6 mice and in HepG2 cells [34, 35]. Thus, ACE and AGTR1 could be involved in diabetes and dyslipidaemia. The *ACE* I/D polymorphism and *AGTR1* rs5182 polymorphism are associated with lipid levels, but the findings are also contradictory [21, 22]. Therefore, investigating the effects of the interactions of the *ACE* I/D and *AGTR1* rs5182 polymorphisms with diabetes and the subsequent effects on lipid profiles has the potential to elucidate the possible mechanism underlying the inconsistency among genetic variations, diabetes and lipid levels.

In the present study, diabetes mellitus was a predictor of ApoA and LDL-C levels in the whole study population but not a predictor of other lipid profiles or ratios. Moreover, although diabetes mellitus was identified as a predictor of HDL-C, ApoA, LDL-C and glucose, neither *ACE* I/D nor *AGTR1* rs5182 contributed to changes in lipid and lipid ratios in males (Tables 2-4). In contrast, both *ACE* I/D and *AGTR1* rs5182 were identified as predictors of HDL-C and LDL-C exclusively in females (Tables 3 and 4). Furthermore, in the present study, *ACE* I/D, not *AGTR1* rs5182, was identified as a predictor of the TG/HDL-C ratio (Table 2), which might be attributable to the increased expression levels and increased activity of ACE in DD homozygotes [36, 37]. In female subjects, diabetes mellitus and *ACE* I/D were predictors of TG and TG/HDL-C levels (Table 3), whereas diabetes mellitus and *AGTR1* rs5182 were predictors of TG/HDL-C ratios (Table 4). Thus, the interactions among *ACE* I/D variation or the *AGTR1* rs5182

polymorphism with gender and diabetes mellitus are likely involved in the heterogeneous relationships between TG metabolism and diabetes.

There are limited data from previous studies concerning the interplay of *ACE* I/D and *AGTR1* rs5182, as well as their associations with diabetes mellitus in terms of lipid profiles in Chinese subjects. These results revealed that the combination of *ACE* I/D and *AGTR1* rs5182 contributed to the TG/HDL-C ratio in the whole study population; TG levels in males; and TC, ApoB, LDL-C, ApoA and HDL-C levels in females (Tables 5-6). The association of *ACE* I/D with lipid levels has been inconsistently reported [38, 39]. In addition, a significant correlation was observed between the *AGTR1* rs5186 variant and TG levels, but the mechanism has not yet been fully elucidated [40, 41]. Therefore, the combined effect of *ACE* I/D and *AGTR1* rs5182 on lipid levels in the current study provides valuable insights into the intricate relationships between the RAS and dyslipidaemia.

Previous studies have shown an association between gender and blood lipids. For example, adult females had lower LDL-C levels and higher HDL-C levels compared with adult males [42]. Moreover, the prevalence of TC, TG, and LDL-C at borderline high or greater levels increased with age in females, but it remained stable or even decreased in males [42]. Although diabetes is often accompanied by hyperlipidaemia [2], TG is associated with a decreased risk of diabetes when increased genetic susceptibility is considered [6]. As a potential predictive marker of insulin resistance (IR), the TG/HDL-C ratio is related to diabetes mellitus [5]. However, a previous study reported that no differences in TG/HDL-C were found between obese normal glucose-

330 tolerant individuals and ²patients with type 2 diabetes [43]. Interestingly, the
 331 combination of genetic variations and diabetes mellitus was demonstrated to be a
 332 predictor of TG and ¹³TG/HDL-C only in the female subjects (Table 6). Thus, the gender-
 333 dependent associations with the combination of genetic variations found in the present
 334 study potentially provide an additional explanation for the inconsistent changes in TG
 335 and other indicators related to diabetes mellitus and provide new ideas to target blood
 336 lipids clinically.

337 Strengths and limitations

338 The current study ¹is the first to assess the correlations between the *ACE I/D* variant and
 339 the *AGTR1* rs5182 polymorphism both independently and synergistically with diabetes
 340 mellitus in a Chinese elderly population. A potential limitation ¹⁷of the current study was
 341 that serum ACE levels were not detected. However, the combination of different
 342 genetic variations was taken into consideration. Moreover, analysis of the younger
 343 Chinese population with diabetes is highly recommended in future studies because of
 344 the elevated prevalence of diabetes in elderly subjects [44]. Additionally, given the
 345 diverse effects of different antidiabetic and lipid-lowering drugs on metabolism [45],
 346 the identification of the associations among specific medications, diabetes and genetic
 347 backgrounds in China will be valuable.

348

349 Conclusion

350 The findings of the present study suggest potential interactions among gender, *ACE I/D*,
 351 *AGTR1* rs5182 and diabetes mellitus in terms of lipid and lipid ratios, especially in

352 terms⁹ of TG levels and the TG/HDL-C ratio. This information provides possible
353 explanations for the contradictory associations between diabetes mellitus and lipid
354 metabolism. Furthermore, in elderly Chinese females, TG and TG/HDL-C levels might
355 be more susceptible to¹ the cumulative effect of *ACE* I/D and *AGTR1* rs5182 as well as
356 their combined effect with diabetes. Such an understanding may suggest the
357 development of personalized treatments based on *ACE* and *AGTR1* genetic
358 polymorphisms to lower elevated TG levels in elderly diabetic female patients that have
359 the potential to normalize the dyslipidaemia induced by diabetes mellitus.