Elevated Homocysteine Levels Associated with Atrial Fibrillation and Recurrent Atrial Fibrillation

Hao Rong, MD, Lei Huang, MD, Nake Jin, MD, Jun Hong, MD, Jianan Hu, MD, Shanshan Wang, MD, Yuquan Xie, PhD and Jun Pu, PhD

Summary

There is increasing evidence linking plasma homocysteine levels and atrial fibrillation (AF). The association between an elevated level of plasma homocysteine and AF was examined by meta-analysis in this study. The PubMed and ScienceDirect databases until August 2019 were utilized to collect previous literature on homocysteine and the potential relation to AF. The pooled effects were evaluated depending on standardized mean differences (SMDs) or odds ratios (ORs) with 95% confidence intervals (CIs), and the calculation was performed using Stata 12 software.

A total of 11 validated articles were included in the meta-analysis. For pooled effect, the results confirmed that AF patients had higher homocysteine levels than control subjects (SMD: 0.58, 95% CI: 0.09-1.06). Compared with control subjects, homocysteine levels were higher in paroxysmal AF (SMD: 0.45, 95% CI: 0.18-0.72) and persistent AF patients (SMD: 1.21, 95% CI: 0.50-1.92). The pooled analysis suggested that patients with elevated homocysteine levels had markedly higher risk of AF compared with lower homocysteine levels in the categorical variable (OR: 2.21, 95% CI: 1.16-4.21) and continuous variable analyses (OR: 1.13, 95% CI: 1.00-1.27), respectively. In addition, the pooled analysis indicated that recurrent AF patients had significantly higher homocysteine levels than those without recurrence (SMD: 0.65, 95% CI: 0.42-0.88). The pooled analysis of the categorical variables indicated that elevated homocysteine levels were associated with increased risk of AF recurrence (OR: 3.81, 95% CI: 3.11-4.68). However, the association was weak in the pooled analysis of continuous variables (OR: 1.88, 95% CI: 0.74-4.81).

Our meta-analysis identified that plasma homocysteine levels were significantly elevated in AF and recurrent AF patients. Elevated homocysteine is associated with increased risk of AF and AF recurrence. (Int Heart J 2020; 61: 705-712)

Key words: Hyperhomocysteinemia, Cardiac arrhythmias, Atrial fibrillation recurrence, Meta-analysis

Homocysteine (Hcy) is a sulfhydryl-containing amino acid that is the byproduct in the conversion of methionine to cysteine. It does not occur in the diet but is the derivative of dietary methionine due to its demethylation, becoming an essential intermediate for normal mammalian metabolism of methionine. Some studies have reported that moderately elevated total homocysteine levels showed increasing significance for the pathogenesis of atherosclerotic sequelae, including cardiovascular mortality, coronary artery disease (CAD), and stroke. The potential mechanisms of the adverse effects of homocysteine include endothelial dysfunction and death, increased oxidative stress, inflammation, altered collagen metabolism, and pro-atherothrombotic.

Editorial p.631

As the most common sustained cardiac arrhythmia encountered in clinical practice, atrial fibrillation (AF) is linked with increased risks of morbidity and mortality. In recent years, various similar studies have reported a possible association between elevated homocysteine levels and AF. Several clinical studies also have shown the positive association of homocysteine with AF and recurrent AF risk. However, there are inconsistent results on the association between homocysteine and AF. Therefore, a comprehensive and critical meta-analysis of previous studies was designed and conducted to clarify evidence-based conclusions concerning the significance of homocysteine for AF.

Methods

Search strategy: A systematic search was conducted by two investigators (Hao Rong, Lei Huang) independently through the PubMed and ScienceDirect databases until
August 2019. To locate all relevant publications which documented the association between homocysteine and AF, medical subject headings or free text words were checked with the following rule of keywords: “Homocysteine” (or “Hcy”) plus “Atrial fibrillation”. The search strategy also covered the references of the selected articles to find out additional works which were neglected in the database search. The search strategy also required the formal publication of the selected works and the availability of a full-text.

**Inclusion and exclusion criteria:** The literature search and review were independently conducted by 3 investigators (Hao Rong, Jianan Hu, Shanshan Wang). First, screening of titles and abstracts was conducted to confirm the relevance and decide upon the selection of some studies. The inclusion or exclusion of some other studies might not have been decided at this step, meaning they would be forwarded to full-text screening for a final decision, which was made by two investigators (Lei Huang, Yuquan Xie). If a discrepancy in opinions occurred between the two investigators, a third investigator (Lei Huang) independently examined the study in order to make a final decision.

The content of the selected literature was examined. The criteria for inclusion were: (1) cross-sectional or case-control or cohort studies in humans; (2) focus on the significance of plasma homocysteine for AF; (3) written in English and approved by standard peer review; (4) provides a considerable amount of valid data on homocysteine levels for both the AF-positive group and AF-negative controls; and (5) provides odds ratios (ORs) or hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) for homocysteine levels. The exclusion criteria were: (1) studies with inadequate relevance; (2) review articles or case reports; (3) animal studies; and (4) failure to provide data on homocysteine levels for either the AF-positive group or AF-negative controls.

**Data extraction and quality assessment:** According to the inclusion and exclusion criteria, data extraction from the included studies was conducted independently by 2 investigators (Nake Jin, Jun Hong). Specifically, the following data were collected: first author, year of publication, nationality, number of participants, plasma homocysteine levels, and adjusted variables. In cases of discrepant information, two investigators discussed each case in detail to decide how it should be processed. The Newcastle-Ottawa Scale (NOS) was used to evaluate the studies included. This assessment tool focused on three aspects: participant selection, comparability, and exposure. Studies that satisfied all the items on the scale were given nine stars. Two authors (Lei Huang, Jun Pu) conducted the quality assessment independently.

**Statistical analysis:** Stata version 12 was used for the statistical analyses and statistical significance was set at $P < 0.05$. The standardized mean differences (SMDs) and 95% confidence intervals (CIs) were determined to compare homocysteine levels between the AF patients and control subjects. As in previous studies, HR values using the multivariate Cox proportional hazards model in each primary study were directly considered as ORs. Multivariate ad-
justed HRs or ORs and the corresponding 95% CIs were used to evaluate the association between homocysteine and risk of AF. The random effects model or fixed effects model was used to evaluate the pooled analysis with or without heterogeneity, respectively. The statistical estimation of heterogeneity was performed with I² tests. Studies with an I² value higher than 50% were considered to have high heterogeneity and the model of random effects was applied; otherwise, the fixed effects model was used. Moreover, for the variation caused by any individual study, sensitivity analysis was also conducted. Publication bias was not performed because the number of pooled meta-analyses was less than 10 for each comparison.

**Results**

**Search results:** A flow diagram of the literature search and study selection is shown in Figure 1. A total of 1766 citations were found in the initial search according to the search strategy as stated in the previous section. Depending on the above-described criteria of evaluation, 11 validated articles were included for analysis in our study. The main characteristics of the included studies are shown in the Table.

**Comparisons of homocysteine levels between AF patients and control subjects:** Four studies were included in the comparisons of homocysteine levels between AF patients and control subjects. The random-effects model was used since heterogeneity existed (I² = 92.7%, P = 0.000). The results indicated that AF patients showed significantly higher homocysteine levels than control subjects (SMD: 0.58, 95%CI: 0.09-1.06; Figure 2).

**Comparisons of homocysteine levels between paroxysmal AF and persistent AF patients:** Homocysteine levels in patients with paroxysmal AF or persistent AF and control subjects were detected from 3 eligible studies. We chose a fixed-effect model in the pooled analysis for comparison of homocysteine levels between persistent AF patients and control subjects (I² = 78.7%, P = 0.030). Compared with control subjects, ho-
Homocysteine levels were higher in paroxysmal AF (SMD: 0.45, 95% CI: 0.18-0.72; Figure 3A) and persistent AF patients (SMD: 1.21, 95% CI: 0.50-1.92; Figure 3B), respectively. In addition, persistent AF patients had a higher level of homocysteine compared with paroxysmal AF patients (SMD: 0.59, 95% CI: 0.35-0.83; Figure 3C).

**Relationship between homocysteine and incidence of AF:** The overall pooled analysis of the estimates as a categorical variable indicated that patients with elevated homocysteine levels had an approximately 121% higher risk of AF compared with the lowest homocysteine level (OR: 2.21, 95% CI: 1.16-4.21; Figure 4A) with a significant heterogeneity ($I^2 = 94.5\%$, $P = 0.000$) among three studies. This association distinctly remained in a pooled analysis comparing the highest homocysteine quartile and lowest homocysteine quartile (Q4 versus Q1: OR: 4.79, 95% CI: 1.06-21.57; Figure 4A). However, there appears to be no association between other homocysteine quartiles and the lowest homocysteine quartile (Q2 versus Q1: OR: 1.20, 95% CI: 0.54-2.70; Q3 versus Q1: OR: 1.92, 95% CI: 0.60-6.13, respectively; Figure 4A). Moreover, the pooled analysis of the estimates as continuous variable also indicated that higher homocysteine levels were also associated with increased risk of AF (OR: 1.13, 95% CI: 1.00-1.27; Figure 4B) without indication of heterogeneity ($I^2 = 27.1\%$; $P = 0.241$).

**Association between homocysteine levels and recurrence of AF:** Four studies were identified in the comparison of homocysteine levels between AF with or without recurrence. The pooled analysis indicated that AF patients with recurrence had significantly higher homocysteine levels than those without recurrence (SMD: 0.65, 95% CI: 0.42-0.88; Figure 5A) in a fixed-effect model with no evidence of obvious heterogeneity ($I^2 = 27.4\%$; $P = 0.252$). The pooled analysis of two studies reporting the estimates as categorical variables indicated that homocysteine was associated with an increased risk of recurrence of AF (OR: 3.81, 95% CI: 3.11-4.68; Figure 5B) in a fixed-effect model without indication of heterogeneity ($I^2 = 43.2\%$; $P = 0.184$). Two studies reported the estimates as continuous variables and a significant heterogeneity was observed ($I^2 = 95.4\%$; $P = 0.000$). Our meta-analysis with a random-effect model showed that elevated homocysteine was associated with a 1.88-fold increase in the risk of the recurrence of AF. The calculation of risk showed no statistical significance (OR: 1.88, 95% CI: 0.74-4.81; Figure 5C).

**Discussion**

In this study, we found for the first time that a meta-analysis indicated that homocysteine levels in AF patients were higher than those in control subjects. The pooled analysis suggested that patients with AF had an approximate 58% increase in homocysteine levels compared with control subjects. However, the cutoff values of homocysteine have not been established because the measuring method and its standardization are different among the studies in the literature. Furthermore, this meta-analysis indicated homocysteine levels were higher in both paroxysmal and persistent AF patients compared with control subjects. Compared with paroxysmal AF patients and control subjects, persistent AF patients had markedly increased homocysteine levels, which suggests that homocysteine levels may be time-dependent. Secondarily, the findings of our meta-analysis provide prospective evidence that homocysteine was prospectively associated with a significantly increased risk of AF in both categories and continuous variable analyses. In categorical variable analysis, the pooled analysis suggested that patients with elevated homocysteine levels had a higher risk of AF and this association distinctly remains in the highest homocysteine quartile, increasing 1.2 and 3.8 times, respectively. Thirdly, we evaluated the association of homocysteine levels and recurrence of AF. The pooled analysis indicated

![Figure 2: Forest plot of studies in homocysteine levels for patients with AF versus control subjects.](image-url)
that AF patients with recurrence had significantly higher homocysteine levels than those without recurrence, with the increase being approximately 65%. The pooled analysis of the categorical variables suggested that higher homocysteine levels are associated with increased risk of AF recurrence. However, the association is weak in the pooled analysis of the continuous variables. Thus, further in-depth studies are required to determine whether homocysteine-lowering therapy with vitamins B6 and B12 and folic acid supplementation could prevent new-onset AF and AF recurrence.

The molecular mechanisms contributing to the significance of plasma homocysteine levels in AF remain unclear. Several pathophysiological mechanisms have been
Figure 4. Forest plot of the association between homocysteine and the risk of AF. A: Homocysteine levels as a categorical variable and risk of AF. B: Homocysteine levels as a continuous variable and risk of AF. Q indicates quartile (Q4 represents the highest homocysteine quartile and Q1 represents the lowest homocysteine quartile).

reported with respect to the association between elevated homocysteine and AF. Some studies have shown that elevated homocysteine levels play key roles in cardiac remodeling by resulting in significantly increased myocyte size, the proliferation of mast cells, cardiac fibrosis, and the activation of matrix metalloproteinases. Shimano, et al proved a positive correlation between elevated homocysteine and left atrial diameter and the carboxy-terminal telopeptide of collagen type I, a collagen type I degradation marker, which could be a potential mechanism responsible for atrial structural remodeling. Secondly, elevated homocysteine also could cause atrial electrophysiological remodeling by causing abnormalities in sodium and potassium currents, such as inhibiting potassium channels and slowing the inactivation and promoting the recovery of sodium channels. Law, et al showed that elevated homocysteine resulted in marked changes in atrial action potentials, such as a more hyperpolarized resting potential, elevated plateau potential during the early stages of repolarization, and abbreviated action po-
Figure 5. Forest plots of the association of homocysteine levels and recurrence of AF. 

A: Forest plot of studies in homocysteine levels for AF without recurrence patients versus AF with recurrence. 

B: Homocysteine levels as a categorical variable and risk of AF with recurrence. 

C: Homocysteine levels as a continuous variable and risk of AF with recurrence.

tential duration (APD). They also found that simulated re-entrant scroll waves were sustained under high homocysteine conditions compared with self-terminated under control conditions, demonstrating the pro-arrhythmic effects of elevated homocysteine in promoting and sustain-
ing AF. Thus, these studies likely reflect progressive electrophysiological and structural remodeling in both atria, making sources of the arrhythmia more stable and long-lasting. Some clinical studies have shown that aging influences the development and prognosis of patients with
Homocysteine has also been reported to gradually increase with age.\(^{20}\) Thus, we speculated that it is plausible that the expression of homocysteine and its association with AF may be age-related.

**Study limitations:** There are some limitations that should be considered in our meta-analysis. First, there was significant heterogeneity because the studies used diverse methodologies; thus, conclusions should be drawn with caution. Second, studies published in other languages and unpublished studies were not included in our meta-analysis, which can result in publication bias. Third, homocysteine levels were analyzed as either a continuous or categorical variable in the individual studies and the meta-analysis could not pool all of the studies together. Thus, the number of eligible studies for each sub-analysis was relatively small. More large prospective studies are needed to further examine this association. Fourth, funnel plots and the Beg and Egger test should have been used for the assessment of any publication bias, however, we did not perform the latter because the number of pooled meta-analyses was less than 10 for each comparison. Publication bias is an inevitable problem in a meta-analysis of methodologies; thus, conclusions should be drawn with caution.

**Conclusion**

The present meta-analysis identified that plasma homocysteine levels were significantly elevated in AF and recurrent AF patients. Elevated homocysteine is associated with an increased risk of AF and AF recurrence. Meanwhile, further in-depth studies are required to determine whether there is a direct causal relationship between elevated homocysteine and AF, as well as whether homocysteine-lowering therapy with vitamins B6 and B12 and folic acid supplementation could prevent new-onset AF and AF recurrence.

**Disclosures**

**Conflicts of interest:** None.

References

1. Finkelstein JD, Martin JJ. Homocysteine. Int J Biochem Cell Biol 2000; 32: 385-9.
2. May HT, Alharethi R, Anderson JL, et al. Homocysteine levels are associated with increased risk of congestive heart failure in patients with and without coronary artery disease. Cardiology 2007; 107: 178-84.
3. Vizzardi E, Bon adei I, Zanini G, et al. Homocysteine and heart failure: an overview. Recent Pat Cardi ovasc Drug Discov 2009; 4: 15-21.
4. Gutierrez C, Blanchard DG. Diagnosis and treatment of atrial fibrillation. Am Fam Physician 2016; 94: 442-52.
5. Cingorhay BY, Yiginer O, Cебeci BS, Kardesoglu E, Demiralp E, Dinçturk M. Role of homocysteine for thromboembolic complication in patients with non-valvular atrial fibrillation. Blood Coagul Fibrinolysis 2002; 13: 609-13.
6. Shi D, Meng Q, Zhou X, et al. Factors influencing the relation-ship between atrial fibrillation and artery stiffness in elderly Chinese patients with hypertension. Aging Clin Exp Res 2016; 28: 653-8.
7. Schnabel RB, Larson MG, Yamamoto JF, et al. Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community. Circulation 2010; 121: 200-7.
8. Huang L, Shen R, Huang L, Yu J, Rong H. Association between serum copper and heart failure: a meta-analysis. Asia Pac J Clin Nutr 2019; 28: 761-9.
9. Zhang Z, Yang Y, Ng CY, et al. Meta-analysis of vitamin d deficiency and risk of atrial fibrillation. Clin Cardiol 2016; 39: 537-43.
10. Marcuschi R, Betti L, Cecchi E, et al. Hyperhomocysteinemia and vitamin b6 deficiency: New risk markers for nonvalvular atrial fibrillation? Am Heart J 2004; 148: 456-61.
11. Giusti B, Gori AM, Marcuschi R, et al. Role of c677t and a1298 c mthr, a2756g mtr and -786 c/t enox gene polymorphisms in atrial fibrillation susceptibility. PLoS One 2007; 2: e495.
12. Shimano M, Inden Y, Tsuji Y, et al. Circulating homocysteine levels in patients with radiofrequency catheter ablation for atrial fibrillation. Europace 2008; 10: 961-6.
13. Naji F, Suran D, Kanic V, Vokac D, Sabovic M. High homocysteine levels predict the recurrence of atrial fibrillation after successful electrical cardioversion. Int Heart J 2010; 51: 30-3.
14. Nasso G, Boni fazi R, Romano V, et al. Increased plasma homocysteine predicts arrhythmia recurrence after minimally invasive epicardial ablation for nonvalvular atrial fibrillation. J Thorac Cardiovasc Surg 2013; 146: 848-53.
15. Yao Y, Gao LJ, Zhou Y, et al. Effect of advanced age on plasma homocysteine levels and its association with ischemic stroke in non-valvular atrial fibrillation. J Geriatr Cardiol 2017; 14: 745-9.
16. Yao Y, Yao W, Bai R, et al. Plasma homocysteine levels predict early recurrence after catheter ablation of persistent atrial fibrillation. Europace 2017; 19: 66-71.
17. Kubota Y, Alonso A, Heckbert SR, Norby FL, Folsom AR. Homocysteine and incident atrial fibrillation: The atherosclerosis risk in communities study and the multi-ethnic study of atherosclerosis. Heart Lung Circ 2019; 28: 615-22.
18. Joseph I, Joseph L, Shekhawat NS, et al. Hyperhomocysteinemia leads to pathological ventricular hypertrophy in normotensive rats. Am J Physiol Heart Circ Physiol 2003; 285: H679-86.
19. Miller A, Mujumdar V, Palmer L, Bower JD, Tyagi SC. Reversal of endocardial endothelial dysfunction by folate acid in homocysteinemic hypertensive rats. Am J Hypertens 2009; 22: 876-81.
20. Cai BZ, Gong DM, Liu Y, et al. Homocysteine inhibits potassium channels in human atrial myocytes. Clin Exp Pharmacol Physiol 2007; 34: 851-5.
21. Cai B, Shan L, Gong D, et al. Homocysteine modulates sodium channel currents in human atrial myocytes. Toxicology 2009; 256: 201-6.
22. Law P, Kharche S, Stott J, Zhang H. Effects of elevated homocysteine hormone on electrical activity in the human atrium: a simulation study. Conf Proc IEEE Eng Med Biol Soc 2009; 2009: 3936-9.
23. Yao Y, Shang MS, Dong JZ, Ma CS. Homocysteine in non-valvular atrial fibrillation: Role and clinical implications. Clin Chim Acta 2017; 475: 85-90.
24. Wolff A, Shantsila E, Lip GY, Lane DA. Impact of advanced age on management and prognosis in atrial fibrillation: Insights from a population-based study in general practice. Age Ageing 2015; 44: 874-8.
25. Taskin G, Yilmaz Sipahi E, Yildirimkaya M, et al. Plasma total homocysteine levels in a healthy turkish population sample. Acta Cardiol 2006; 61: 35-42.