Interactions between genetic variants involved in the folate metabolic pathway and serum lipid, homocysteine levels on the risk of recurrent spontaneous abortion

Zhong Lin1†, Qianxi Li2†, Yifan Sun3†, Jingchun Huang4†, Wan Wang4, Jinjian Fu5, Jianhua Xu4,6* and Dingyuan Zeng1*

Abstract

Background: The interaction between folate pathway gene polymorphisms and homocysteine, serum lipid levels are poorly understood in patients with recurrent spontaneous abortion (RSA). The aim of this study is to explore the effects of folate pathway gene polymorphisms (the 5–10-methylenetetrahydrofolate reductase, MTHFR C677T, MTHFR A1298C and the methionine synthase reductase, MTRR A66G) and their interactions with homocysteine on serum lipid levels in patients with RSA.

Methods: A total of 403 RSA women and 342 healthy women were randomly selected. Genotyping of the MTHFR C677T, A1298C and MTRR A66G were performed by TaqMan-MGB technique. Serum homocysteine, folate, fasting glucose, fasting insulin, Interleukin 6, Tumor necrosis factor (TNFα) and lipid profiles were measured according to the kits. Continuous variables were analyzed using 2-sample t-tests. Categorical variables were analyzed and compared by χ² or Fisher’s exact tests. Unconditional logistic regression model was applied to test the interactions of gene polymorphisms on RSA.

Results: The distribution of genotype of CC, CT TT and T allele of MTHFR C677T, genotype of AA and C allele of MTHFR A1298C, and genotype of AA, AG and G allele of MTRR A66G were different between cases and controls (all p were < 0.05). There were significant interactions between MTHFR C677T-A1298C and MTHFR A1298C-MTRR A66G in RSA group and control group, with ORs of 1.62 (95%CI: 1.28–2.04, p < 0.001) and 1.55 (95%CI: 1.27–1.88, p < 0.001), respectively. Serum TNFα level and insulin resistant status (HOMA-IR) were higher in RSA group than in control group (p = 0.038, 0.001, respectively). All the three gene SNPs except MTRR 66AG gene variant had detrimental effects on HOMA-IR (all p were < 0.05). RSA group who carried the MTHFR 677CT, TT, CT/TT genotypes and MTRR 66AG, AG/GG genotypes had detrimental effects on serum homocysteine levels, the MTHFR 677CT, CT/TT genotype carriers had favorable effects on serum folate levels, the MTHFR 677TT, CT/TT, 1298 AC, AC/CC genotype carriers had detrimental effects on serum low-density lipoprotein cholesterol (LDL-C) levels, and the MTRR 66AG genotype carriers had lower high-density lipoprotein cholesterol (HDL-C) levels than the AA genotype carriers (all p were < 0.05).

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Conclusions: Interaction between the MTHFR C677T, A1298C and MTHFR A1298C, MTRR A66G are observed in our RSA group. Besides, all the three gene SNPs except MTRR 66AG gene variant had detrimental effects on HOMA-IR. MTHFR C677T and MTRR A66G gene variants had detrimental effects on serum homocysteine levels and insulin resistance status, while MTHFR C677T, A1298C and MTRR A66G gene variants had detrimental effects on certain serum lipid profiles.

Keywords: MTHFR C677T, MTHFR A1298C, MTRR A66G, Homocysteine, Lipid profiles, Recurrent spontaneous abortion

Background
Recurrent spontaneous abortion (RSA) is a common health problem, defined as the loss of two or more consecutive pregnancies before 20 weeks of gestation which is challenging for both the patients and obstetricians [1]. RSA is a complex multi-factorial disorder and caused very often by genetic disorders, uterine pathologies, endocrine dysfunctions, autoimmune diseases, and environmental factors [1]. Dyslipidemia has been postulated as association with adverse pregnancy outcome, including RSA [2].

Dyslipidemia, as mainly defined by increased serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels, serving as a crucial risk factor for some medical diseases such as cardiovascular diseases, diabetes and insulin resistance, has become a serious public health problem worldwide because of its high prevalence [3–5]. It was reported that the prevalence of dyslipidemia among Chinese adults increases yearly and the prevalence of dyslipidemia was 52.72% among adults in northwestern China in 2010 [6]. The etiology of dyslipidemia is complicated, both genetic and environmental factors as well as their interactions are considered to be the contributors for the cause of dyslipidemia [7, 8].

The 5–10-methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C and methionine synthase reductase (MTRR) A66G gene, may contribute to the risk of the development of hyperhomocysteinemia and are now believed to be good candidate for susceptibility to dyslipidemia and insulin resistance [9, 10]. Numerous epidemiological studies revealed that high homocysteine levels have been suggested to be associated with changing serum lipid levels.

Recent attention has focused on certain gene polymorphism and biomarkers interaction that may predispose to an increased risk of severe pregnancy complications, including RSA [11]. Only recently genetic analyses of affected patients was it discovered that C677T, A1298C polymorphisms of MTHFR and A66G of MTRR may represent the important candidates for exploration of the risk of developing disease as their key roles for not only in gene expression but also in modifications of serum lipid and homocysteine concentrations [12].

Few studies so far have investigated the effect of homocysteine, insulin resistance, TNFα and lipid levels and the MTHFR, MTRR gene polymorphisms on RSA risk. Mtiraoui et al. [13] have demonstrated that MTHFR gene polymorphisms were associated with progression of recurrent miscarriage through elevations of plasma homocysteine levels. Ikruthi et al. [14] have revealed that hyperhomocysteinemia was associated with hyperlipoproteinemia. Li et al. [9] identified that MTHFR C677T, A1298C and MTRR A66G gene polymorphisms combined with low folate were the major determinant of plasma lipid levels.

In summary, elevated plasma levels of homocysteine may cause RSA and dysregulation of cholesterol and triglyceride biosynthetic pathways, with changed expression by DNA methylation. As a consequence, we hypothesize that the MTHFR and MTRR gene polymorphisms associated with higher levels of homocysteine may be related with different serum lipid levels in the RSA populations. The aim of this study is to explore the interactions of these three gene polymorphisms (MTHFR C677T, MTHFR A1298C and MTRR A66G), homocysteine and serum lipid profiles with RSA in Chinese population.

Methods
Study population
This investigation was carried out as a case–control study conducted between January 1, 2013 and November 12, 2015, in the Gynecology clinic of Liuzhou Maternity and Child Healthcare Hospital. A total of 403 women who had 2 or more consecutive spontaneous abortions were diagnosed as RSA and recruited as case group. Control group consisted of 342 healthy women of reproductive age with at least 1 delivery and no history of abortion. Women who had chromosomal abnormalities, personal or family history of thrombosis, induced abortions, infection or systemic diseases were excluded from this study. A questionnaire detailing age, ethnic, education level, gynecological history, smoking, drinking, X-ray contact, chemical exposure, folate supplement, multivitamin supplement were asked to fill and consent form indicating their acceptance to participate were signed and obtained. This study was approved by the
Institutional Review Board at Liuzhou Maternity and Child Healthcare Hospital.

Laboratory tests
EDTA-anticoagulated blood (5 ml sample) and buccal cell samples were obtained from participants and was processed within 30 min of collection for biochemical analysis and genetic analysis, respectively. The levels of triglyceride (TG), TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, total protein, homocysteine and fast glucose in blood samples were measured by enzymatic method on a Hitachi Autoanalyzer (Type 7600; Hitachi Ltd., Tokyo, Japan). The levels of folate, vitamin B12 and fast insulin in blood samples were measured by chemi-luminescence method on an Abbott Autoanalyzer (Type i4000SR; Abbott Ltd., America). The levels of IL6 and TNFα were measured by liquid suspension chip on luminex200 (Austin, Texas, America).

Genotyping
Genomic DNA was extracted from buccal samples using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). The TaqMan-MGB technique was used for detecting gene polymorphisms of the MTHFR C677T, A1298C and MTRR A66G. The primers and probes were showed in Table 1. Universal reaction conditions in a final volume of 10 μl for each genotyping are as follows: 1 μl of 20 ng/μl DNA, 5 μl of 2 × Taqman Universal Master Mix, 0.5 μl of 20 × TaqMan-MGB assay locus-specific probe, with 3.5 μl of sterile water. All PCR reagents were purchased from ABI Company. The PCR cycling conditions were 1 cycle of 95 °C for 10 min; then 20 cycles of 96 °C for 15 s, 60 °C for 60 s; then 30 cycles of 89 °C for 15 s, 60 °C for 60 s. After PCR amplification, an endpoint plate read was performed using an Applied Biosystems Real-Time PCR System. The Sequence Detection System (SDS) Software uses the fluorescence (Rn) values based on the signals from each measurements made during the plate read to plot the fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicate the alleles that are present in each sample. All cycling protocols were performed on a ABI 7900.

Statistical analysis
SAS version 9.4 (Cary, NC, USA) was used to perform the statistical analysis. Continuous variables were analyzed using 2-sample t-tests. Categorical variables were analyzed and compared by χ² or Fisher’s exact tests. For the main effect of gene-gene variants interactions, unconditional logistic regression was conducted to calculate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). A p-value less than 0.05 was considered indicative of statistical significance.

Results
General characteristics, serum lipid levels and allelic frequencies
Table 2 examined the characteristics, homocysteine, serum lipid levels and allelic frequencies of MTHFR C677T, A1298C and MTRR A66G between the RSA group and healthy group. A χ² analysis has found that folate supplement was higher in control group than in case group (p = 0.048). HOMA-IR index was higher in the RSA group than in the control group (p = 0.018). The levels of homocysteine, serum total protein, LDL-C and TNFα were higher in RSA group than in control group (all p < 0.05), whereas the level of HDL-C was lower in RSA group than in control group (p = 0.018).

The frequency of MTHFR C677T, A1298C and MTRR A66G alleles and genotypes are shown in Table 2. The frequencies of CC, CT and TT genotypes and ‘T’ allele of C677T were 0.529, 0.380, 0.092 and 0.282 in cases, compared with 0.740, 0.228, 0.032 and 0.146 in controls, respectively (p < 0.001–0.0001). The distribution of genotype of AA and ‘C’ allele of MTHFR A1298C were slightly different between cases and controls (p = 0.042 and 0.046, respectively). The distribution of genotype of AA, AG and G allele of MTRR A66G were different between cases and controls (p = 0.004, 0.0031 and 0.003, respectively).

The two-factor gene-gene interaction analyses by logistic regression analysis revealed significant interactions between MTHFR C677T-A1298C and MTHFR A1298C-MTRR A66G in RSA group and control group, with ORs of 1.62 (95% CI: 1.28–2.04, p < 0.001) and 1.55 (95% CI, 1.27–1.88, p < 0.001), respectively (Table 3).

MTHFR C677T genotypes and serum homocysteine, inflammatory factor and lipid levels
Table 4 shows the interaction of MTHFR C677T gene polymorphism with RSA risk on serum homocysteine, inflammatory factor and lipid levels. All the three gene

| SNPs          | Primers Forward | Primers Reverse | Probes Forward | Probes Reverse |
|---------------|-----------------|-----------------|---------------|---------------|
| MTHFR C677T   | GAAAAGCTGCCTGATGATG | TGGAGGAAAGAAGTGC | AATCG [G]CTCCCGC | AATCG [A]CTCCCGC |
| MTHFR A1298C  | AAGAAGGAAAGACCTC | TGGGGGGAGAGGTGAG | ACACTT [G]CTCCTG | ACACTT [T]CTCCTG |
| MTRR A66G     | AGGCAAGGCGCATCGCA | ATCCATGTACACAGCCTT | AAGAAT [A]TTGAG | AAGAAT [G]TGTGAG |
| Variable                              | Case (n = 403) | Control (n = 342) | $t$/$\chi^2$ | $p$ |
|--------------------------------------|----------------|------------------|--------------|-----|
| Ethnic                               |                |                  |              |     |
| Han                                  | 198 (49.1)     | 160 (46.8)       | 0.43         | 0.805 |
| Zhuang                               | 178 (44.2)     | 159 (46.5)       |              |     |
| Minority                             | 27 (6.7)       | 23 (6.7)         |              |     |
| Education level                      |                |                  | 1.20         | 0.550 |
| ≤ 9 years of school                  | 157 (39.0)     | 120 (35.1)       |              |     |
| 10–12 years of school                | 83 (20.6)      | 74 (21.6)        |              |     |
| ≥ 13 years of school                 | 163 (40.4)     | 148 (43.3)       |              |     |
| Gynecological surgery history        |                |                  | 1.09         | 0.298 |
| Yes                                  | 42 (10.4)      | 28 (8.2)         |              |     |
| No                                   | 361 (91.8)     | 314 (91.8)       |              |     |
| Current smoking                      |                |                  | 0.10         | 0.756 |
| Yes                                  | 7 (1.7)        | 7 (2.0)          |              |     |
| No                                   | 396 (98.3)     | 335 (98.0)       |              |     |
| Passive smoking history              |                |                  | 0.003        | 0.960 |
| Yes                                  | 89 (22.1)      | 75 (21.9)        |              |     |
| No                                   | 314 (77.9)     | 267 (78.1)       |              |     |
| Drinking history                     |                |                  | 0.04         | 0.847 |
| Yes                                  | 43 (10.7)      | 38 (11.1)        |              |     |
| No                                   | 360 (90.3)     | 304 (88.9)       |              |     |
| X-ray contact history                |                |                  | 0.52         | 0.596 |
| Yes                                  | 1 (0.2)        | 2 (0.6)          |              |     |
| No                                   | 402 (99.8)     | 340 (99.4)       |              |     |
| Chemical exposure history            |                |                  | 3.41         | 0.065 |
| Yes                                  | 1 (0.2)        | 5 (1.5)          |              |     |
| No                                   | 402 (99.8)     | 337 (98.5)       |              |     |
| Folic acid supplement                |                |                  | 3.91         | 0.048 |
| Yes                                  | 131 (32.5)     | 135 (39.5)       |              |     |
| No                                   | 272 (67.5)     | 207 (60.5)       |              |     |
| Multivitamin supplement              |                |                  | 1.38         | 0.240 |
| Yes                                  | 17 (4.2)       | 9 (2.6)          |              |     |
| No                                   | 386 (95.8)     | 333 (97.4)       |              |     |
| Age, year                            | 29.58 ± 5.48   | 29.88 ± 5.28     | 0.85         | 0.673 |
| Folic acid, nmol/L                   | 32.45 ± 8.86   | 34.35 ± 18.98    | -1.79        | 0.072 |
| Vitamin B12, pg/ml                   | 352.70 ± 124.01| 347.48 ± 124.10 | 0.57         | 0.567 |
| Homocysteine, umol/L                 | 11.89 ± 4.62   | 11.20 ± 3.40     | 2.36         | 0.018 |
| Total protein, g/L                   | 72.17 ± 6.79   | 70.67 ± 9.47     | 2.52         | 0.012 |
| Total cholesterol, mmol/L            | 5.43 ± 20.10   | 4.42 ± 1.91      | 0.92         | 0.359 |
| Triglyceride, mmol/L                 | 1.06 ± 0.69    | 1.03 ± 0.55      | 0.62         | 0.536 |
| High-density lipoprotein cholesterol, mmol/L | 1.66 ± 0.38 | 1.73 ± 0.38 | -2.38 | 0.018 |
| Low-density lipoprotein cholesterol, mmol/L | 2.61 ± 0.77 | 2.41 ± 0.68 | 3.62 | < 0.001 |
| Fasting glucose, mmol/L              | 4.93 ± 0.38    | 4.92 ± 0.19      | 0.15         | 0.884 |
| Fasting insulin, pmol/L              | 71.13 ± 14.82  | 67.60 ± 13.84    | 3.35         | 0.001 |
| HOMR-IR                              | 2.23 ± 0.45    | 2.12 ± 0.41      | 3.48         | 0.001 |
variants had detrimental effects on HOMA-IR (all $p$ were < 0.05). The CT genotype carriers had higher serum homocysteine levels and lower folate levels in the RSA group than that in the control group ($p < 0.001$ and 0.047, respectively). For those RSA group who carried CT/TT genotype, they had higher serum homocysteine and LDL-C levels and lower folate levels than that in the control group ($p = 0.003$, 0.018 and 0.012, respectively).

**Discussion**

We demonstrated that patients carrying the MTHFR 677CT, TT and MTRR 66AG genotypes, as well as

Table 2 General characteristics and genotype distribution (Continued)

| Variable        | Case (n = 403) | Control (n = 342) | $t$/$\chi^2$ | $p$   |
|-----------------|----------------|-------------------|--------------|-------|
| IL6, pg/ml      | 75.12 ± 311.20 | 40.58 ± 202.95    | 1.82         | 0.069 |
| TNFα, pg/ml     | 28.40 ± 92.16  | 18.28 ± 31.02     | 2.08         | 0.038 |
| MTHFR C677T     |                |                   |              |       |
| CC              | 213 (52.9)     | 253 (74.0)        | 35.24        | < 0.001|
| CT              | 153 (38.0)     | 78 (22.8)         | 19.87        | < 0.001|
| TT              | 37 (9.2)       | 11 (3.2)          | 10.92        | 0.001 |
| C allele        | 579 (71.8)     | 584 (85.4)        | –            | –     |
| T allele        | 227 (28.2)     | 100 (14.6)        | 39.62        | < 0.001|
| MTHFR A1298C    |                |                   |              |       |
| AA              | 231 (57.3)     | 221 (64.6)        | 4.13         | 0.042 |
| AC              | 144 (35.7)     | 102 (29.8)        | 2.92         | 0.088 |
| CC              | 28 (6.9)       | 19 (5.6)          | 0.61         | 0.436 |
| A allele        | 606 (75.2)     | 544 (79.5)        | –            | –     |
| C allele        | 200 (24.8)     | 140 (20.5)        | 3.97         | 0.046 |
| MTRR A66G       |                |                   |              |       |
| AA              | 225 (55.8)     | 226 (66.1)        | 8.14         | 0.004 |
| AG              | 148 (36.7)     | 100 (29.2)        | 4.67         | 0.031 |
| GG              | 30 (7.4)       | 16 (4.7)          | 2.44         | 0.118 |
| A allele        | 598 (74.2)     | 552 (80.7)        | –            | –     |
| G allele        | 208 (25.8)     | 132 (19.3)        | 8.00         | 0.003 |

MTHFR A66G genotypes and serum homocysteine, lipid levels

Table 6 shows the interaction of MTRR A66G gene polymorphism with RSA risk on serum homocysteine and lipid levels. The AA genotype carriers had higher HOMA-IR, total protein and LDL-C levels in the RSA group than that in the control group ($p = 0.011$, 0.008 and < 0.001, respectively). The RSA group who carrying AG genotype had higher serum homocysteine levels and lower serum HDL-C levels than that in the control group ($p = 0.047$ and 0.010, respectively). For RSA patients who carried AG/GG genotype, they had higher HOMA-IR, serum homocysteine levels than that in the control group ($p = 0.020$ and 0.030, respectively).

**Discussion**

We demonstrated that patients carrying the MTHFR 677CT, TT and MTRR 66AG genotypes, as well as

Table 3 Interactions between genetic variants in the folate pathway on the risk of recurrent spontaneous abortion

| Gen-gen interactions | B   | SE  | Wald | $p$   | OR   | 95%CI          |
|----------------------|-----|-----|------|-------|------|---------------|
| C677T-A1298C         | 0.48| 0.12| 16.23| 0.000 | 1.62 | 1.28–2.04     |
| C677T-A66G           | 0.02| 0.08| 0.07 | 0.799 | 1.02 | 0.87–1.20     |
| A1298C-A66G          | 0.44| 0.10| 18.84| 0.000 | 1.55 | 1.27–1.88     |
Table 4 Interaction of MTHFR C677T polymorphism with recurrent spontaneous abortion on serum folate and lipid levels

| Variable                                      | Case          | control        | t   | p    |
|-----------------------------------------------|---------------|----------------|-----|------|
| **CC genotype**                              | n = 213       | n = 253        |     |      |
| Folic acid, nmol/L                           | 36.49 ± 6.47  | 35.29 ± 21.53  | 0.78| 0.434|
| Vitamin B12, pg/ml                           | 347.23 ± 114.82| 346.84 ± 124.51| 0.03| 0.976|
| Homocysteine, umol/L                         | 10.33 ± 4.34  | 10.83 ± 3.38   | −1.39| 0.165|
| Total protein, g/L                           | 72.87 ± 4.78  | 70.69 ± 9.99   | 2.93| 0.004|
| Total cholesterol, mmol/L                    | 4.48 ± 1.09   | 4.51 ± 2.13    | −0.16| 0.873|
| Triglyceride, mmol/L                         | 1.06 ± 0.81   | 1.03 ± 0.57    | 0.68| 0.494|
| High-density lipoprotein cholesterol, mmol/L | 1.68 ± 0.42   | 1.74 ± 0.36    | −1.81| 0.071|
| Low-density lipoprotein cholesterol, mmol/L  | 2.65 ± 0.84   | 2.16 ± 0.60    | 1.78| 0.082|
| Fasting glucose, mmol/L                      | 4.93 ± 0.38   | 4.92 ± 0.29    | 0.26| 0.798|
| Fasting insulin, pmol/L                      | 70.64 ± 14.43 | 67.90 ± 14.03  | 2.08| 0.038|
| HOMR-IR                                      | 2.21 ± 0.45   | 2.12 ± 0.42    | 2.12| 0.035|
| IL6, pg/ml                                   | 81.84 ± 339.77| 33.09 ± 215.37| 1.89| 0.058|
| TNFa, pg/ml                                  | 29.11 ± 88.72 | 18.86 ± 30.27  | 1.75| 0.081|
| **CT genotype**                              | n = 153       | n = 78         |     |      |
| Folic acid, nmol/L                           | 32.73 ± 7.85  | 38.43 ± 9.29   | −3.55| 0.000|
| Vitamin B12, pg/ml                           | 359.98 ± 131.88| 353.94 ± 115.88| 0.33| 0.745|
| Homocysteine, umol/L                         | 12.65 ± 3.97  | 11.71 ± 3.06   | 1.99| 0.047|
| Total protein, g/L                           | 71.68 ± 8.05  | 70.61 ± 8.01   | 0.92| 0.361|
| Total cholesterol, mmol/L                    | 6.99 ± 32.56  | 4.17 ± 0.81    | 0.72| 0.475|
| Triglyceride, mmol/L                         | 1.02 ± 0.52   | 0.95 ± 0.37    | 0.97| 0.332|
| High-density lipoprotein cholesterol, mmol/L  | 1.67 ± 0.33   | 1.72 ± 0.48    | −0.92| 0.358|
| Low-density lipoprotein cholesterol, mmol/L  | 2.59 ± 0.78   | 2.39 ± 0.74    | 1.82| 0.070|
| Fasting glucose, mmol/L                      | 4.95 ± 0.37   | 4.88 ± 0.39    | 0.62| 0.532|
| Fasting insulin, pmol/L                      | 72.28 ± 15.42 | 66.89 ± 12.57  | 2.86| 0.005|
| HOMR-IR                                      | 2.27 ± 0.47   | 2.11 ± 0.36    | 2.83| 0.005|
| IL6, pg/ml                                   | 72.60 ± 275.52| 41.08 ± 147.43| 1.09| 0.276|
| TNFa, pg/ml                                  | 29.34 ± 106.30| 16.52 ± 34.81  | 1.25| 0.210|
| **TT genotype**                              | n = 37        | n = 11         |     |      |
| Folic acid, nmol/L                           | 25.72 ± 7.67  | 24.54 ± 7.84   | 0.45| 0.658|
| Vitamin B12, pg/ml                           | 354.14 ± 142.24| 322.82 ± 167.57| 0.62| 0.541|
| Homocysteine, umol/L                         | 17.80 ± 2.86  | 15.93 ± 2.08   | 2.39| 0.026|
| Total protein, g/L                           | 70.17 ± 9.96  | 70.42 ± 3.79   | −0.08| 0.933|
| Total cholesterol, mmol/L                    | 4.34 ± 1.01   | 3.96 ± 0.80    | 1.13| 0.265|
| Triglyceride, mmol/L                         | 1.11 ± 0.57   | 1.49 ± 0.73    | −1.85| 0.071|
| High-density lipoprotein cholesterol, mmol/L  | 1.58 ± 0.33   | 1.62 ± 0.26    | −0.31| 0.761|
| Low-density lipoprotein cholesterol, mmol/L  | 2.61 ± 0.76   | 2.43 ± 0.67    | 2.78| 0.006|
| Fasting glucose, mmol/L                      | 4.95 ± 0.43   | 4.79 ± 0.36    | 1.38| 0.175|
| Fasting insulin, pmol/L                      | 70.52 ± 16.13 | 67.86 ± 17.26  | 0.55| 0.583|
| HOMR-IR                                      | 2.21 ± 0.45   | 2.07 ± 0.54    | 0.96| 0.341|
| IL6, pg/ml                                   | 14.94 ± 43.01 | 104.44 ± 273.61| −1.58| 0.126|
| TNFa, pg/ml                                  | 15.72 ± 29.57 | 20.42 ± 19.78  | −0.65| 0.521|
| **CT/TT genotype**                           | n = 190       | n = 89         |     |      |
| Folic acid, nmol/L                           | 31.31 ± 8.99  | 35.11 ± 21.36  | −2.96| 0.003|
MTHFR C677T, MTHFR A1298C and MTRR A66G alleles had a significantly higher risk of experiencing RSA. In the current study, interaction between the MTHFR C677T and A1298C polymorphism, and interaction between the MTHFR A1298C and the MTRR A66G polymorphism were associated with increased RSA risk. All the three gene SNPs except MTRR 66AG gene variant had detrimental effects on HOMA-IR. We found that compared with control group, RSA group who carried the MTHFR 677CT, TT, CT/TT genotypes and MTRR 66AG, AG/GG genotypes had favorable effects on serum folate and the MTHFR 677TT, CT/TT, 1298 AC, AC/CC genotype carriers had detrimental effects on serum LDL-C levels, the MTRR 66AG genotype carriers had lower HDL-C levels than the AA genotype carriers.

In our main effect analysis, the MTHFR C677T and MTRR A66G were the two SNPs exhibited a statistically significant association with increased recurrent spontaneous abortion risk. Besides support from biologically functional evidence, elevated plasma of homocysteine has been proven to damage the vascular endothelium and involve in placental vascular risk and endothelial dysfunction, thus lead to RSA [19].

The association between recurrent spontaneous abortion and insulin resistance is in argue. It was reported that increased inflammatory cytokine levels such as TNFα and plasma hyperhomocysteinemia were associated with insulin resistance and endocrine abnormalities [20, 21]. Insulin resistance may have positive association with an increase of plasma hyperhomocysteinemia which may damage pregnancy by interfering with endometrial blood flow and vascular integrity leads to increase the risk of early pregnancy abortion [21].

In Mexico general populations [22] it is observed that people who carried 677 T allele may need more folate intake than those carried the C allele. Our results revealed that the MTHFR 677CT, CT/TT genotype carriers had favorable effects on serum folate, which was in accordance with the previous study demonstrated that folate deficiency related with hyperhomocysteinemia was the risk associated with recurrent abortion [23].

Besides the modest main effect of MTHFR C677T, we also observed significant effect of gene-gene interactions, which were able to amplify the modest effect of the single genetic variant, and enhance the predictive power. Individual patients with the combination of MTHFR 677T and MTHFR 1298C had a significantly higher risk for RSA than those with the combination of MTHFR 677C and MTHFR 1298A (OR = 1.62, 95% CI: 1.28–2.04,  \( p = 0.004 \)). Logistic regression analysis showed that certain gene-gene interactions among MTHFR 1298C and MTRR 66G predict a higher risk for RSA (OR = 2.36, 95% CI: 1.22–5.297,  \( p = 0.005 \)) compared to those with the combination of MTHFR 1298A and MTRR66A.

### Table 4 Interaction of MTHFR C677T polymorphism with recurrent spontaneous abortion on serum folate and lipid levels (Continued)

| Variable                        | Case          | control       | t   | p    |
|---------------------------------|---------------|---------------|-----|------|
| Vitamin B12, pg/ml              | 358.84 ± 133.59 | 349.61 ± 123.50 | 0.53 | 0.598|
| Homocysteine, umol/L            | 12.37 ± 4.57  | 11.52 ± 3.65  | 2.52 | 0.012|
| Total protein, g/L              | 71.39 ± 8.45  | 70.59 ± 7.55  | 0.73 | 0.465|
| Total cholesterol, mmol/L       | 6.48 ± 29.22  | 4.14 ± 0.81   | 0.71 | 0.478|
| Triglyceride, mmol/L            | 1.04 ± 0.53   | 1.03 ± 0.47   | 0.14 | 0.887|
| High-density lipoprotein cholesterol, mmol/L | 1.66 ± 0.34  | 1.71 ± 0.45   | −1.08| 0.281|
| Low-density lipoprotein cholesterol, mmol/L | 2.61 ± 0.79  | 2.36 ± 0.72   | 2.38 | 0.018|
| Fasting glucose, mmol/L         | 4.95 ± 0.38   | 4.94 ± 0.39   | 0.15 | 0.158|
| Fasting insulin, pmol/L         | 71.99 ± 15.50 | 67.08 ± 13.55 | 2.78 | 0.006|
| HOMA-IR                         | 2.26 ± 0.47   | 2.11 ± 0.40   | 2.96 | 0.003|
| IL6, pg/ml                      | 63.25 ± 253.50| 53.54 ± 119.70 | 0.37 | 0.714|
| TNFα, pg/ml                     | 15.72 ± 29.57 | 20.42 ± 19.78 | −0.65| 0.521|

**HOMA-IR** Homeostatic model assessment of insulin resistance, **IL6** Interleukin 6, **TNFα** Tumor necrosis factor α.
| Variable                                                                 | Case          | control        | t     | p    |
|-------------------------------------------------------------------------|---------------|----------------|-------|------|
| **AA genotype**                                                         | n = 231       | n = 221        |       |      |
| Folic acid, nmol/L                                                      | 31.28 ± 9.77  | 33.89 ± 23.08  | −1.58 | 0.115|
| Vitamin B12, pg/ml                                                      | 352.21 ± 125.06 | 347.58 ± 124.71 | 0.39  | 0.694|
| Homocysteine, umol/L                                                    | 11.93 ± 4.63  | 11.27 ± 2.75   | 1.81  | 0.071|
| Total protein, g/L                                                      | 71.91 ± 7.99  | 70.26 ± 10.93  | 1.83  | 0.067|
| Total cholesterol, mmol/L                                              | 4.32 ± 0.84   | 4.47 ± 2.08    | −1.05 | 0.295|
| Triglyceride, mmol/L                                                    | 1.07 ± 0.77   | 1.06 ± 0.59    | 0.13  | 0.894|
| High-density lipoprotein cholesterol, mmol/L                            | 1.67 ± 0.39   | 1.76 ± 0.40    | −2.51 | 0.012|
| Low-density lipoprotein cholesterol, mmol/L                             | 2.56 ± 0.74   | 2.45 ± 0.71    | 1.65  | 0.101|
| Fasting glucose, mmol/L                                                | 4.92 ± 0.37   | 4.92 ± 0.38    | 0.02  | 0.987|
| Fasting insulin, pmol/L                                                | 72.88 ± 14.93 | 67.83 ± 14.42  | 3.55  | <0.001|
| HOMR-IR                                                                | 2.28 ± 0.46   | 2.12 ± 0.43    | 3.74  | <0.001|
| IL6, pg/ml                                                              | 58.24 ± 239.93| 36.23 ± 138.03 | 1.19  | 0.235|
| TNFα, pg/ml                                                             | 29.47 ± 108.57| 19.73 ± 35.54  | 1.29  | 0.198|
| **AC genotype**                                                         | n = 144       | n = 102        |       |      |
| Folic acid, nmol/L                                                      | 34.18 ± 6.80  | 35.24 ± 6.50   | −1.23 | 0.218|
| Vitamin B12, pg/ml                                                      | 354.39 ± 129.77| 350.37 ± 116.89| 0.25  | 0.804|
| Homocysteine, umol/L                                                    | 11.85 ± 4.63  | 11.06 ± 4.35   | 1.49  | 0.137|
| Total protein, g/L                                                      | 72.69 ± 4.52  | 71.63 ± 6.12   | 1.52  | 0.129|
| Total cholesterol, mmol/L                                              | 7.41 ± 33.55  | 4.21 ± 1.23    | 0.96  | 0.337|
| Triglyceride, mmol/L                                                    | 1.07 ± 0.59   | 0.96 ± 0.44    | 1.54  | 0.126|
| High-density lipoprotein cholesterol, mmol/L                            | 1.65 ± 0.36   | 1.70 ± 0.35    | −1.15 | 0.252|
| Low-density lipoprotein cholesterol, mmol/L                             | 2.73 ± 0.83   | 2.34 ± 0.65    | 3.88  | 0.000|
| Fasting glucose, mmol/L                                                | 4.95 ± 0.39   | 4.94 ± 0.42    | 0.16  | 0.873|
| Fasting insulin, pmol/L                                                | 68.94 ± 14.64 | 67.46 ± 13.49  | 0.03  | 0.409|
| HOMR-IR                                                                | 2.16 ± 0.43   | 2.11 ± 0.40    | 0.89  | 0.372|
| IL6, pg/ml                                                              | 109.82 ± 422.09| 51.87 ± 305.40 | 1.26  | 0.208|
| TNFα, pg/ml                                                             | 28.91 ± 69.92 | 15.59 ± 21.63  | 2.16  | 0.032|
| **CC genotype**                                                         | n = 28        | n = 19         |       |      |
| Folic acid, nmol/L                                                      | 33.18 ± 9.09  | 34.99 ± 8.43   | −0.69 | 0.494|
| Vitamin B12, pg/ml                                                      | 348.11 ± 80.49| 330.79 ± 157.10| 0.50  | 0.622|
| Homocysteine, umol/L                                                    | 12.43 ± 5.99  | 11.84 ± 4.58   | 0.36  | 0.717|
| Total protein, g/L                                                      | 71.67 ± 4.22  | 70.19 ± 4.62   | 1.14  | 0.262|
| Total cholesterol, mmol/L                                              | 4.27 ± 0.74   | 4.93 ± 2.66    | −1.23 | 0.226|
| Triglyceride, mmol/L                                                    | 0.89 ± 0.37   | 1.00 ± 0.49    | −0.91 | 0.366|
| High-density lipoprotein cholesterol, mmol/L                            | 1.78 ± 0.42   | 1.62 ± 0.32    | 1.44  | 0.156|
| Low-density lipoprotein cholesterol, mmol/L                             | 2.41 ± 0.67   | 2.38 ± 0.53    | 0.16  | 0.876|
| Fasting glucose, mmol/L                                                | 4.93 ± 0.38   | 5.00 ± 0.35    | −0.81 | 0.420|
| Fasting insulin, pmol/L                                                | 68.09 ± 13.33 | 66.68 ± 11.63  | 0.45  | 0.657|
| HOMR-IR                                                                | 2.14 ± 0.46   | 2.12 ± 0.36    | 0.24  | 0.809|
| IL6, pg/ml                                                              | 37.96 ± 81.36 | 30.61 ± 91.73  | 0.34  | 0.735|
| TNFα, pg/ml                                                             | 17.62 ± 22.33 | 18.33 ± 27.97  | −0.11 | 0.910|
| **AC /CC genotype**                                                     | n = 172       | n = 121        |       |      |
| Folic acid, nmol/L                                                      | 34.02 ± 7.20  | 35.20 ± 6.80   | −1.42 | 0.155|
Our results were consistent with the previous studies which reported that the folate pathway gene variants and gene-gene interactions could significantly impact the occurrence of RSA [18, 24, 25]. Several studies have reported the association between the MTHFR C677T polymorphism, high homocysteine and serum lipid profiles in humans, with some indicating that the T allele was associated with unfavorable lipid profiles [26–28]. One study indicated the positive relationship between the MTHFR C677T polymorphism and the lipoprotein level in unexplained recurrent miscarriages [29]. We found that the MTHFR 677TT, CT/TT genotypes and MTHFR 1298 AC, AC/CC genotypes had detrimental effects on serum LDL-C levels and the MTRR 66GG genotype had favorable effects on serum HDL-C levels in RSA group. Our study was consistent with the study conducted by Frelut et al. who reported that MTHFR C677T gene variant was significantly increased LDL-C level [30]. Recently, Westerbuck et al. reported that sterol regulatory element binding proteins (SREBPs) can be activated by endoplasmic reticulum stress which induced by homocysteine [31]. This SREBPs was crucial for the genes responsible for cholesterol biosynthesis, uptake and intracellular accumulation. Besides support from biologically functional evidence, MTHFR-deficient mice presented hyperhomocysteinemia in mice fed control or folate-deficient diets [32]. Moreover, homocysteine was reported inversely correlated with HDL-C [9].

Publications about the influence of MTHFR A1298C mutant on serum lipid metabolic profiles were relatively rare. Chang et al. [33] found no significant associations exist between lipid profiles and MTHFR A1298C gene variants. Li et al. [9] demonstrated that MTHFR C677T and A1298C with low folate showed higher risk of low levels of high-density lipoprotein cholesterol (<p for trend: 0.008 and 0.031). Unlike the previous studies, our data showed that MTHFR A1298C mutant was associated with higher level of LDL-C in RSA group than the healthy controls. Based on the positive association between MTHFR C677T, A1298C and serum homocysteine level [9, 24–26], and the favorable effect of homocysteine level on lipid metabolism [9], we speculate that MTHFR C677T and A1298C polymorphism and high homocysteine level interactively increased the prevalence of dyslipidemia in RSA patients.

MTRR is responsible for homocysteine remethylation. The MTRR A66G polymorphism results in its enzyme expression and affecting plasma homocysteine levels [34]. Homocysteine levels further affects serum lipid profiles [9, 34]. Many previous studies have explored the relationship between the MTRR gene polymorphisms and serum or plasma lipid profiles in humans, but with no consistent results [34–37]. For example, Misiak et al. [35] found there was no significant association between MTRR 66GG and TG or HDL-C levels in schizophrenia patients and healthy controls. But Jiang et al. [34] revealed that hypertensive patients who carried the MTRR 66GG genotype had lower serum TC and LDL-C levels than patients carried MTRR 66AA genotype. Zhi et al. [37] revealed that MTRR 66GG genotype was associated with increased risk of high TG (TG ≥1.7 mmol/L), while no significant association was found between this polymorphism and low HDL-C levels. Our data revealed that the MTRR 66AG genotype carriers had lower HDL-C levels than the AA genotype carriers, which was consistent with the previous studies reported that MTRR gene variants can affect the lipid metabolisms via plasma homocysteine levels [34–37].

### Table 5 Interaction of MTHFR A1298C polymorphism with recurrent spontaneous abortion on serum folate and lipid levels

| Variable                      | Case         | Control       | t    | p       |
|-------------------------------|--------------|---------------|------|---------|
| Vitamin B12, pg/ml            | 353.37 ± 122.93 | 347.30 ± 23.50 | 0.42 | 0.678   |
| Homocysteine, umol/L          | 11.86 ± 4.63   | 11.06 ± 4.35   | 1.49 | 0.137   |
| Total protein, g/L            | 72.52 ± 4.73   | 71.40 ± 5.92   | 1.80 | 0.073   |
| Total cholesterol, mmol/L     | 6.92 ± 30.79   | 4.32 ± 1.55    | 0.93 | 0.356   |
| Triglyceride, mmol/L          | 1.04 ± 0.57    | 0.96 ± 0.45    | 1.14 | 0.253   |
| High-density lipoprotein cholesterol, mmol/L | 1.67 ± 0.37 | 1.68 ± 0.35 | −0.42 | 0.672 |
| Low-density lipoprotein cholesterol, mmol/L | 2.67 ± 0.81 | 2.35 ± 0.63 | 3.69 | 0.000 |
| Fasting glucose, mmol/L       | 4.94 ± 0.39    | 4.95 ± 0.40    | −0.22 | 0.826   |
| Fasting insulin, pmol/L       | 68.79 ± 14.39  | 67.27 ± 13.02  | 0.98 | 0.325   |
| HOMR-IR                       | 2.16 ± 0.43    | 2.11 ± 0.39    | 0.93 | 0.351   |
| IL6, pg/ml                    | 97.57 ± 386.58 | 46.67 ± 269.04 | 1.37 | 0.170   |
| TNFa, pg/ml                   | 269.8 ± 644.3 | 16.26 ± 23.26  | 2.04 | 0.042   |

HOMA-IR: Homeostatic model assessment of insulin resistance, IL6: Interleukin 6, TNFa: Tumor necrosis factor α.
Table 6 Interaction of MTRR A66G polymorphism with recurrent spontaneous abortion on serum folate and lipid levels

| Variable                                      | Case            | control         | t    | p    |
|-----------------------------------------------|-----------------|-----------------|------|------|
| **AA genotype**                               |                 |                 |      |      |
| Folic acid, nmol/L                           | 33.20 ± 9.06    | 33.58 ± 8.42    | −0.57| 0.572|
| Vitamin B12, pg/ml                           | 362.49 ± 134.76 | 346.12 ± 126.65 | 1.33 | 0.185|
| Homocysteine, umol/L                         | 11.38 ± 4.59    | 11.05 ± 3.49    | 0.86 | 0.393|
| Total protein, g/L                           | 72.83 ± 5.21    | 71.08 ± 8.41    | 2.64 | 0.008|
| Total cholesterol, mmol/L                    | 6.27 ± 26.85    | 4.48 ± 2.25     | 0.99 | 0.320|
| Triglyceride, mmol/L                         | 1.17 ± 0.55     | 1.05 ± 0.56     | 1.75 | 0.081|
| High-density lipoprotein cholesterol, mmol/L | 1.68 ± 0.41     | 1.72 ± 0.39     | −1.15| 0.251|
| Low-density lipoprotein cholesterol, mmol/L  | 2.67 ± 0.79     | 2.39 ± 0.72     | 3.97 | 0.000|
| Fasting glucose, mmol/L                      | 4.94 ± 0.39     | 4.92 ± 0.41     | 0.24 | 0.820|
| Fasting insulin, pmol/L                      | 71.08 ± 15.23   | 67.63 ± 14.54   | 2.20 | 0.028|
| HOMR-IR                                      | 2.23 ± 0.47     | 2.11 ± 0.42     | 2.56 | 0.011|
| IL6, pg/ml                                    | 67.27 ± 259.61  | 45.69 ± 255.26  | 0.79 | 0.426|
| TNFα, pg/ml                                   | 26.26 ± 85.51   | 16.97 ± 22.95   | 1.47 | 0.143|
| **AG genotype**                               |                 |                 |      |      |
| Folic acid, nmol/L                           | 31.89 ± 9.38    | 33.84 ± 7.62    | −1.86| 0.064|
| Vitamin B12, pg/ml                           | 335.09 ± 107.21 | 340.27 ± 117.81 | −0.36| 0.720|
| Homocysteine, umol/L                         | 12.29 ± 4.31    | 11.29 ± 3.07    | 1.99 | 0.047|
| Total protein, g/L                           | 71.31 ± 7.81    | 69.38 ± 11.93   | 1.54 | 0.126|
| Total cholesterol, mmol/L                    | 4.31 ± 1.06     | 4.24 ± 0.68     | 0.58 | 0.561|
| Triglyceride, mmol/L                         | 0.93 ± 0.37     | 0.98 ± 0.52     | −1.00| 0.317|
| High-density lipoprotein cholesterol, mmol/L | 1.65 ± 0.33     | 1.77 ± 0.37     | −2.60| 0.010|
| Low-density lipoprotein cholesterol, mmol/L  | 2.52 ± 0.73     | 2.45 ± 0.61     | 0.74 | 0.462|
| Fasting glucose, mmol/L                      | 4.94 ± 0.37     | 4.94 ± 0.38     | −0.05| 0.960|
| Fasting insulin, pmol/L                      | 71.43 ± 14.34   | 68.49 ± 13.09   | 1.84 | 0.067|
| HOMR-IR                                      | 2.24 ± 0.43     | 2.16 ± 0.40     | 1.82 | 0.070|
| IL6, pg/ml                                    | 102.91 ± 403.61 | 34.88 ± 120.71  | 2.02 | 0.044|
| TNFα, pg/ml                                   | 28.02 ± 71.11   | 20.87 ± 40.68   | 1.08 | 0.281|
| **GG genotype**                               |                 |                 |      |      |
| Folic acid, nmol/L                           | 30.15 ± 9.40    | 48.46 ± 80.78   | −1.24| 0.222|
| Vitamin B12, pg/ml                           | 366.17 ± 110.06 | 411.75 ± 114.65 | −1.32| 0.194|
| Homocysteine, umol/L                         | 13.84 ± 5.66    | 12.71 ± 3.86    | 0.71 | 0.480|
| Total protein, g/L                           | 71.54 ± 10.64   | 72.81 ± 4.08    | −0.46| 0.649|
| Total cholesterol, mmol/L                    | 4.56 ± 1.46     | 4.69 ± 1.92     | −0.27| 0.792|
| Triglyceride, mmol/L                         | 0.87 ± 0.33     | 1.03 ± 0.56     | −1.17| 0.250|
| High-density lipoprotein cholesterol, mmol/L | 1.67 ± 0.36     | 1.69 ± 0.32     | −0.22| 0.828|
| Low-density lipoprotein cholesterol, mmol/L  | 2.59 ± 0.87     | 2.48 ± 0.73     | 0.43 | 0.670|
| Fasting glucose, mmol/L                      | 4.87 ± 0.33     | 4.94 ± 0.31     | −0.92| 0.360|
| Fasting insulin, pmol/L                      | 70.39 ± 14.95   | 63.61 ± 12.91   | 2.19 | 0.032|
| HOMR-IR                                      | 2.18 ± 0.46     | 2.00 ± 0.42     | 1.81 | 0.074|
| IL6, pg/ml                                    | 18.62 ± 36.34   | 38.53 ± 180.82  | −0.62| 0.543|
| TNFα, pg/ml                                   | 37.97 ± 155.97  | 14.07 ± 15.96   | 1.08 | 0.283|
| **AG/GG genotype**                           |                 |                 |      |      |
| Folic acid, nmol/L                           | 31.59 ± 8.56    | 35.86 ± 30.44   | −1.76| 0.079|
But the molecular mechanism of these metabolites under conditions of folate pathway gene polymorphisms with dyslipidemia in different diseases especially RSA is not fully understood, and is worthy to be explored in the future.

**Limitation**

There are some limitations to our study. First of all, the single-center design may limit the generalizability of our study results. Secondly, this case-control study is hospital-based and selection bias may exist, however, since the controls were from the same region with cases and were randomly selected from health examination population, which may reduce the effect of selection bias.

**Conclusions**

In conclusion, we present the first study to date in the interactions of the MTHFR C677T, A1298C and MTRR A66G polymorphisms with the RSA risk on some serum lipid profiles. Interaction between the MTHFR C677T, A1298C and MTHFR A1298C, MTRR A66G are observed in our RSA group. Besides, all the three gene SNPs except MTRR 66AG gene variant had detrimental effects on HOMA-IR. MTHFR C677T and MTRR A66G gene variants had detrimental effects on serum homocysteine levels, while MTHFR C677T, A1298C and MTRR A66G gene variants had detrimental effects on certain serum lipid profiles. Further studies are in urgent to confirm or refute our findings in the future.

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**Authors’ contributions**

ZL and QL designed the study and drafted an outline. YS, WW, JH and JF participated in data analysis, JF draft of initial manuscript, ZL, QL and DZ participated in diagnosing RSA and collected the data; JX and DZ critically reviewed the manuscript and all of authors approved the final content off this manuscript.

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**Availability of data and materials**

We declare that the data supporting the conclusions of this article are fully described within the article, and the database is available from the first author (uters@126.com) upon reasonable request.

**Ethics approval and consent to participate**

This study was approved by the Institutional Review Board of Liuzhou Maternity and Child Healthcare Hospital.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1Department of Obstetrics and Gynecology, Liuzhou Maternity and Child Health Care Hospital, 50 Yingshan Road, Liuzhou 545001, Guangxi, China. 2Department of Obstetrics and Gynecology, The Maternal & Child Health Hospital of Guangxi Zhuang Autonomous Region, Guangxi 530003, China. 3Department of Clinical Laboratory, Affiliated Liute Central Hospital of Guangxi Medical University, Liuzhou 545001, Guangxi, China. 4Department of Laboratory Science, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, Guangdong, China. 5Department of Laboratory, Liuzhou Maternity and Child Health Care Hospital, Liuzhou

### Table 6 Interaction of MTRR A66G polymorphism with recurrent spontaneous abortion on serum folate and lipid levels (Continued)

| Variable                        | Case         | Control          | t     | p    |
|---------------------------------|--------------|------------------|-------|------|
| Vitamin B12, pg/ml              | 340.33 ± 108.01 | 350.13 ± 119.48  | −0.73 | 0.467|
| Homocysteine, umol/L            | 12.55 ± 4.58  | 11.49 ± 3.21     | 2.18  | 0.030|
| Total protein, g/L              | 71.34 ± 8.32  | 69.85 ± 11.24    | 1.31  | 0.193|
| Total cholesterol, mmol/L       | 4.36 ± 1.14   | 4.31 ± 0.95      | 0.39  | 0.699|
| Triglyceride, mmol/L            | 0.92 ± 0.36   | 0.98 ± 0.52      | −1.38 | 0.108|
| High-density lipoprotein chol.   | 1.65 ± 0.34   | 1.76 ± 0.37      | −2.50 | 0.113|
| Low-density lipoprotein chol.    | 2.52 ± 0.75   | 2.46 ± 0.62      | 0.87  | 0.383|
| Fasting glucose, mmol/L         | 4.93 ± 0.36   | 4.94 ± 0.37      | −0.48 | 0.640|
| Fasting insulin, pmol/L         | 71.18 ± 14.46 | 67.56 ± 13.16    | 2.54  | 0.011|
| HOMA-IR                         | 2.23 ± 0.43   | 2.13 ± 0.41      | 2.34  | 0.020|
| IL6, pg/ml                      | 82.54 ± 333.49| 35.57 ± 133.61   | 1.78  | 0.077|
| TNFα, pg/ml                     | 30.42 ± 98.18 | 19.58 ± 37.31    | 1.48  | 0.141|

*HOMA-IR: Homeostatic model assessment of insulin resistance, IL6: Interleukin 6, TNFα: Tumor necrosis factor α*
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