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Research article

Homocysteine levels, H-Hypertension, and the MTHFR C677T genotypes: A complex interaction

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ABSTRACT

Background and objectives: High homocysteine levels are associated with increased risk of hypertension and stroke. Homocysteine is metabolized by the methylenetetrahydrofolate reductase (*MTHFR*). We aimed to investigate the levels of homocysteine and their association with hypertension, stroke, and antihypertensive medication usage in patients with different *MTHFR* C677T genotypes.

Methods and results: Genotype frequency of *MTHFR* polymorphism was performed, and plasma homocysteine levels were measured in 2,640 adult Lebanese patients. Hypertension, history of stroke, and list of medications were documented, among other clinical and demographic parameters. The TT mutant genotype and the T mutant allele of *MTHFR* were more prevalent in hyperhomocysteinemia (HHcy) and H-hypertensive (H-HTN, defined as hypertension with hyperhomocysteinemia) patients when compared to non-HHcy subjects and non H-HTN patients respectively. Homocysteine levels were significantly higher in hypertensive patients specifically

Abbreviations: HTN, Hypertension; H-HTN, H-type hypertension; MTHFR, methyltetrahydrofolate reductase; HHcy, Hyperhomocysteinemia; NHANES III, the Third National Health and Nutrition Examination Survey; SHEP, Systolic Hypertension in the Elderly Program; CSPPT, Chinese stroke primary prevention trial; MEIA, Microparticle enzyme immunoassay; HWE, Hardy–Weinberg equilibrium; ACE, Angiotensin-converting enzyme.

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among those on diuretics. A higher level of homocysteine was found in hypertensive patients with the *MTHFR* T allele compared to patients carrying the C allele. Among the T allele carriers, the average plasma homocysteine level was 13.3 ± 0.193 μmol/L for hypertensive subjects compared to 11.9 ± 0.173 μmol/L (non-hypertensives). Furthermore, homocysteine levels significantly correlated with stroke risk in patients with the T alleles.

Conclusions: We found an association of homocysteine with hypertension, hypertensive medication, and stroke risk among patients with the *MTHFR* T allele and the TT genotype. The association of diuretics therapy with higher homocysteine levels calls for routine measurements and therapeutic control of homocysteine in patients on diuretic, to improve health-related outcomes.

1. Introduction

H-type hypertension (H-HTN) is characterized by increased blood pressure associated with Hyperhomocysteinemia (HHcy), which is defined as plasma homocysteine level ≥10 μmol/L, due to defective metabolism of plasma homocysteine [1].

H-HTN has been under extensive investigation in recent years. Despite some controversy, elevated levels of plasma homocysteine are an emerging factor associated with several conditions such as congenital heart defect, cardiovascular disease, and neurodegenerative disorders (i.e, Alzheimer's) [2,3]. The relationship between HHcy and HTN has come to contrasting conclusions across several studies. Multiple investigations, such as the Framingham study, failed to establish HHcy as a culprit for HTN [4,5]. Other reports convincingly linked HHcy to HTN and crystallized it as a new risk factor in multiple other findings [6,7]. A large observational trial, conducted as part of the NHANES III (The Third National Health and Nutrition Examination Survey), established an association between HHcy and HTN [8]. Similar findings were provided by the SHEP (Systolic Hypertension in the Elderly Program) study, which showed a direct correlation between HHcy and elevated systolic blood pressure [7]. Of interest, the prevalence of HTN in China is 29.6%, and approximately 75% of hypertensive patients have elevated homocysteine levels [9]. Similarly, the Chinese stroke primary prevention trial (CSPPT) showed that lowering homocysteine levels in patients with H-HTN, through folate intake, had a significant impact on primary prevention of stroke in these patients [10]. H-HTN was associated with an increased risk of all-cause mortality (ACM) in Chinese and in older American patients [11–13]. Therefore, for every 1 μmoL/l increment of homocysteine, hypertensive patients had a 7% higher risk of cardiovascular disease mortality and an 8% higher risk of ACM.

High homocysteine levels are linked to an increased risk of ischemic stroke, mainly involving large but also small arteries. However, high homocysteine levels are not believed to be associated with the cardioembolic and other stroke subtypes [14,15].

There are contradictory reports in the literature regarding the association of genetically determined elevations in plasma homocysteine with risk for cerebrovascular and cardiovascular disease [16–19]. Zhao et al. investigated the impact of the *MTHFR* C677T polymorphism on the association of homocysteine with stroke. *MTHFR* codes for an enzyme involved in the breakdown of methionine, using folate and vitamin B12 [20,21]. They reported that in hypertensive patients with the CC/CT genotype, homocysteine levels were associated with an increased risk of stroke. However, this association was not seen in patients with the TT genotype, highlighting a significant role for the genetic background of patients in such association [22]. A meta-analysis involving more than 11,000 *MTHFR* homozygous patients and nearly 13,000 matched controls from 40 observational studies found that the *MTHFR* TT genotype is associated with a higher risk for cardiovascular disease [16]. On the contrary, another meta-analysis of genome-wide association studies reported no association between genetic variants that influence plasma total homocysteine concentrations and cardiovascular disease [18]. The perceived associations between mild hyperhomocysteinemia and cardiovascular diseases may be due to an overlook of confounding risk factors [23–25].

Although the prevalence of H-HTN is reaching an epidemic proportion, its management is still poorly explored in many populations, including the current study population. In fact, even observational studies on the prevalence and prognosis of H-HTN in Lebanon are inexistent. Previous studies targeted Lebanese adults and concluded a prevalence of HTN of 35.9%, with only 27% having controlled blood pressure, while another study yielded similar results with the following values 31.2% and 28.7%, respectively [26,27]. Nonetheless, there is a paucity of information on the types of HTN and their associated treatment within the Lebanese population, particularly H-HTN. The plasma levels of homocysteine and consequently H-HTN are under the control of exogenous and endogenous factors [28]. We previously described the frequency of the *MTHFR* 677C > T polymorphism in the Lebanese population [20,21,28]. However, the correlation between the 677C > T polymorphism, HHcy, HTN, and its management was not evaluated. This study investigates the levels of homocysteine and their association with HTN, stroke, and antihypertensive medication in patients with different *MTHFR* C677T genotypes. The prevalence of HHcy and H-HTN is also determined.

2. Methods

2.1. Study subjects

A total of 2,640 Lebanese subjects were recruited for this study: out of which 1,589 were hypertensive and 1,051 were not hypertensive and served as control group. The subjects are a subset of a larger group (8,000) enrolled as part of a multicenter cross-sectional study for the FGENTCARD Consortium [29], now part of the CARDIoGRAMplusC4D consortium (http://www.cardiogramplusc4d.org). These patients were recruited at two major University Hospitals in Lebanon, one located in Beirut and one in

the Northern part of the country. Only patients with documented homocysteine levels and other biochemical and clinical data listed in Table 1, were included in this study. The genotyping of the samples was available for a subset of 1,337 subjects. The study was approved by the Institutional Review Board committee at the Lebanese American University. Potential participants were briefed on the purpose and the details of what is expected from them in this study; it was also highlighted that their participation is voluntary; those participants who agreed to enroll in this project signed a written informed consent prior to their participation. All protocols were performed according to the Helsinki Declaration of 1975.

Data collection forms were completed on site to record information about the patients' risk factors (hypertension, lifestyle, and family history). Data on clinical conditions, laboratory tests, and current use of medications were annotated following a medical chart/codes as previously described [28,29].

2.2. Description of variants

The description of variants was done in line with our previous report [28]. Homocysteine concentration >10 μmol/L was defined as hyperhomocysteinemia. Clinical conditions (i.e. diabetes, hypertension, hyperlipidemia and stroke) of the participants were recorded according to the patient's treatment regimen or when an ascertained physician documented the condition in the medical chart. The family history (Fx) of hypertension was included when a close family member to the participant (parent, sibling, or second degree relative) was hypertensive. Medications intake, lipid and other metabolic profiles were also recoded from the patient's records. We calculated the body mass index (BMI) following standard measurement methods. Participants with previous and/or current history of smoking cigarettes were considered Smokers.

2.3. Genotyping and immunoassay

Sampling blood was performed by collecting 20 ml of blood (EDTA and gel separating tubes) from each participant. Blood in EDTA tubes was used for plasma collection and DNA extraction. Blood in gel separator tubes was used to collect serum. For the measurement of homocysteine and the evaluation of lipid profile, samples were centrifuged (15 min at RT), and both the plasma and serum aliquots were stored at −20 °C for further handling, as previously described [28]. Using a microparticle enzyme immunoassay (MEIA) method, we determined the plasma level of homocysteine for 2,640 participants and this according to the manufacturer protocol. Cholesterol levels were measured on COBAS INTEGRA 400 Plus using fasting blood samples collected from the participants.

The genomic DNA from our group of patients was extracted using the phenol extraction method as described previously [28]. The genotyping of the extracted DNA was performed using the Illumina Human610-Quad BeadChip (Illumina, San Diego, CA, USA) and the Illumina Human660W-Quad BeadChip (Illumina, San Diego, CA, USA). Genotyping was performed according to manufacturer's

Table 1
Descriptive statistics of the 2640 subjects.

	Not Hypertensive (%)	Hypertensive (%)	Total	P-value
	1,051 (39.8%)	1,589 (60.2%)	2,640	
Gender				
Male	788 (75.0%)	986 (62.1%)	1,774 (67.2%)	<0.0001*
Female	263 (25.0%)	603 (37.9%)	866 (32.8%)	
Age	57.037 (11.876)	62.556 (10.688)	60.358 (11.496)	<0.0001*
Diabetes				
No	845 (80.4%)	988 (62.2%)	1,833 (69.4%)	<0.0001*
Yes	206 (19.6%)	601 (37.8%)	807 (30.6%)	
Hyperlipidemia				
No	648 (61.8%)	740 (46.6%)	1,388 (52.7%)	<0.0001*
Yes	401 (38.2%)	847 (53.4%)	1,248 (47.3%)	
Total Cholesterol (mg/dL)	188.512 (48.072)	182.147 (46.243)	184.665 (47.069)	<0.0001*
Hyperhomocysteinemia				
No	260 (24.7%)	331 (20.8%)	591 (22.4%)	0.021*
Yes	791 (75.3%)	1,258 (79.2%)	2,049 (77.6%)	
Smoking				
No	346 (33.0%)	605 (38.1%)	951 (36.0%)	0.008*
Yes	704 (67.0%)	984 (61.9%)	1,688 (64.0%)	
Family history of Hypertension				
No	551 (52.5%)	579 (36.6%)	1,130 (42.9%)	<0.0001*
Yes	498 (47.5%)	1,003 (63.4%)	1,501 (57.1%)	
Weight (kg)	79.448 (15.395)	80.263 (15.217)	79.939 (15.290)	0.186
BMI (kg/m^2)	28.310 (4.704)	29.810 (5.073)	29.215 (4.983)	<0.0001*
Stroke				
No	1,042 (99.2%)	1,550 (97.7%)	2,592 (98.3%)	0.004*
Yes	8 (0.8%)	37 (2.3%)	45 (1.7%)	

For categorical variables, the number of individuals and percentages are reported. For scale variables, data are mean ± standard deviation (SD). The P is generated using a chi-square test (categorical variables) and an independent *t*-test (scale variables). P is significant (*) if P-value <0.05. Hyperhomocysteinemia: plasma homocysteine is ≥ 10 μmol/L.

instructions for the Illumina 610 and 660W-Quad arrays.

2.4. Statistical analysis

We processed our data by applying chi-square tests for ordered and unordered categorical measures, and independent *t*-test for continuous variables. We applied generalized linear model (GLM) binomial logistic regression to evaluate the association of HTN and the demographic and clinical data such as gender, age, diabetes, hyperlipidemia, Fx HTN, stroke, total cholesterol and BMI. We also applied binomial regression to evaluate the association between homocysteine and the different medications used for HTN such as beta blockers, calcium channel blockers, Angiotensin-Converting Enzyme (ACE), Angiotensin II antagonists, diuretics, nitrates, and vasodilators. Adjusted odds ratios were obtained using the same regression, after controlling for age and/or sex. P value of <0.05 indicated statistical significance. R package was used to perform the above-mentioned statistical analyses. We performed allele and genotype frequency of *MTHFR* polymorphism (rs1801133) to relate the presence of 677C > T mutation of *MTHFR* with HHcy. Grouped Barplots by the percentage of *MTHFR* alleles and genotypes in HHcy and non-HHcy groups were plotted. The Hardly-Weinberg equilibrium (HWE) was tested with Chi-square (X^2) goodness of Fit test. The distribution of genotypes agrees with HWE if the p value > 0.05. In addition, Fisher's exact test using R package was applied to test the differences between frequencies. Differences were considered significant at p value < 0.05. We evaluated homocysteine levels among T or C allele carriers according to HTN, diuretics intake, and stroke. Two-way ANOVA with Tukey posthoc test was used to determine whether *MTHFR* variants and genotypes, HTN, diuretics or stroke impact homocysteine levels and to determine whether there is an interaction between *MTHFR* alleles/genotypes and HTN, diuretics or stroke, with homocysteine levels.

3. Results

Our study targeted a group of Lebanese adults with a mean age of 60.358 (±11.496) years, a mean body mass weight of 79.939 (±15.290)Kg, a mean BMI of 29.215 (±4.983) Kg/m^2, and a mean total cholesterol 184.665 (±47.069) mg/dL (Table 1). In our groups of patients, 1,774 of the subjects were males (67.2%) and 866 were females (32.8%). A total of 1,589 (60.2%) of the subjects were hypertensive, 807 subjects were diabetic (30.6%), 1,248 were hyperlipidemic (47.3%), 2,049 had hyperhomocysteinemia (77.6%) (HHcy: homocysteine >10 μmol/L), 1,501 had family history (Fx) of hypertension (57.1%), 45 had history of stroke (1.7%), and 1,688 subjects were smokers (64%). Significant differences between hypertension patients and control individuals for age, gender, stroke, diabetes, hyperlipidemia, HHcy, smoking, Fx HTN, total cholesterol, BMI and homocysteine levels were detected. About 37.9% of the hypertensive subjects were females and 62.1% were males, while 25% of the non-hypertensive subjects were females and 75% were males. This gender-linked component was statistically significant ($P < 0.001$). Diabetes and hyperlipidemia were both significantly elevated in people with HTN when compared with non-hypertensive subjects, from 19.6% to 37.8% and from 38.2% to 53.4%, respectively. HHcy were significantly higher in hypertensive patients compared to non-hypertensive patients (79.2% vs 75.3%). It is worth noting that 48.8% of our participants suffer from H-hypertension. Stroke history was also significantly higher in hypertensive patients compared to non-hypertensives (2.3% vs 0.8%). Family history of hypertension was significantly increased from 47.5% to 63.4% in hypertensive patients compared to non-hypertensives. BMI levels were significantly different between HTN and non-HTN groups ($P < 0.0001$).

Following adjustment for age and sex, we identified significant association between HTN and the clinical and biochemical features (Table 2). Most notably diabetes, hyperlipidemia, and Fx HTN were shown to be significantly associated with HTN, with adjusted odds ratio (OR) of 2.32 [95% C.I. 1.92–2.80], 1.89 [1.60–2.23], and 2.21 [1.86–2.61], respectively. The risk of HTN was significantly correlated with HHcy with OR of 1.28 [1.06–1.56]. BMI was also positively correlated with HTN with OR of 1.06 [1.04–1.08]. Stroke was negatively associated with HTN with OR of 0.41 [0.17–0.85].

We explored the relationship between antihypertensive medications intake and plasma homocysteine levels. The intake of beta-blockers ($P = 0.014$) or ACE (Angiotensin-Converting Enzyme) inhibitors ($P = 0.03$) was significantly associated with HHcy (Table 3). The intake of diuretics showed the strongest association (OR = 2.03 [1.54–2.70]; $P < 0.0001$) with plasma homocysteine

Table 2
Association analysis between hypertension and different sample metadata.

	P-value	OR	95%C.I.	
Gender	<0.0001*	1.69	1.42	2.02
Age	<0.0001*	1.04	1.04	1.05
Diabetes	<0.0001*	2.32	1.92	2.80
Hyperlipidemia	<0.0001*	1.89	1.60	2.23
Total Cholesterol	0.016*	1.00	1.00	1.00
Hyperhomocysteinemia	0.011*	1.29	1.06	1.57
Fx Hypertension	<0.0001*	2.21	1.86	2.61
BMI	<0.0001*	1.07	1.05	1.09
Stroke	0.025*	0.41	0.17	0.85

After adjusting for age and/or sex, logistic regression was used to assess the association between hypertension and different clinical and biochemical features. OR >1 indicates increased occurrence of HTN. Association with HTN is significant (*) if P-value <0.05. OR: odd ratio; Fx: family history; 95%C.I.: 95% confidence interval. A patient is considered Hyperhomocysteinemic if plasma homocysteine is ≥ 10 μmol/L.

levels. In contrast, the intake of calcium channel blockers, angiotensin II receptor antagonists, nitrates, or vasodilators did not significantly correlate with plasma homocysteine levels.

We sought to compare the genotypes and allele frequency in HHcy and non-HHcy groups. Fisher's exact test was used to examine the difference in the genotype distribution among HHcy groups ($P = 0.3757$) and the difference in the allelic distribution among HHcy groups ($P = 0.583$) (Fig. 1 A & B). The T allele frequency was 35% in the HHcy group compared to 33.1% in non-HHcy, while the C allele frequency was 65% in the HHcy group compared to 66.9% in the non-HHcy group (Fig. 1A). *MTHFR* genotype frequency distribution in the HHcy group was: CC 43.6%, CT 42.8% and TT 13.6%. In the non-HHcy group, the *MTHFR* genotypes frequency distribution was: CC 46.8%, CT 40.6%, and TT 12.9%. Moreover, The T allele frequency was 36.1% in the H-HTN group compared to 30.1% in non the H-HTN group, while the C allele frequency was 63.9% in the H-HTN group compared to 69.9% in the non H-HTN group (Supplementary Fig. 1). *MTHFR* genotypes frequency distribution in the H-HTN group was: CC 42.9%, CT 42.1%, and TT 15.1% while in the non H-HTN group was: CC 50.3%, CT 39.3%, and TT 10.4%.

Homocysteine levels varied significantly with HTN and *MTHFR* alleles ($P < 0.001$), though the interaction between HTN and *MTHFR* was not significant ($P = 0.2445$). The results showed that homocysteine levels for C allele carriers with HTN were higher than that for C allele carrier without HTN ($P < 0.0001$). In addition, homocysteine levels for T allele carriers with HTN were higher than that for T allele carriers without HTN ($P < 0.0001$), 13.3 ± 0.193 μmol/L, 11.9 ± 0.173 μmol/L, respectively (Fig. 2a). Higher levels of homocysteine were found in hypertensive patients carrying the T allele (13.3 ± 0.193 μmol/L) compared with patients carrying the C allele (12.8 ± 0.127 μmol/L). The Two-way ANOVA analysis showed that there was a significant difference in the levels of homocysteine between diuretics and non-diuretics groups ($P < 0.001$). In the C allele carriers, the homocysteine level was significantly higher in those who are on diuretics compared to those who are not on diuretics ($P < 0.001$). Increased levels of homocysteine in T carriers with diuretics treatment were observed when compared to the non-diuretics treatment group (14.1 ± 0.385 vs 13.1 ± 0.230 μmol/L, $P < 0.001$). In the diuretics group, the average plasma homocysteine level for the T allele carriers was 14.1 ± 0.385 μmol/L compared to 13.6 ± 0.262 μmol/L for the C allele carriers (Fig. 2b).

Moreover, since stroke was associated with HTN, we sought to evaluate the levels of homocysteine in the different alleles and genotypes of *MTHFR*. The levels of homocysteine correlated significantly with stroke and the different *MTHFR* alleles ($P < 0.05$). However, no significant interaction was found between stroke and the *MTHFR* alleles ($P = 0.99$). For the C allele carriers, homocysteine levels in subjects with stroke were higher than those without stroke ($P < 0.0001$). For the T allele carriers, the homocysteine levels for patients with stroke were higher than those without stroke, 15.2 ± 1.42 μmol/L vs 12.4 ± 0.124 μmol/L ($P < 0.0001$) (Fig. 2c). Higher levels of homocysteine were found in stroke patients carrying the T allele compared with patients carrying the C allele (15.2 ± 1.42 vs. 14.9 ± 0.761 μmol/L, respectively).

In the TT genotypes, an average plasma homocysteine level of 13.6 ± 0.424 μmol/L was observed in hypertensives compared with 12.3 ± 0.442 μmol/L for the non-hypertensive subjects. Among hypertensive patients, higher levels of homocysteine were found in those carrying the TT genotype compared to the CC carriers (13.6 ± 0.424 vs 12.3 ± 0.442 μmol/L). Increased levels of homocysteine in the TT carriers with diuretic treatments were observed when compared to the non-diuretic treatment group (14.5 ± 0.857 vs 13.1 ± 0.491 μmol/L). The average plasma homocysteine level for the TT genotype in the diuretics group was 14.5 ± 0.857 μmol/L compared to 13.4 ± 0.419 μmol/L and 13.8 ± 0.517 μmol/L for the CC and CT genotypes, respectively. In addition, higher levels of homocysteine were found in stroke patients carrying the TT genotype compared with patients carrying the CC genotype (16.7 vs. 15.1, respectively) (Supplementary Fig. 2).

4. Discussion

Elevated homocysteine is a predictor of cardiovascular disease, but its role in HTN remains controversial [30,31]. While HTN has been associated with HHcy, the role of HHcy in the development of HTN and its complication has been contentious for the past decade. Despite the fact that studies showed a causal link between HHcy and HTN [7,8,32], others failed to reproduce similar results and came to contrasting conclusions [33]. The potential role of HHcy in the pathogenesis of high blood pressure was supported by the reduction in both systolic and diastolic blood pressures, which correlated with homocysteine-lowering treatment [10,34,35]. Our study brings additional information to the ongoing debate, while addressing the causal link between HTN and HHcy, and shows that high serum levels of homocysteine are associated with HTN.

Table 3
Association analysis between hyperhomocysteinemia and different medications for hypertension.

	P-value	OR	95% C.I.	
Beta blockers	0.014*	1.28	1.05	1.57
Calcium channel blockers	0.47	0.91	0.70	1.19
ACE inhibitors	0.036*	1.28	1.02	1.63
Angiotensin II antagonists	0.66	1.06	0.81	1.42
Diuretics	<0.0001*	2.03	1.54	2.70
Nitrates	0.12	1.42	0.93	2.27
Vasodilators	0.17	1.22	0.92	1.64

After adjusting for age and sex, logistic regression was used to assess the association between hyperhomocysteinemia and different medications used for hypertension. OR >1 indicates increased occurrence of hyperhomocysteinemia (homocysteine >10 μmol/L). Association with hyperhomocysteinemia is significant (*) if P-value <0.05. ACE: Angiotensin-Converting Enzyme.

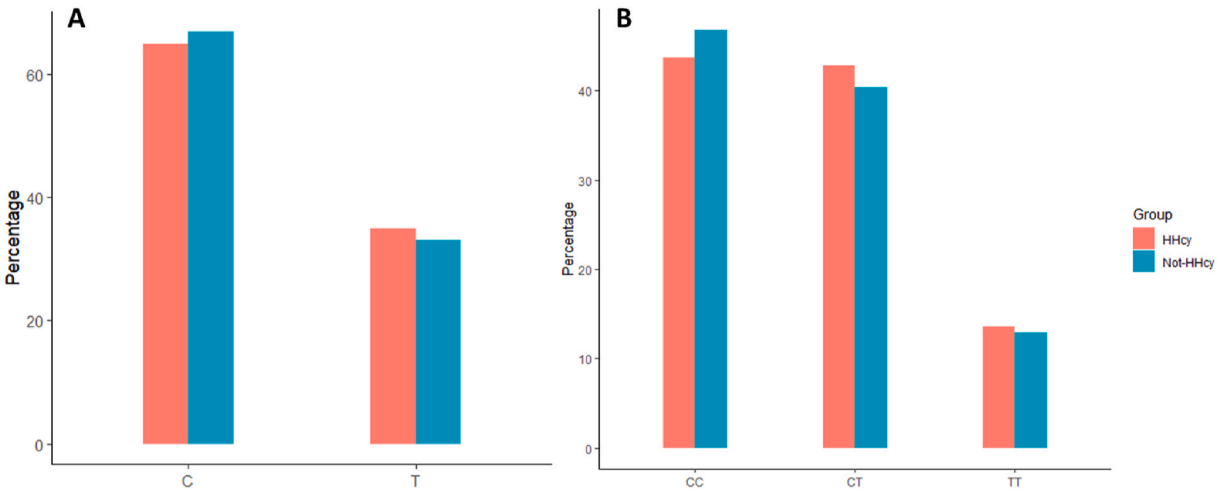

Fig. 1. Genotypes and allele frequencies (in %) of *MTHFR* C677T according to hyperhomocysteinemia (HHcy) groups.

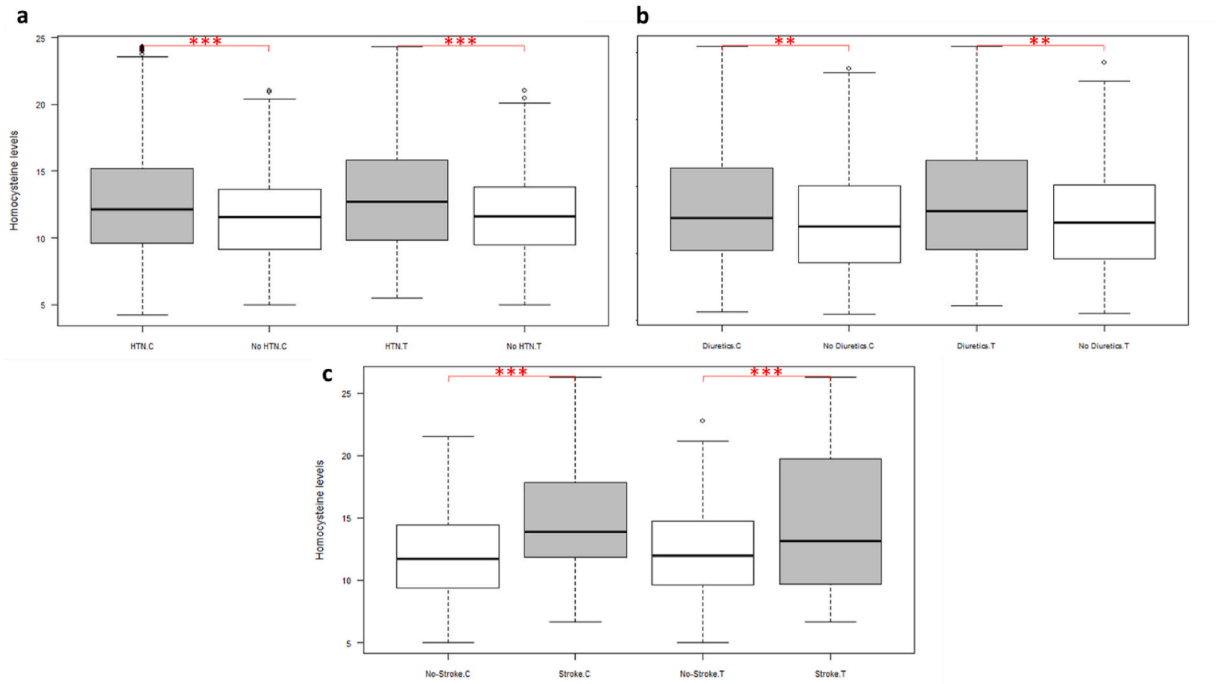

Fig. 2. Homocysteine levels and *MTHFR* alleles according to **(a)** hypertension status, **(b)** diuretics intake and **(c)** stroke.

Our data show an association between high homocysteine levels and HTN, HTN medication, and stroke risk among patients with the *MTHFR* T allele, particularly the TT genotype. The association strengthened further after correction for several confounding predisposing factors, thus confirming that the seen effect is not due to an indirect association between HHcy and other risk factors. The *MTHFR* 677C > T is associated with elevated plasma levels of homocysteine. The *MTHFR* enzyme plays an important role in homocysteine metabolism. Thus, the mutant allele 677C > T, which results in a reduced activity of the enzymes involved in homocysteine metabolism, is now known to be the most prevalent genetic cause of hyperhomocysteinemia [36–38]. We found an increase in the prevalence of the TT mutant genotype and the T mutant allele in the HHcy group, compared to non-HHcy group. Previous studies reported a median homocysteine value of 11.5 μmol/L for people with HTN, and 9.9 μmol/L for control subjects [7]. Although the *MTHFR* 677C > T polymorphism was not genotyped, these values are in line with our recordings for hypertensive patients with a CC genotype. Genotyping this polymorphism in other populations is crucial to test whether a TT genotype would correlate with higher systolic and/or diastolic blood pressure levels and with higher homocysteine levels.

Our results also indicate a positive association of HHcy with the use of beta blockers, and ACE inhibitors. Both of these medications

were shown to decrease plasma homocysteine levels in hypertensive patients [39]. Studies investigating the impact of antihypertensive medications on plasma homocysteine levels remain however scarce.

Patients on diuretics carrying the TT genotype or the T allele have higher levels of homocysteine than those carrying the CC genotype or the C allele. In fact, patients carrying the C allele who are on diuretics have almost similar homocysteine levels (13.6 μmol/L) than those carrying the T allele and are not on diuretic therapy (13.1 μmol/L). These findings demonstrate the complex and non-linear interaction between the *MTHFR* genotype, HTN, homocysteine levels and diuretic use. Previous studies have shown a significant increase in serum homocysteine concentration associated with the chronic use of diuretics [40,41], while others showed a modest association between these two factors [42]. Our data show that elevated levels of serum homocysteine are seen in correlation with the TT genotype and the T allele, regardless of the intake of diuretics. We posit that the TT genotype is directly correlated with increased levels of homocysteine with no evidence for any association between diuretics intake and HHcy, thus ruling out a causation effect of diuretics on HHcy.

The *MTHFR* 677C > T polymorphism is reported to be associated with high levels of homocysteine and stroke [43], we therefore investigated the association between stroke, homocysteine and the *MTHFR* C677T polymorphism. High levels of homocysteine were found in patients with stroke regardless of the *MTHFR* genotype. In addition, a higher level of homocysteine was found to be associated with the TT genotype and the T allele in stroke patients, compared to the CC genotype and the C allele. This suggested that the risk of stroke might increase with high homocysteine level in the presence of TT mutant genotype in our population.

Our data suggest that patients with high levels of homocysteine who are carriers of the TT genotype are at a higher risk of developing severe HTN, necessitating treatment by a combination of antihypertensive medications including diuretics. These patients are likely to be at higher risk of developing stroke and may be requiring homocysteine lowering medications. Furthermore, HHcy has been linked to deficiencies in folate, vitamins B6 and B12. In fact, B-vitamins supplementation has been shown to reduce homocysteine [44]. A recent meta-analysis of randomized trials showed that folic acid supplementation could efficiently decrease the risk of cardiovascular diseases, including stroke [45]. Folic acid and/or a vitamin B complex might be useful in the prevention of morbidity associated with H-HTN.

Our study has several limitations, the most prominent one being the low number of individuals genotyped for the *MTHFR* 677C > T polymorphism. Despite the fact that only 1.7% of our population experienced stroke, there was a strong statistical significance ($p = 0.004$) between stroke and hypertension. Unfortunately, due to this lower number we could not directly evaluate the association between H-Hypertension and stroke after adjustment for anti-hypertensive medication use. Our study demonstrated however, that H-Hypertension, a phenotype that has not been previously addressed in this population, is prevalent and is a major modulator of health risks that are associated with elevated blood pressure including stroke.

In conclusion, we have shown that high levels of plasma homocysteine are partly associated with the *MTHFR* 677C > T polymorphism. We also showed that a high risk of stroke might be associated with high levels of homocysteine and the presence of the mutant T allele of *MTHFR* in our subjects. Further, we showed that the T allele is correlated with high homocysteine levels irrespective of the intake of diuretics, thus ruling out the causation effect of diuretics on HHcy. Our findings promote our knowledge on the etiology of HHcy with direct impact on public health. Finally, we posit that early detection of HHcy may lead to the prevention of the high levels morbidity related to HTN and its complications, namely stroke.

Author contribution statement

Cynthia Al Hageh, Eman Alefishat, Michella Ghassibe-Sabbagh: Analyzed and interpreted the data; Wrote the paper.
Daniel E Platt: Analyzed and interpreted the data.
Hamdan Hamdan, Raya Tcheroyan, Siobhán O'Sullivan: Performed the experiments.
Elie Chammas, Antoine Abchee: Contributed reagents, materials, analysis tools or data.
Binyan Wang, Xiping Xu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Moni Nader, Pierre Zalloua: Conceived and designed the experiments.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16444.

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