Sex differences in risk factors for stroke in patients with hypertension and hyperhomocysteinemia

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Data on the sex-specific differences in risk of stroke among patients with H-type hypertension are limited. We aimed to analyze interactions between sex and other risk factors on stroke, including the sex-methylenetetrahydrofolate reductase (MTHFR) interaction. A retrospective analysis of baseline data from 2040 patients with hypertension and hyperhomocysteinemia (HHcy) included demographic characteristics, biomarkers, history of chronic diseases and lifestyle factors. Polymerase chain reaction-restriction fragment length polymorphism method was used to investigate the C677T polymorphism of MTHFR gene. We examined independent effects and interactions between sex and stratified factors on the risk of stroke by logistic regression model. A total of 1412 patients suffered stroke, and the prevalence of stroke was 70.65% in men and 66.53% in women. Both men and women had independent risk factors for stroke, including diabetes mellitus, atrial fibrillation, smoking, increased level of systolic blood pressure (SBP) and plasma total homocysteine (tHcy), as well as the decreased level of high-density lipoprotein cholesterol. Diastolic blood pressure (DBP) -specific risk of stroke was unique to men. Interactions between sex and other risk factors on stroke risk were statistically significant: age, fasting plasma glucose (FPG), SBP, DBP, triglycerides (TG) and tHcy. Furthermore, tHcy interacted with age, SBP and DBP in men, and age, SBP, DBP, FPG, and TG in women to modulate the risk of stroke. Although TT genotype did not have an independent effect on stroke, it could interact with sex and FPG, TG and SBP to increase stroke. In conclusion, sex-specific differences are useful to stratify the risk of stroke and assist clinicians in the decision to select a reasonable therapeutic option for high-risk patients.

Stroke, a type of cerebral impairment syndrome induced by acute blood circulation in brain, is one of the leading causes of mortality and morbidity, with Asian disproportionately affected. To date, the global burden of management for patients with stroke is mainly coming from Asia due to rise in the prevalence of vascular risks. In China, stroke featured by high prevalence, mortality, morbidity and recurrence, is considered as the leading cause for death and disability. In general, the male population are prone to develop stroke compared to the female counterparts, however, a higher incidence of disability is reported in women and the possibility of functional independence is comparatively lower. Sex differences in stroke risk and prognosis may be associated with the genetic backgrounds, including immune system, coagulation, endocrine system, reproductive system, as well as social factors.

Hyperhomocysteinemia (HHcy) defined as the concentration of plasma total homocysteine (tHcy) of ≥10 μmol/L, is often a consequence of reduced enzymatic activity involved in homocysteine metabolism, such as a point mutation of methylenetetrahydrofolate reductase (MTHFR) in which cytosine is replaced by thymidine at position 677 (MTHFR C677T). H-type hypertension, which refers to concurrence of primary hypertension and HHcy, is an important factor for increased prevalence and persistent progression of stroke in China mainland. Previous studies have shown that hyperhomocysteinemia (HHcy) and hypertension often coexist. HHcy

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and hypertension are two independent, modifiable risk factors for incident stroke and stroke death. Moreover, coexistence of HHcy and hypertension could act additively on a multiplicative scale, which contributed to the risk of stroke and stroke-related death.

Many studies have shown sex differences in the association between Hcy and different diseases. A study from the Korea that included 150 men and 132 women showed that Hcy was associated with the prevalence of non-alcoholic fatty liver disease in men but not in women. Chen et al. conducted a cross-sectional study, and observed that Hcy was negatively associated with estimated glomerular filtration rate, which was more profound in women. Sex differences in the association between traditional risk factors besides MTHFR C677T genotype and stroke in patients with H-type hypertension remain unsettled and include the possibility of a sex interaction. The objective of this study was to investigate sex differences in independent effect of risk factors on stroke and important interactions between individual factors by subgroup analysis.

**Subjects and Methods**

**Study design.** This was a retrospective study among 2040 hospitalized patients with H-type hypertension (men: 1329; women: 711; age: 61.02 ± 8.68 years) recruited continuously from October 2013 to October 2014 in Brain Hospital, Affiliated Hospital of Xuzhou Medical University and department of Cardiology, Xuzhou Central Hospital. Individuals with a systolic blood pressure (SBP) of 140 mm Hg or higher or a diastolic blood pressure (DBP) of 90 mm Hg or higher at rest, or with definite medical records for hypertension and current administration with antihypertensive drugs, were all diagnosed as hypertension. It includes graded 1 (140–159 mmHg SBP and/or 90–99 mmHg DBP), 2 (160–179 mmHg SBP and/or 100–109 mmHg DBP), and 3 (≥180 mmHg SBP and/or ≥110 mmHg DBP). Types of stroke included cerebral ischemic stroke, hemorrhagic stroke and transient ischemic attack (TIA). Stroke is based on strict neurological examination and computer tomography scans or magnetic resonance imaging. Confirmation of dyslipidemia is in accordance with the 2017 American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for the management of dyslipidemia and prevention of cardiovascular disease. Triglyceride (TG) levels <1.7 mmol/L are normal, 1.7 to 2.3 mmol/L are borderline-high and >2.3 mmol/L are high. Total cholesterol (TC) levels <5.2 mmol/L are normal, ≥5.2 and <6.2 mmol/L are borderline-high and ≥6.2 mmol/L are high. Diabetes mellitus (DM) is diagnosed based on the fasting plasma glucose (FPG) ≥7.0 mmol/L. When the FPG concentration is ≥6.1 and <7.0 mmol/L, impaired fasting glucose (IFG) should be considered. Body mass index (BMI) level <24.0 kg/m² is considered “normal” for adults, with 24.0 to 27.9 kg/m² considered “overweight” and 28.0 kg/m² or greater considered “obesity.” The cutoff values for age are based on recommendations from the Global Burden of Disease Study 2010. Those with secondary hypertension, acute or chronic inflammation, primary nephritis, arthritis, and systemic lupus erythematosus were excluded from the study. Study protocols were approved by the Ethics Committee of Xuzhou Central Hospital, and each patient signed the informed consent. All methods were performed in accordance with relevant guidelines and regulations.

**Table 1.** Sex differences in demographic characteristics of patients with H-type hypertension. Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; Lg tHcy, Log(10) total homocysteine; DM, diabetes mellitus; CAD, coronary artery disease; AF, atrial fibrillation.
C677T Polymorphism of the MTHFR Gene.

Data collection and blood chemical assay. The collected clinical data included age, sex, BMI, cigarette smoking, alcohol consumption, SBP, DBP, as well as history of chronic diseases, such as stroke, coronary artery disease (CAD), DM, hyperlipidemia, and atrial fibrillation (AF). Fasting venous blood was collected from each participant within 24 hours after admission to measure FPG, TG, TC, high-density lipoprotein cholesterol (HDL-C) and tHcy. tHcy was determined using fluorescence polarization immunonoassay method. Commercial kits used for the determination were purchased from the Abbott Corporation (CA, US).

C677T Polymorphism of the MTHFR Gene. Genomic DNA was extracted from peripheral venous blood anticoagulated by EDTA using a GenElute™ Blood Genomic DNA Kit (Sigma-Aldrich Chemical Corporation, St. Louis, MO, US), according to manufacturer’s protocol. Genotyping C677T polymorphism of MTHFR gene was investigated using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A 198 bp fragment was amplified by PCR using oligonucleotide primer sequences forward primer 5′- TGAAGGAGAAGGTGTCTGCGGGA-3′ and reverse 5′-AGGACGGTGCGGTGA GAGTG-3′. Amplified PCR products were digested with HinfI restriction enzyme (Promega Corporation, Madison, US). Mutation at position 677, where C was replaced by T, created a restriction site for the enzyme. The wild-type (CC) genotype produced a 198 bp fragment. Heterozygous (CT) genotype produced two fragments: 198 bp and 175 bp, and the homozygous mutant (TT) produced a 175 bp fragment. For verifying the genotyping results of PCR-RFLP, 600 subjects with different genotypes were randomly selected for DNA sequencing analysis, with 100% agreement.

Statistical analysis. Analyses were performed by using SPSS 20.0 software. Continuous variables were presented as mean ± standard deviation. Categorical variables were presented as number of cases (n) and percentage. tHcy was log transformed to reduce skewness and heteroscedasticity. Sex differences in demographic characteristics of the participants were compared using Chi-square tests for categorical factors and Student’s t-tests for continuous variables. Using Kruskal-Wallis test, we can assess intergroup significance among the stratified factors. Multiple binary logistic regression models were constructed using the forced entry method to evaluate the associations between stroke risk and risk factors, interactions between tHcy and other risk factors on stroke risk by sex in patients with H-type hypertension. The models had adjustment for age, BMI, SBP, DBP, FPG, TG, TC, HDL-C, tHcy, smoking, alcohol consumption, MTHFR C677T polymorphism, and comorbidity. We defined comorbidity as a history of a diagnosis of 1 of the following previously reported concomitant risk factors: DM, hyperlipidemia, CAD, or AF. Binary variables for each of these factors and MTHFR C677T polymorphism were included as dummy variables in the models. Interactions between sex and age, BMI, FPG, SBP, DBP, TG, TC, tHcy and MTHFR C677T polymorphism on stroke risk were also individually tested by adding multiplicative term of sex and stratified factors besides all baseline variables to multiple binary logistic regression models. Interaction between each subgroup factor and sex was tested for differences in stroke risk among a subset of individuals with known CT/TT subtypes compared with CC subtype. Factors of subgroup analyses included age (≥ 55 years

| Variables | Men, n = 1329 | Coefficient | SE | OR (95%CI) | P-value | Women, n = 711 | Coefficient | SE | OR (95%CI) | P-value |
|-----------|--------------|-------------|----|------------|---------|----------------|-------------|----|------------|---------|
| Age       | −0.014       | 0.009       | 0.986 (0.969–1.003) | 0.108 | 0.012 | 0.013 | 1.012 (0.987–1.038) | 0.346 |
| BMI       | 0.024        | 0.042       | 1.024 (0.944–1.112) | 0.563 | 0.016 | 0.073 | 1.016 (0.881–1.172) | 0.823 |
| SBP       | 0.045        | 0.005       | 1.047 (1.036–1.057) | <0.001 | 0.035 | 0.006 | 1.036 (1.024–1.048) | <0.001 |
| DBP       | 0.025        | 0.008       | 1.025 (1.009–1.042) | 0.002 | 0.020 | 0.011 | 1.020 (0.999–1.041) | 0.068 |
| FPG       | −0.026       | 0.053       | 0.975 (0.879–1.080) | 0.623 | 0.101 | 0.072 | 1.107 (0.962–1.274) | 0.157 |
| TG        | −0.071       | 0.092       | 0.932 (0.778–1.117) | 0.445 | 0.130 | 0.125 | 1.139 (0.892–1.454) | 0.297 |
| TC        | 0.163        | 0.139       | 1.177 (0.896–1.545) | 0.242 | 0.037 | 0.172 | 1.037 (0.741–1.453) | 0.831 |
| HDL-C     | −1.708       | 0.288       | 0.181 (0.103–0.318) | <0.001 | −1.479 | 0.343 | 0.228 (0.116–0.446) | <0.001 |
| tHcy      | 0.019        | 0.006       | 1.019 (1.007–1.031) | 0.001 | 0.049 | 0.014 | 1.050 (1.022–1.080) | <0.001 |
| Cigarette smoking | 0.949 | 0.143       | 2.583 (1.952–3.417) | <0.001 | 1.119 | 0.484 | 3.061 (1.185–7.907) | 0.021 |
| Alcohol consumption | 0.238 | 0.178       | 1.269 (0.896–1.796) | 0.180 | −0.400 | 0.516 | 0.670 (0.244–1.842) | 0.438 |
| DM        | 0.847        | 0.239       | 2.333 (1.461–3.726) | <0.001 | 0.925 | 0.319 | 2.322 (1.351–4.709) | 0.004 |
| Hyperlipidemia | −0.238 | 0.178       | 0.788 (0.557–1.116) | 0.180 | 0.038 | 0.242 | 1.039 (0.646–1.670) | 0.876 |
| CAD       | 0.052        | 0.193       | 1.053 (0.722–1.537) | 0.788 | 0.125 | 0.250 | 1.134 (0.694–1.852) | 0.616 |
| AF        | 1.741        | 0.677       | 5.702 (1.513–21.494) | 0.010 | 2.193 | 1.108 | 8.964 (1.022–78.603) | 0.048 |

MTHFR C677T Polymorphism

| TT (vs. CC) | 0.019 | 0.0214 | 1.019 (0.670–1.550) | 0.930 | −0.067 | 0.263 | 0.935 (0.558–1.565) | 0.798 |
| CT (vs. CC) | 0.042 | 0.167 | 1.042 (0.752–1.445) | 0.803 | 0.283 | 0.229 | 1.327 (0.847–2.079) | 0.218 |

Table 2. Associations between stroke risk and risk factors by sex in patients with H-type hypertension. Abbreviations: SE, standard error; OR, odds ratio; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; tHcy, total homocysteine; DM, diabetes mellitus; CAD, coronary artery disease; AF, atrial fibrillation. Logistic regression models were adjusted for age, BMI, SBP, DBP, FPG, TG, TC, HDL-C, tHcy, cigarette smoking, alcohol consumption, MTHFR C677T polymorphism, and comorbidity.
Results

Sex differences in patient demographics. Among the 2040 patients with H-type hypertension, FPG, TG, TC, and HDL-C levels in women were significantly higher than those of men, and the prevalence of hyperlipidemia was 52.32% and 45.82% for women and men, respectively. However, plasma tHcy (logarithmic value, Lg tHcy) level in women was significantly lower than that in men, as was also their exposure to environmental cigarette smoking and alcohol consumption. No statistical sex differences were observed in the age, SBP, DBP, BMI, genotype distributions of the MTHFR C677T polymorphism, and prevalence of stroke, CAD, DM and AF (P > 0.05, Table 1).

Sex differences in stroke risk factors. Binary Logistic regression analysis was carried out to assess the associations between stroke risk and risk factors by sex in patients with H-type hypertension, after adjustment for MTHFR C677T polymorphism and other conventional risk factors. Independent risk factors for stroke in both men and women included DM, AF, smoking, increased level of SBP and tHcy, as well as the decreased level of HDL-C. However, DBP elevation was an independent risk factor for stroke in men, but not in women (Table 2).
**Table 4.** Analysis of tHcy interaction with risk factors in both men and women with H-type hypertension. Abbreviations: tHcy, total homocysteine; SE, standard error; OR, odds ratio; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides. Logistic regression models were adjusted for age, body mass index, SBP, DBP, FPG, TG, total cholesterol, high-density lipoprotein cholesterol, tHcy, cigarette smoking, alcohol consumption, MTHFR C677T polymorphism, and comorbidity.

**Interactions between sex and other risk factors on stroke risk.** Data were reported separately for men and women, and stratified in terms of age-, BMI-, FPG-, blood pressure (BP)-, lipid-, and genotype-specific rates of stroke prevalence. Interactions between sex and other risk factors on stroke risk were statistically significant: age (P = 0.005), FPG (P = 0.002), SBP (P < 0.001), DBP (P < 0.001), TG (P = 0.004) and tHcy (P = 0.038) after adjustment for sex, age, BMI, SBP, DBP, FPG, TG, TG, HDL-C, tHcy, MTHFR C677T polymorphism and history of cigarette smoking, alcohol consumption, DM, hyperlipidemia, CAD, and AF. Subgroup analyses of each risk factor revealed that risk of stroke in those with age ≥55 years, FPG ≥6.1 mmol/L, and TG ≥1.978 mmol/L (Fig. 2) with TT genotype was the strongest, and men interacted with TT genotype and SBP ≥160 mmHg (Fig. 1) or ≥5.54 mmol/L of tHcy, a tHcy-FPG (P = 0.038) and tHcy (P = 0.009) interaction could also increase stroke risk in women (Table 4).

MTHFR genotype-sex-risk factors interactions revealed that stroke risk in women ≥6.1 mmol/L of FPG (Fig. 1) or ≥2.3 mmol/L of TG (Fig. 2) with TT genotype was the strongest, and men interacted with TT genotype and SBP ≥160 mmHg (Fig. 3) on increased risk of stroke (Table 5).

**Discussion**

The present study has shown that most independent risk factors for stroke in women were similar to those in men among patients with H-type hypertension, including DM, AF, smoking, increased level of SBP and tHcy, as well as decreased HDL-C. DBP-specific risk of stroke was unique to men. Interactions between sex and other risk factors on stroke risk were statistically significant: age, FPG, SBP, DBP, TG and tHcy. Furthermore, an interaction between tHcy and other factors also modulated the risk of stroke, including age, SBP and DBP in men, and age, SBP, DBP, FPG, and TG in women. At the same time, although TT genotype did not have an independent effect on stroke, it could interact with sex and FPG, TG and SBP to increase stroke.

H-type hypertension has a large negative impact on society. Besides an independent effect, a multiplicative effect of hypertension and HHcy substantially increases the risk for stroke\(^4\). Sex differences in incident and
recurrent stroke, temporal patterns of stroke, and outcomes after stroke have been reported in published studies. As noted previously, females have a lower ischemic stroke incidence over much of their life span than their age-matched male counterparts, which was changed in those aged >85 years. Stroke risk factors include reproductive factors that are unique to women and obesity, metabolic syndrome, and AF that are more common in women than men. Data on the epidemiology of stroke in South, East, and South-East Asia indicated that a high prevalence of hypertension, DM and tobacco smoking was seen among men, whereas a high prevalence of hypercholesterolemia, inactivity and obesity was seen among women.

Our study found effects of DM, AF, smoking, increased level of SBP and tHcy, as well as decreased HDL-C on stroke risk were independent, after multivariate adjustment for risk factors. However, DBP-specific risk of stroke was independent in men but not in women. As the independent risk factors for stroke were similar in men and women, an in-depth analysis should be considered to investigate the interaction between sex and other stroke risk factors in patients with H-type hypertension. As a result, interactions between sex and other risk factors

**Figure 1.** A MTHFR genotype-sex-fasting plasma glucose (FPG) interaction on stroke risk with H-type hypertension.

**Figure 2.** A MTHFR genotype-sex-triglycerides (TG) interaction on stroke risk with H-type hypertension.

**Figure 3.** A MTHFR genotype-sex-systolic blood pressure (SBP) interaction on stroke risk with H-type hypertension.
Sex differences in BP control rates were observed between women and men. Specifically, women tended to have lower BPs than men across much of their lifespan. However, the prevalence of hypertension becomes higher in postmenopausal women than men after 55 years. Among participants with hypertension after 80 years in the Framingham Heart Study, 38% of men but only 23% of women showed a BP of ≥160 mmHg. A higher risk of stroke in women was associated with higher age, FPG, TG and tHcy levels. These risk factors included age, SBP and DBP in men, and age, SBP, DBP, FPG, and TG in women.

Table 5. MTHFR genotype-sex-risk factors interactions on stroke risk in 2040 patients with H-type hypertension. Abbreviations: SE, standard error; OR, odds ratio; CI, confidence interval; BMI, body mass index; FPG, fasting plasma glucose; DBP, diastolic blood pressure; TC, total cholesterol; tHcy, total homocysteine; SBP, systolic blood pressure. Logistic regression models were adjusted for sex, age, BMI, SBP, DBP, FPG, TG, TC, high-density lipoprotein cholesterol, tHcy, cigarette smoking, alcohol consumption, MTHFR C677T polymorphism, and comorbidity.
MTHFR C677T mutation is associated with many diseases, such as stroke32, CAD33, and thrombosis34. Homozygous form (TT) of the MTHFR C677T is prevalent in 10% of the population. This genotype has also been shown to correlate with elevated Hcy levels35. Our study observed that TT genotype rates were higher both in men and women than the above investigation demonstrated, and stroke rates of TT subgroup were higher in men than women among patients with H-type hypertension. However, there was no association between TT genotype and stroke risk after adjustment for multiple risk factors both in men and women. We assessed the complexities of the interactions of risk factors with a stratified analysis. Differences in interactions between TT genotype and risk factors in stroke risk among men and women are recognized. The association of higher SBP with stroke risk was the strongest in male patients with TT genotype, and certain interactions between TT genotype and FPG, or TT genotype and TG are specific to women's health. Both sex and genotype are non-modifiable risk factors for stroke. Therefore, successful reduction of SBP, FPG and TG may be much more important to decrease stroke risk.

Study Limitations. This retrospective study had several limitations. First, although logistic regression model was performed as a means of controlling for differences in characteristics of the patients and their diseases, we had no medication data on admission, such as antihypertensive, lipid-lowering, hypoglycemic and even anti-platelet agents. That might have had an influence on distinguishing the relationship between sex and stroke risk. Second, due to nature of a cross-sectional study, the time of stroke onset was unknown in our study. Data were analyzed retrospectively after onset of stroke. Precise information about the pre-stroke status was unavailable. Both of these factors limit conclusions of a causal relationship between risk factors and outcome, which will be illustrated by the ongoing follow-up phase of our study. Third, because this was a hospital-based study based on data from a single medical center, characteristics of the present subjects might differ from those of the general community. Thus, a prospective multicenter registry trial with a large sample population is needed to clarify our results.

Conclusions
Risk stratification in H-type hypertension that account for sex-specific differences is useful to stratify risk of stroke and assist clinicians in the decision to select a reasonable therapeutic option for high-risk patients. Our study shows that, although most independent risk factors for stroke in women are similar to those in men, sex differences in interactions between individual factors that need to be considered in predicting overall risk are much more important. For example, tHcy, but not MTHFR C677T, is an independent risk factor for stroke. However, MTHFR C677T can interact with sex and other factors to increase stroke risk.

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Author Contributions

H.P. conceived and designed the study, collected data, and supervised all the work. Q.C. and Q.F. collected data and planned the analyses. L.H. did all calculations and interpreted the data. Z.Z. supervised the statistical analyses and drafted the manuscript. All authors contributed intellectually to the manuscript and all reviewed drafts and accepted the final draft.

Additional Information

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