Association between MTHFR Polymorphisms and Acute Myeloid Leukemia Risk: A Meta-Analysis

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Abstract

Previous observational studies investigating the association between methylenetetrahydrofolate reductase (MTHFR) polymorphisms and acute myeloid leukemia risk (AML) have yielded inconsistent results. The aim of this study is to derive a more precise estimation of the association between MTHFR (C677T and A1298C) polymorphisms and acute myeloid leukemia risk. PubMed and Embase databases were systematically searched to identify relevant studies from their inception to August 2013. Odds ratios (ORs) with 95% confidence intervals (CIs) were the metric of choice. Thirteen studies were selected for C677T polymorphism (1838 cases and 5318 controls) and 9 studies (1335 patients and 4295 controls) for A1298C polymorphism. Overall, pooled results showed that C677T polymorphism was not significant associated with AML risk (OR: 0.98–1.04; 95% CI: 0.86–0.92 to 1.09–1.25). Similar results were observed for the A1298C polymorphism and in subgroup analysis. All comparisons revealed no substantial heterogeneity nor did we detect evidence of publication bias. In summary, this meta-analysis provides evidence that MTHFR polymorphisms were not associated with AML risk. Further investigations are needed to offer better insight into the role of these polymorphisms in AML carcinogenesis.

Citation: Qin Y-T, Zhang Y, Wu F, Su Y, Lu G-N, et al. (2014) Association between MTHFR Polymorphisms and Acute Myeloid Leukemia Risk: A Meta-Analysis. PLoS ONE 9(2): e88823. doi:10.1371/journal.pone.0088823

Methods

Search Strategy and Selection Criteria

Eligible studies were identified by searching electronic literature databases PubMed and Embase (from inception to August 2013). The search strategy used the following keywords: MTHFR, polymorphism, acute myeloid leukemia or acute myeloblastic leukemia. We did not apply language restrictions. References of reviews or original studies identified in the literature search were also examined.

Search Results

A total of 13 studies met the inclusion criteria for the C677T polymorphism and 9 studies for the A1298C polymorphism. The studies were conducted in different countries, including China, the United States, and the United Kingdom, among others.

Evaluation of the Studies

All studies were evaluated for methodological quality using the Newcastle-Ottawa Scale.

Data Synthesis

The primary outcome was the association between MTHFR polymorphisms and acute myeloid leukemia risk. The secondary outcome was the association between MTHFR polymorphisms and specific subtypes of acute myeloid leukemia.

Results

The pooled analysis of 13 studies for the C677T polymorphism showed no significant association with acute myeloid leukemia risk (OR: 0.98–1.04; 95% CI: 0.86–0.92 to 1.09–1.25). Similarly, the analysis of 9 studies for the A1298C polymorphism also showed no significant association (OR: 0.98–1.04; 95% CI: 0.86–0.92 to 1.09–1.25).

Subgroup Analysis

Subgroup analysis was conducted based on different patient populations, such as different age groups and ethnicities. The results showed no significant association in any subgroup.

Publication Bias

No evidence of publication bias was found in the meta-analysis.

Conclusion

The meta-analysis provides evidence that MTHFR polymorphisms were not associated with acute myeloid leukemia risk. However, further studies are needed to confirm these findings and to explore the role of MTHFR polymorphisms in the development of specific subtypes of acute myeloid leukemia.

PLOS ONE | www.plosone.org 1 February 2014 | Volume 9 | Issue 2 | e88823
hand searched for additional studies. Studies were included if they met the following inclusion criteria: (1) explored the association of \textit{MTHFR} (C677T and A1298C) polymorphisms with AML risk; (2) used a case-control design; (3) provided available genotype or allele frequency of the cases and control to calculate ORs with 95% CIs. The exclusion criteria also applied: the data from study were repeated or overlapped; there was no genotype or allele frequency; the patients were about therapy-related AML; the studies were review, case report, or comment.

Data Extraction

Two investigators (YTQ and FW) independently extracted data using a standardized data-collection form. Study characteristics extracted from each article were as follows: first author, year of publication, country of origin, racial decent, participant age, number of participants, source of controls, genotype studied, and available genotype frequency information for \textit{MTHFR} C677T and A1298C. Any disagreements were resolved by consensus and a third author (YZ). All data were extracted from the published studies and no authors were contacted to require further information.

Statistical Analysis

The strength of the association between \textit{MTHFR} (C677T and A1298C) polymorphisms and AML risk was measured by using crude odds ratio (OR) with 95% confidence interval (CI). The pooled ORs were estimated in following models: allele contrast (T vs. C), codominant model (CT vs. CC; TT vs. CC), dominant model (TT+CT vs. CC), and recessive model (TT vs. CT+CC), respectively. For \textit{MTHFR} A1298C polymorphism, we assessed the same association. The Cochran Q test was used to test statistical heterogeneity. The \( I^2 \) statistics [31] was also calculated to quantify the proportion of the variations across studies. A \( P \) value of less than 0.1 for the \( Q \) statistic was considered as heterogeneity across studies, allowing for the use of a random-effects model (DerSimonian and Laird method [32]). Otherwise, a fixed-effects model (Mantel–Haenszel method [33]) was applied.

Subgroup analysis based on ethnicity (Caucasian, Asian, and Brazilian), sample size (large sample size \( \geq 100 \), and small sample size \( < 100 \), and HWE was performed to assess the source of heterogeneity. We also assessed the influence of individual studies on the combined risk estimate by sequentially omitting one study each time.

Potential publication bias was assessed both by visually inspecting of the Begg funnel plot and statistically via Egger’s unweighted regression tests [34]. All statistical analyses were conducted using Stata version 11.0 (Stata Corporation, College Station, TX). All \( P \) values are tailed where 0.05 was considered statistically significant except in the test for heterogeneity.

Results

Identification of Eligible Studies

The search strategy yielded 35 potential studies from PubMed and Embase databases. However, most of them were excluded after reviewing titles and abstracts, leaving 19 for full-text review. The literature search and detailed study selection procedures were presented in Figure S1. Six studies were excluded (two studies [26,27] were conference articles, and two [28,29] with patients who were about therapy-related AML, one [11] was review article, and one [30] was supplementary material). Finally, 13 studies [7,14–25] were included in this meta-analysis.

Table 1. Characteristics of studies included in this meta-analysis.

| First author | Year | Country | Racial decent | Cases, n | Controls, n | Source of controls | HWE | Studied \textit{MTHFR} genotypes |
|--------------|------|---------|---------------|----------|-------------|-------------------|-----|-------------------|
| Hussain [14] | 2012 | India   | Asian         | 112      | 251         | Population        | yes | C677T             |
| Lightfoot [19]| 2010 | United Kingdom | Caucasian | 89      | 824         | Population        | yes | C677T and A1299C  |
| Vahid [20]   | 2010 | Iran    | Caucasian     | 106      | 97          | Population        | yes | C677T and A1299C  |
| Amorim [15]  | 2008 | Brazil  | Brazilian     | 50       | 248         | Population        | yes | C677T and A1299C  |
| Kim [24]     | 2008 | Korea   | Asian         | 389      | 1700        | Population        | yes | C677T and A1299C  |
| Barbosa [7]  | 2008 | Brazil  | Brazilian     | 27       | 100         | Population        | yes | C677T and A1299C  |
| Bolufere [22]| 2007 | Spain   | Caucasian     | 302      | 454         | Population        | yes | C677T             |
| Moon [23]    | 2007 | South Korea | Asian | 200      | 434         | Population        | yes | C677T and A1299C  |
| Chen [25]    | 2006 | China   | Asian         | 40       | 157         | Population        | yes | C677T             |
| Costa Ramos [16]| 2006 | Brazil  | Brazilian     | 182      | 315         | Population        | yes | C677T and A1299C  |
| Hur [18]     | 2006 | Korea   | Asian         | 55       | 200         | Population        | no  | C677T and A1299C  |
| Deligezer [17]| 2003 | Turkey  | Caucasian     | 49       | 161         | Population        | yes | C677T             |
| Skibola [21] | 1999 | United Kingdom | Caucasian | 237      | 377         | Hospital          | yes | C677T and A1299C  |

HWE, Hardy-Weinberg equilibrium; \textit{MTHFR}, Methylenetetrahydrofolate reductase.

doi:10.1371/journal.pone.0088823.t001

The main characteristics of the included studies were shown in Table 1. These studies were published between 1999 and 2012. Sample size ranged from 27 to 1,700 (including 1,838 patients with AML and 5,318 healthy controls). Among these, five studies were in Caucasian descent [17,19–22], five studies of Asian descent [14,18,23–25] and three studies of Brazilian descent [7,15,16]. Thirteen studies including 1838 cases and 4295 controls investigated the association between \textit{MTHFR} A1298C polymorphism with AML risk, and 9 studies with a total of 1335 patients and 4295 controls investigated the association between \textit{MTHFR} C677T and A1299C polymorphism with AML risk.
polymorphism and AML risk. Of these, 12 studies were population-based and one was hospital-based.

**MTHFR C677T**

Figure 1 showed the results from a fixed-effects model combining the ORs for the association of MTHFR C677T polymorphism and AML risk. Overall, the pooled results showed that the MTHFR C677T polymorphism was not associated with the development of AML (OR, 0.98–1.04; 95% CI, 0.86–0.92 to 1.09–1.25; P, 0.750–0.976), without statistically significant between-study heterogeneity (I², 0.0%–26.4%; P for heterogeneity, 0.178–0.573). Table 2 shows that the Asian and Brazilian subgroups were at increased risk in some genetic models. Caucasians may even have some low-level protection in some models (OR, 0.81–0.89).

**MTHFR A1298C**

Figure 2 presented the results from a fixed-effects model combining the ORs for the association of MTHFR A1298C polymorphism and AML risk. Overall, the estimate results indicated non-significant increased risk association of MTHFR A1298C polymorphism with AML risk in some genetic models (OR, 1.11–1.13), without zero heterogeneity (P for heterogeneity, 0.562–0.955). Table 3 shows that the Brazilian subgroup are at increased risk in all genetic models (OR, 1.1–1.4), and in two genetic models, so are the Asians (OR, 1.23–1.25) as well as the HWE studies (OR, 1.11) and even small sample size studies (OR, 1.36–1.50).

**Publication Bias**

The Begg rank correlation test and Egger linear regression tests for publication bias in the meta-analysis indicated no obvious publication bias among studies (Begg’s test, P = 0.360; Egger’s test, P = 0.659; Figure 3).

**Discussion**

To the best of our knowledge, this is the first meta-analysis to assess the association between MTHFR polymorphisms and AML risk. Thirteen studies (1838 cases and 5318 controls) and 9 studies (1335 patients and 4295 controls) explored the association between the C677T and A1298C polymorphisms and AML risk, respectively. Results of this study suggested that MTHFR (C677T and A1298C) polymorphisms were not significantly associated
with AML risk. Moreover, similar results were observed in subgroup analyses based on ethnicity, sample size, and HWE in controls.

Nowadays, several meta-analyses have been performed to clarify the association between MTHFR (C677T and A1298C) polymorphisms and risk of several cancers. For instance, You et al have demonstrated that the MTHFR C677T and A1298C polymorphisms were associated with bladder cancer risk [35]. Wei et al provided evidence that the MTHFR C677T polymorphism increased the risk for developing colorectal cancer [36]. However, a meta-analysis by Ding et al indicated that no significant association was observed between MTHFR C677T polymorphism and susceptibility to ovarian cancer [37]. Besides, Niu et al suggested that no significant association between MTHFR A1298C polymorphism head and neck cancer [38], which were consistent with our results. These inconsistent and confusing conclusions can be attributed to several factors. Different selection criteria and selection bias might account for the diversity of the results. In addition, the reason might be the complexity of the folate metabolic pathway because MTHFR is only one of many enzymes involved in the pathway. Moreover, the studies with small sample size will have a lower statistical

| Genetic comparisons | Population and subgroups under analysis | Studies | Fixed-effects model |
|---------------------|----------------------------------------|--------|-------------------|
|                     |                                        | OR (95% CI) | p-value | I², % | p for heterogeneity |
| T vs. C             | All                                    | 1.00 (0.92–1.09) | 0.976 | 0.0 | 0.559 |
|                     | Caucasian                              | 0.89 (0.76–1.03) | 0.119 | 0.0 | 0.573 |
|                     | Asian                                  | 1.07 (0.95–1.20) | 0.279 | 0.0 | 0.417 |
|                     | Brazilian                              | 1.04 (0.83–1.31) | 0.720 | 0.0 | 0.951 |
|                     | Large sample size                      | 1.01 (0.92–1.11) | 0.862 | 15.4 | 0.312 |
|                     | Small sample size                      | 0.97 (0.80–1.18) | 0.776 | 0.0 | 0.629 |
|                     | All in HWE                             | 1.00 (0.92–1.09) | 0.976 | 0.0 | 0.473 |
| CT vs. CC           | All                                    | 0.98 (0.86–1.11) | 0.750 | 10.5 | 0.340 |
|                     | Caucasian                              | 0.81 (0.66–1.01) | 0.056 | 26.0 | 0.248 |
|                     | Asian                                  | 1.14 (0.95–1.36) | 0.169 | 0.0 | 0.680 |
|                     | Brazilian                              | 0.94 (0.69–1.30) | 0.722 | 0.0 | 0.824 |
|                     | Large sample size                      | 0.99 (0.86–1.14) | 0.873 | 0.0 | 0.578 |
|                     | Small sample size                      | 0.95 (0.73–1.24) | 0.704 | 42.1 | 0.125 |
|                     | All in HWE                             | 0.96 (0.84–1.09) | 0.530 | 0.0 | 0.455 |
| TT vs. CC           | All                                    | 1.04 (0.87–1.25) | 0.648 | 2.9 | 0.417 |
|                     | Caucasian                              | 0.88 (0.64–1.21) | 0.427 | 0.0 | 0.411 |
|                     | Asian                                  | 1.12 (0.88–1.42) | 0.370 | 41.7 | 0.143 |
|                     | Brazilian                              | 1.20 (0.72–1.97) | 0.484 | 0.0 | 0.997 |
|                     | Large sample size                      | 1.05 (0.86–1.29) | 0.606 | 28.8 | 0.209 |
|                     | Small sample size                      | 1.00 (0.66–1.51) | 0.985 | 0.0 | 0.553 |
|                     | All in HWE                             | 1.05 (0.88–1.27) | 0.570 | 7.9 | 0.367 |
| TT+CT vs. CC        | All                                    | 0.99 (0.88–1.12) | 0.913 | 0.0 | 0.573 |
|                     | Caucasian                              | 0.83 (0.66–1.01) | 0.061 | 0.0 | 0.433 |
|                     | Asian                                  | 1.14 (0.96–1.35) | 0.143 | 0.0 | 0.933 |
|                     | Brazilian                              | 0.99 (0.74–1.34) | 0.965 | 0.0 | 0.875 |
|                     | Large sample size                      | 1.00 (0.88–1.15) | 0.956 | 0.0 | 0.585 |
|                     | Small sample size                      | 0.96 (0.74–1.23) | 0.737 | 12.3 | 0.336 |
|                     | All in HWE                             | 0.98 (0.87–1.11) | 0.762 | 0.0 | 0.580 |
| TT vs. CT+CC        | All                                    | 1.02 (0.86–1.20) | 0.836 | 26.4 | 0.178 |
|                     | Caucasian                              | 0.95 (0.71–1.29) | 0.748 | 15.4 | 0.316 |
|                     | Asian                                  | 1.01 (0.82–1.26) | 0.892 | 63.3 | 0.028 |
|                     | Brazilian                              | 1.23 (0.76–1.99) | 0.398 | 0.0 | 0.985 |
|                     | Large sample size                      | 1.02 (0.86–1.23) | 0.797 | 42.2 | 0.110 |
|                     | Small sample size                      | 0.99 (0.67–1.46) | 0.950 | 16.6 | 0.306 |
|                     | All in HWE                             | 1.04 (0.88–1.23) | 0.631 | 24.3 | 0.205 |

MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval; vs., versus; HWE, Hardy-Weinberg equilibrium.

doi:10.1371/journal.pone.0088823.t002
power than those with large sample size. Furthermore, the different mechanisms of carcinogenesis of different cancers might due to gene-variant associations vary in different kinds of diseases.

Several studies have demonstrated that individuals with MTHFR 677 TT genotype, lack of vitamins B6 and B12, methionine and folate, and high consumption of alcohol are at increased risk of developing colorectal tumors [39–42]. However, no studies have reported these gene-nutrient interactions with the risk of AML. The present study was lack of data to estimate the association of gene-nutrient and risk of AML. These interesting clues may be useful for future research.

Dietary intake of several nutrients could influence the distribution of intracellular folate metabolites. Vitamins B6 and B12 may affect DNA synthesis and MTHFR enzyme activity. Moreover, high consumption of alcohol might take place of more nutritious foods, which may lead to the intake deficiency of folate and B vitamins [43]. Deficiency of folate is associated with carcinogenesis mainly in two ways [8]: (1) The conversion of dUMP to dTMP, using for DNA synthesis and repair, demands methyl group donated by 5, 10-methylene THF, so lack of folate can intervene thymidylate biosynthesis and then lead to errors in DNA synthesis, strand breakage, and chromosomal repair. (2) Low-level 5-methyl THF may result in DNA hypomethylation and cause proto-oncogene expression due to cellular S-adenosylmethionine used up. Thus, cohort studies are needed to focus on gene-nutrient interactions in the future.

In order to better estimate the association of MTHFR (C677T and A1298C) polymorphisms with AML risk, subgroup analysis based on ethnicity, sample size and HWE, was performed. Although Asian and Brazilian subgroups were at increased risk in some genetic models, no significant associations between MTHFR (C677T and A1298C) polymorphisms and AML risk were found in samplesize subgroups or all in HWE, which indicated that the results of our analysis was reliable and stable. The real effect of MTHFR (C677T and A1298C) polymorphisms may be concealed by the causal genes in AML. Moreover, different ethnicity of genotypic milieu and living surroundings might have an effect on AML risk, which may led to an effect in our results.

Several limitations might be acknowledged in this meta-analysis. First, we only selected the published articles to acquire data for analyses, and the unpublished article's effect was unknown. Thus, it is necessary to conduct a system review to avoid the potential effect in analysis. Second, our study was based on single-factor estimate, which explained the effects of two polymorphisms on AML risk respectively and lack of combination of two

![Figure 2. Meta-analysis for the association of acute myeloid leukemia risk with MTHFR A1299C polymorphism (C vs. A).](doi:10.1371/journal.pone.0088823.g002)
polymorphisms analysis. So, conducting a meta-analysis to investigate the combination of these two functional polymorphisms may offer better insight into MTHFR (C677T and A1298C) polymorphisms on AML risk. Third, there were no significant effects for both polymorphisms. Fourth, gene-gene and gene-environment interactions might also be considered in future studies. In spite of these, our meta-analysis also has two advantages as follows: (1) there was no significant absence of evidence of publication bias in the present study, which highlighted further, ensured the reliability of association analysis our findings. (2) There was no evidence of statistical heterogeneity between the analyses of two polymorphisms and AML risk underpins the combinaibility of the component studies.

In conclusion, our meta-analysis indicates that MTHFR C677T polymorphism is not associated with AML risk, as well as A1298C polymorphism. Future well-design study is warranted to estimate the effect of combination of two polymorphisms and gene-environment interactions. If epidemiologic study confirms the role of gene-environment interactions, additional studies will be needed to further elucidate the potential biological mechanisms involved.

Table 3. Distribution of MTHFR A1298C genotypes and allelic frequencies in acute myeloid leukemia patients.

| Genetic comparisons | Population and subgroups under analysis | Studies | Fixed-effects model |
|---------------------|----------------------------------------|---------|---------------------|
|                     |                                        |         | OR (95% CI)       | p-value | I², % | p for heterogeneity |
| C vs. A             | All                                    | 9       | 1.02 (0.91–1.14)  | 0.733   | 0.0   | 0.955 |
|                     | Caucasian                              | 3       | 0.97 (0.81–1.16)  | 0.717   | 0.0   | 0.926 |
|                     | Asian                                  | 3       | 1.00 (0.85–1.18)  | 0.993   | 0.0   | 0.625 |
|                     | Brazilian                              | 3       | 1.16 (0.91–1.48)  | 0.216   | 0.0   | 0.995 |
|                     | Large sample size                      | 5       | 1.02 (0.90–1.15)  | 0.808   | 0.0   | 0.746 |
|                     | Small sample size                      | 4       | 1.03 (0.82–1.31)  | 0.785   | 0.0   | 0.878 |
|                     | All in HWE                             | 8       | 1.02 (0.91–1.14)  | 0.736   | 0.0   | 0.916 |
| AC vs. AA           | All                                    | 9       | 0.98 (0.85–1.13)  | 0.760   | 0.0   | 0.801 |
|                     | Caucasian                              | 3       | 0.97 (0.74–1.26)  | 0.795   | 36.7  | 0.206 |
|                     | Asian                                  | 3       | 0.95 (0.78–1.15)  | 0.593   | 0.0   | 0.880 |
|                     | Brazilian                              | 3       | 1.09 (0.79–1.49)  | 0.614   | 0.0   | 0.723 |
|                     | Large sample size                      | 5       | 1.01 (0.87–1.19)  | 0.857   | 0.0   | 0.859 |
|                     | Small sample size                      | 4       | 0.84 (0.61–1.16)  | 0.291   | 0.0   | 0.523 |
|                     | All in HWE                             | 8       | 0.99 (0.85–1.14)  | 0.838   | 0.0   | 0.732 |
| CC vs. AA           | All                                    | 9       | 1.13 (0.86–1.48)  | 0.378   | 0.0   | 0.792 |
|                     | Caucasian                              | 3       | 0.97 (0.66–1.42)  | 0.860   | 0.0   | 0.666 |
|                     | Asian                                  | 3       | 1.23 (0.74–2.02)  | 0.425   | 3.7   | 0.354 |
|                     | Brazilian                              | 3       | 1.42 (0.82–2.47)  | 0.213   | 0.0   | 0.847 |
|                     | Large sample size                      | 5       | 1.06 (0.78–1.45)  | 0.715   | 0.0   | 0.486 |
|                     | Small sample size                      | 4       | 1.36 (0.81–2.28)  | 0.250   | 0.0   | 0.903 |
|                     | All in HWE                             | 8       | 1.11 (0.85–1.46)  | 0.447   | 0.0   | 0.744 |
| CC+AC vs. AA        | All                                    | 9       | 1.00 (0.88–1.14)  | 0.995   | 0.0   | 0.940 |
|                     | Caucasian                              | 3       | 0.96 (0.75–1.23)  | 0.752   | 0.0   | 0.541 |
|                     | Asian                                  | 3       | 0.97 (0.81–1.17)  | 0.762   | 0.0   | 0.782 |
|                     | Brazilian                              | 3       | 1.14 (0.85–1.54)  | 0.377   | 0.0   | 0.895 |
|                     | Large sample size                      | 5       | 1.02 (0.88–1.18)  | 0.796   | 0.0   | 0.899 |
|                     | Small sample size                      | 4       | 0.93 (0.69–1.25)  | 0.616   | 0.0   | 0.677 |
|                     | All in HWE                             | 8       | 1.00 (0.88–1.15)  | 0.947   | 0.0   | 0.900 |
| CC vs. AC+AA        | All                                    | 9       | 1.11 (0.86–1.44)  | 0.415   | 0.0   | 0.562 |
|                     | Caucasian                              | 3       | 0.95 (0.68–1.38)  | 0.797   | 23.4  | 0.271 |
|                     | Asian                                  | 3       | 1.25 (0.76–2.06)  | 0.379   | 2.9   | 0.357 |
|                     | Brazilian                              | 3       | 1.39 (0.81–2.38)  | 0.234   | 0.0   | 0.762 |
|                     | Large sample size                      | 5       | 1.01 (0.75–1.37)  | 0.939   | 8.7   | 0.357 |
|                     | Small sample size                      | 4       | 1.50 (0.91–2.48)  | 0.113   | 0.0   | 0.896 |
|                     | All in HWE                             | 8       | 1.110 (0.84–1.43) | 0.495   | 0.0   | 0.508 |

MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval; vs., versus; HWE, Hardy-Weinberg equilibrium.
doi:10.1371/journal.pone.0088823.t003
Supporting Information

Figure S1 Flow chart.

Checklist S1 PRISMA checklist.

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Figure 3. Publication bias test (MTHFR C677T: T vs. C).
doi:10.1371/journal.pone.0088823.g003

Author Contributions

Conceived and designed the experiments: R-SW. Performed the experiments: YS G-NL FW. Analyzed the data: YZ. Contributed reagents/materials/analysis tools: Y-TQ YZ YS. Wrote the paper: Y-TQ.
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