Determinants of Blood Pressure in Hypertensive Individuals with the C677T Polymorphism in Methylenetetrahydrofolate Reductase

Submitted in partial fulfilment of the regulations for the Award of the Degree of Bachelor of Science with Honours in Dietetics

Project Supervisor:

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This project report has been prepared in accordance to the instructions to authors for British Journal of Nutrition.
Statement of Student Contribution

This paper reports on data collected as part of an ongoing PhD intervention study. Although the report is focused only on the baseline data I participated in all aspects related to the running of the intervention study. Together with another student and a supervising PhD student, I was involved in meeting and phoning participants, assisting with anthropometry measurements, packing supplements for each treatment group, posting weekly supplement batches, monitoring participant compliance, entering data from questionnaires and assisting with blood processing. The work I carried out was between October-April therefore the overall data which I was permitted to analyse was collected over a longer time period by the PhD student. I was permitted to use all of the baseline data which had been collected from this intervention study but I also accessed the baseline data from a previously completed study at the University of Ulster. I undertook all of the statistical analyses reported in this paper.

I , hereby confirm that I am the major Author of this paper and work described therein and that this work has been conducted using and following humane and ethical procedures, in accordance with the Research Ethics and Governance Policies and Procedures and other Research Policies and Procedures at the University of Ulster including the Code of Practice for Professional Integrity in the Conduct of Research. I also confirm that this work is not plagiarised, does not infringe copyright, and provides a full disclosure of relevant interests.
Abstract

The aim of the investigation was to examine the determinants which significantly contribute to hypertension in individuals with the C677T polymorphism in methylenetetrahydrofolate reductase (MTHFR) who are predisposed to hypertension, and how the TT genotype interacted with established risk factors (age, BMI, gender) and potential interactions with B-vitamin status. A total of 180 individuals with MTHFR TT polymorphism participated in the study of which 60% were male. Data collection involved anthropometric measurements, blood pressure measurements, biochemical analyses for plasma homocysteine, serum and red cell folate and riboflavin status using standardised methods. Logistic regression results showed that gender was significantly associated with systolic hypertension, with female gender at a decreased risk (β value -1.63, OR 0.20, \( p \) value <0.01) this is a novel finding within the TT genotype. Blood pressure control among females was also significantly higher at 63% compared to 36% in males (\( p \) 0.01). No medication type was shown to be associated with significantly decreased systolic hypertension. A non-significant trend was observed for B-vitamin status where increasing homocysteine concentrations (β value 0.40, OR 1.49, \( p \) value 0.39) and decreasing serum folate (β value -0.93, OR 0.39, \( p \) value 0.06) appeared to be associated with a decreased risk of systolic hypertension. Increasing age and BMI were not shown to be significant determinants of hypertension in a TT population. Gender was the most significant determinant of hypertension in this TT cohort, where female gender appeared to be associated with a decreased risk. Further research into the baseline determinants is required.

Keywords: blood pressure, methylenetetrahydrofolate reductase, riboflavin, folate.

Abbreviations: CVD, cardiovascular disease; MTHFR, methylenetetrahydrofolate reductase; BP, blood pressure; FAD, flavin adenine dinucleotide; ACE, angiotensin converting enzyme.
Introduction

Hypertension has a significant global prevalence rate with a third of the population currently having an elevated blood pressure (BP). Hypertension is a growing concern with projected increases in prevalence from 1 billion in 2008 to 1.56 billion in 2025 (WHO 2012). Hypertension significantly contributes to mortality due to a causative association with cardiovascular disease (CVD) and stroke. A recent report estimated that 45% of CVD deaths and 51% of stroke deaths are attributable to hypertension (WHO 2012). An elevated BP has a significant financial impact, with direct costs determined to be 10% of global health expenditure (Gaziano et al. 2009). The indirect costs are estimated to be even more significant due to the association with a number of medical conditions which are exacerbated as a result of hypertension.

There are a number of modifiable risk factors for hypertension along with non-modifiable which includes increasing age, gender (de Simone et al. 2006) and genetic factors. One genetic factor currently attracting interest is a polymorphism in the gene coding for the methylenetetrahydrofolate reductase (MTHFR) enzyme. The polymorphism as noted by Frosst et al. (1995) constitutes a base change from C to T in the MTHFR coding region, and individuals who are homozygous, will have the TT genotype. Individuals with the polymorphism have a reduced enzyme activity level to approximately 50% that of CC individuals (Strain et al. 2004).

The TT genotype for MTHFR has a worldwide prevalence of 10%, yet certain areas have a significantly higher occurrence of the polymorphism such as the USA which has a varying prevalence of up to 18%, China at 20% and Mexico the highest at 32%. (Wilcken et al., 2003).

Individuals with the TT genotype have been shown to have an increased risk of hypertension (Jiang et al., 2005). This has been supported by separate evidence from Newton-Cheh et al. (2009) which highlighted the MTHFR region as one of eight loci which are linked to BP. Further epidemiological evidence from Japanese populations showed significant increases in BP in TT individuals compared to CC individuals with mean increases in systolic and diastolic BP of 14mmHg and 12mHg, respectively (Nishio et al. 1996).
A number of case control studies have highlighted an increase in the presence of the T allele in those with hypertension (Heux et al. 2004; Koupepidou et al. 2005) and this has been supported by a recent meta-analysis which concluded that there was a significant association between TT genotype and hypertension, (Qian et al. 2007). A study conducted in Northern Ireland by Horigan et al. (2010) showed that TT individuals had a significantly higher mean baseline BP (12mmHg for systolic and 6mmHg for diastolic) compared to CC individuals. The results of a 4 year follow up study by Wilson et al. (2012) which re-sampled this population indicated that values for both systolic and diastolic BP were significantly higher than CC individuals, despite changes in the previous years to medication as published in the NICE Guidelines (2006).

Due to the reduced activity of the variant enzyme the focus is now on its correction. MTHFR activity is dependent on a number of B-vitamins, most importantly riboflavin in the co-factor form of flavin adenine dinucleotide (FAD) (Hustad et al. 2000; McNulty et al. 2002). Further research carried out at the University of Ulster by McNulty et al. (2006) showed correction of MTHFR through riboflavin supplementation, demonstrated by a reduction in homocysteine by 22% in the TT group and by 40% in those with the lowest riboflavin baseline status. Further developments at the by Horigan et al. (2010) supported the evidence of MTHFR correction through riboflavin supplementation. This was shown by a significant reduction of systolic and diastolic BP in TT individuals of 13 mmHg and 8 mmHg, respectively. In addition one third of the TT group had a reduction in systolic BP of 20 mmHg through riboflavin supplementation, independent of homocysteine concentrations. Previous work by NICHE (Northern Ireland Centre for Food and Health) has highlighted the role of riboflavin in relation to lowering BP in TT individuals.

There is currently limited evidence linking folate and BP. However folate has a potential role as it is the product of MTHFR therefore when MTHFR activity is corrected the folate levels are increased (Hustad et al. 2000). Supporting the potential role of folate a meta-analysis concluded that supplementation with high dose folic acid caused a slight but significant decrease in systolic blood pressure, the cohort was not genotyped and therefore the effect may have been more pronounced in a TT cohort (McRae et al. 2009).

Other established determinants of hypertension in normal populations such as gender, anti-hypertensive medication use, increasing age and BMI have not been considered to any great extent in the studies which focused on this novel gene nutrient interaction. It may
therefore be possible to examine whether the TT polymorphism interacts with these established determinants.

There are no studies which have focused on the determinants of BP in hypertensive individuals with the C677T polymorphism in MTHFR. This study will use the baseline data from previous studies at to examine the determinants. The aim of the study is to examine the determinants which significantly contribute to hypertension in individuals with the MTHFR TT Genotype who are predisposed to hypertension and how TT genotype interacts with the established risk factors (age, sex, BMI) and the potential interactions with B-vitamins.

**Materials and Methods**

*Study Participants*

Participants were recruited and baseline data was accessed from hypertensive individuals who had participated in two separate interventions at (Figure 1). Both cohorts were combined, giving one cohort for this study (n = 180). Both interventions had similar inclusion criteria and required participants to have been identified as having TT genotype and being hypertensive. The exclusion criteria were history of gastrointestinal, hepatic or renal disease, those who take B-vitamin supplements individually or as part of a multivitamin or those who use medication which is thought to interfere with B-vitamin metabolism such as anticonvulsants. Ethical approval was granted by the Research Ethical Committee of the . Participants provided written informed consent and completed lifestyle questionnaires.

*Study Design and Data Collection*

This study accessed baseline information from participants which included blood pressure measurements, biochemical data and data collected using a food frequency questionnaire. For this study a new excel database was created from merged data. The data was anonymously analysed using participant identification numbers.
Hypertensive individuals pre-screened for MTHFR 677C→T polymorphism

Deceased n = 1
Non-Contactable n = 8
Declined n = 31
Not Suitable n = 32

Agreed to Participate
n = 89

Total Contacted n = 161

Excluded

Hypertensive individuals pre-screened for MTHFR 677C→T polymorphism

Deceased n = 1
Non-Contactable n = 12
Declined n = 37
Not Suitable n = 16

Agreed to Participate
n = 91

Total Contacted n = 157

Excluded

Total Combined Cohort of TT individuals
n = 180

Male
n = 102

Female
n = 78

Figure 1. Recruitment of TT participants from previous and ongoing studies at NICHE.
Blood Pressure

Blood pressure was measured in accordance with the current hypertension NICE Guidelines (2011). The participant was in a seated position with both feet on the floor. The blood pressure was taken from the reference arm. The reference arm is the arm with the naturally higher blood pressure, which varies in all individuals. After the equipment was set up, the participant was left alone for 5-10 minutes to allow them to relax reducing the ‘White Coat’ effect. Between 2 and 5 readings were taken, if a difference of 5mmHg in systolic or 10mmHg in diastolic was noted between the first two readings, further readings were taken up to a maximum of 5. Blood pressure was measured using an Omron 705CP electronic blood pressure monitor (Medisave, Dorset, UK)

Participant Information

General participant information was established from a questionnaire. Part of the questionnaire included a food frequency questionnaire which was used to determine the habitual food consumption of both the natural and fortified sources of B-vitamins. The questions were answered with a selected response, relating to the frequency of consumption of dairy products and fortified breakfast cereals. The questionnaire also asked about current supplement usage. Participants provided information regarding their current medication use with emphasis on hypertension medications.

Anthropometry

Participant’s height (m) was taken using a calibrated stadiometer and weight (kg) taken using calibrated scales. From this information the BMI (weight kg/ height²(m)) was calculated. Waist circumference (cm) was also measured using a tape measure.

Blood Collection and Analysis

A 30ml blood sample was taken by a trained phlebotomist. Blood was collected using 4ml and 9ml EDTA tube and 4ml and 8ml Serum tube. Blood samples were initially processed within 4 hours and then frozen for batch analysis at a later stage. Blood samples were analysed for plasma total homocysteine at Trinity College Dublin by a competitive immunoassay as detailed by Leino (1999). Serum and red cell folate were determined by microbiological assay which was carried out at Trinity College Dublin (Molloy & Scott 1997). Riboflavin status was determined by erythrocyte glutathione reductase activation coefficient (EGRac), by measurement of the function of glutathione reductase before and
after activation with the prosthetic group of riboflavin, FAD (Powers et al. 1983). EGRac is calculated as a ratio of FAD-stimulated and un-stimulated enzyme activity with values equal to or above 1.3 being indicative of sub-optimal riboflavin status. EGRac analyses were carried out at , by an academic researcher. Biochemical analysis was only completed for one of the data sets, as the other study is ongoing.

Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, Version 17.0; SPSS UK Ltd, Chertsey, UK). Data was initially tested for normal distribution and transformed where required. A one-way ANOVA with Tukey posthoc test was carried out to compare the differences in mean blood pressure for males and females depending on anti-hypertension medication use, BMI categories and age categories. Chi square was used to compare the differences between medication use and the percentage achieving goal blood pressure. Logistic regression analysis was carried out to compare systolic hypertension with generic determinants (age, BMI, gender, smoking, alcohol consumption). Further logistic regression was carried out with gender as a control for type of medications and as a control factor for B-vitamin status. Post hoc independent t tests were carried out to assess significance between variables and chi-square was used when t-tests were not appropriate. Data was presented as mean ± SD. In all analyses P values less than 0.05 were considered significant.

Results

Data from 180 white individuals of which 60% were male, was analysed for the purpose of the current study. The mean age was 71 years (range 46-90 years) and the mean BMI was 24.9kg/m². Mean systolic BP was 140 mmHg and diastolic was 78 mmHg. Overall 92% were taking anti-hypertensive medications with 47% achieving goal BP (≤ 140/90 mmHg) (NICE Guidelines 2011). The majority (33%) were taking 1 medication with the most common being diuretics with 47% of participants taking them. The baseline characteristics (Table 1) show that BP was significantly different between males and females. With a difference in systolic BP of 5.3 mmHg ($p = 0.03$) and in diastolic BP of 3.6 mmHg ($p = 0.05$). In terms of achieving goal BP as defined by the NICE Guidelines (2011) as ≤ 140/90 mmHg, over 60% of females were achieving goal BP compared to less than 40% of males. ($p = 0.01$). In terms of B-vitamin status, plasma homocysteine was 3.1 μmmol/L higher in males compared to females ($p = 0.04$).
<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>71.1</td>
<td>71.0</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.9</td>
<td>23.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Blood Pressure (BP)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>142.7</td>
<td>137.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79.8</td>
<td>76.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Antihypertensive medication use (%)</td>
<td>94</td>
<td>73</td>
<td>0.93</td>
</tr>
<tr>
<td>Participants achieving goal BP* (%)</td>
<td>36</td>
<td>63</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>B-vitamin status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGRac</td>
<td>1.32</td>
<td>1.33</td>
<td>0.71</td>
</tr>
<tr>
<td>Plasma homocysteine (µmol/L)</td>
<td>18.0</td>
<td>15.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Red Cell Folate (µmol/L)</td>
<td>738.0</td>
<td>700.0</td>
<td>0.62</td>
</tr>
<tr>
<td>Serum Folate (µmol/L)</td>
<td>8.3</td>
<td>9.1</td>
<td>0.50</td>
</tr>
</tbody>
</table>

BMI, body mass index; EGRac, erythrocyte glutathione reductase activation coefficient (riboflavin biomarker) a higher value is equal to a lower riboflavin status.

Data expressed as mean unless otherwise indicated. Statistical significance between male and female participants by independent t-test or chi-square as appropriate.

*Goal BP is <140/90mmHg (NICE Guidelines, 2011).
The determinants of systolic hypertension (≥140 mmHg) (NICE Guidelines 2011) (Table 2) indicate that female gender was associated with a reduced risk of systolic hypertension (β value -1.63, p<0.01), supporting the differences presented in Table 1. No significant interactions were observed between medication type and BP. In terms of B-vitamins a higher serum folate status was associated with a decreased risk systolic hypertension (β value -0.93, p = 0.06). A high riboflavin status was found to be associated with an increased risk of systolic hypertension (β value 1.19, p = 0.02). Increasing age and BMI were not found to be significantly associated with systolic hypertension.

No significant trends were observed in BP between BMI categories (Figure 2). In the obese category males were found to have a 15.5 mmHg higher mean systolic BP compared to females in the same category (p = 0.05). No significant trends were observed between age categories (Figure 2). Males in the 61-70 (yrs) category had a significantly higher mean systolic BP (p = 0.04) which was 6.81 mmHg higher compared to females in the same category.

Medication usage (Table 3) indicated significant differences between males and females achieving goal BP on diuretics (p = 0.03), calcium channel blockers (p = <0.01) and angiotensin converting enzyme inhibitors (p = <0.01) where an increased percentage of females were achieving goal BP. All females who were taking alpha-blockers were reaching goal BP, it cannot be ascertained if this is the independent effect of this medication or the combined effect of a number of medications. Comparisons between the effectiveness of certain medications could not be determined as participants may be using multiple medications and there also may be other interacting factors.

In terms of the number of medications used, males and females taking 1 anti-hypertensive medication had the highest mean systolic blood pressure, with male and female participants taking 2 anti-hypertensive medications being the lowest, differences were non-significant (male, p = 0.84, female p = 0.59). The highest percentage achieving goal BP were those taking 3 or more anti-hypertensive medications for both male and female, no significant differences were found between those achieving goal blood pressure and number of anti-hypertensive medications.
Table 2: Determinants of Systolic Hypertension* (>140mmHg).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Beta Value</th>
<th>OR</th>
<th>95% C.I</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Generic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (Female)</td>
<td>-1.63</td>
<td>0.20</td>
<td>0.07-0.56</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>-0.02</td>
<td>0.98</td>
<td>0.91-1.06</td>
<td>0.64</td>
</tr>
<tr>
<td>BMI Normal Category (REF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI Overweight Category</td>
<td>-0.44</td>
<td>0.65</td>
<td>0.24-1.75</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Anti-hypertensive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>medication use (yes vs. no)</td>
<td>-0.85</td>
<td>0.43</td>
<td>0.45-4.05</td>
<td>0.46</td>
</tr>
<tr>
<td>Alcohol Consumption (yes vs. no)</td>
<td>-0.25</td>
<td>0.78</td>
<td>0.28-2.14</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Medication Use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretic a (yes vs. no)</td>
<td>0.01</td>
<td>1.01</td>
<td>0.54-1.88</td>
<td>0.99</td>
</tr>
<tr>
<td>CCB a (yes vs. no)</td>
<td>-0.64</td>
<td>0.53</td>
<td>0.27-1.03</td>
<td>0.53</td>
</tr>
<tr>
<td>ACE Inhibitors a (yes vs. no)</td>
<td>-0.37</td>
<td>0.69</td>
<td>0.36-1.44</td>
<td>0.69</td>
</tr>
<tr>
<td>Beta-Blocker a (yes vs. no)</td>
<td>0.39</td>
<td>1.48</td>
<td>0.76-2.89</td>
<td>0.25</td>
</tr>
<tr>
<td>ARB a (yes vs. no)</td>
<td>-0.34</td>
<td>0.71</td>
<td>0.32-1.58</td>
<td>0.71</td>
</tr>
<tr>
<td>Alpha-Blocker a (yes vs. no)</td>
<td>-0.51</td>
<td>0.60</td>
<td>0.23-1.60</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>B-vitamin Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Riboflavin (REF) b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Riboflavin</td>
<td>1.19</td>
<td>0.33</td>
<td>0.13-0.85</td>
<td>0.02</td>
</tr>
<tr>
<td>Low Homocysteine (REF) b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Homocysteine (µmmol/L)</td>
<td>0.40</td>
<td>1.49</td>
<td>0.60-3.69</td>
<td>0.39</td>
</tr>
<tr>
<td>Low Red Cell Folate (REF) b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Red Cell Folate (µmmol/L)</td>
<td>-0.11</td>
<td>0.90</td>
<td>0.36-2.26</td>
<td>0.82</td>
</tr>
<tr>
<td>Low Serum Folate (REF) b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Serum Folate (µmmol/L)</td>
<td>-0.93</td>
<td>0.39</td>
<td>0.15-1.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>

BMI, body mass index; CCB, calcium channel blocker; ACE angiotensin converting enzyme; ARB angiotensin II receptor blocker.

*a, controlled for by gender. b, controlled for by gender.*Systolic Hypertension (>140mmHg) (NICE Guidelines 2011), BMI categories as defined by NICE Guidelines (2006).
Figure 2. Mean Systolic Blood Pressure (mmHg) for male and female participants (a) for each BMI category (b) for each age category.

(a) Systolic Blood Pressure (mmHg) for male and female participants for each BMI category.

(b) Systolic Blood Pressure (mmHg) for male and female participants for each age category.

BMI, body mass index.

*a* represents significance between categories, p-values determined by independent t-test determines significance between gender. Error bars represent standard deviation.

BMI categories as defined by NICE Guidelines (2006).
Table 3. The effectiveness of anti-hypertensive medications between male and female gender.

<table>
<thead>
<tr>
<th>Medication Type</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP mmHg</th>
<th>% Achieving Goal BP*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(m)</td>
<td>(f)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Diuretic (n 84)</td>
<td>143.7</td>
<td>19.9</td>
<td>136.4</td>
</tr>
<tr>
<td>CCB (n 75)</td>
<td>141.7</td>
<td>13.9</td>
<td>132.7</td>
</tr>
<tr>
<td>ACE Inhibitor (n 66)</td>
<td>141.7</td>
<td>17.0</td>
<td>130.2</td>
</tr>
<tr>
<td>Beta-Blocker (n 59)</td>
<td>141.3</td>
<td>17.6</td>
<td>143.5</td>
</tr>
<tr>
<td>ARB (n 46)</td>
<td>142.8</td>
<td>18.5</td>
<td>134.0</td>
</tr>
<tr>
<td>Alpha Blocker (n 23)</td>
<td>149.0</td>
<td>21.4</td>
<td>122.0</td>
</tr>
</tbody>
</table>

Medication Combination

<table>
<thead>
<tr>
<th>Medication Combination</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP mmHg</th>
<th>% Achieving Goal BP*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(m)</td>
<td>(f)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>0 medicationsa (n 13)</td>
<td>143.1</td>
<td>14.1</td>
<td>137.6</td>
</tr>
<tr>
<td>1 medicationa (n 59)</td>
<td>144.6</td>
<td>17.4</td>
<td>139.1</td>
</tr>
<tr>
<td>2 medicationsa (n 54)</td>
<td>141.0</td>
<td>15.6</td>
<td>131.9</td>
</tr>
<tr>
<td>3 &gt; medicationsa (n 54)</td>
<td>142.7</td>
<td>18.9</td>
<td>136.5</td>
</tr>
</tbody>
</table>

CCB, calcium channel blocker; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; BP, blood pressure; (m), male; (f), female.

a denotes significance between groups.

* Goal Blood Pressure is defined as ≤ 140/90 mmHg (NICE Guidelines, 2011)
Discussion

The aim of this study was to examine the determinants of hypertension in individuals with the C677T polymorphism in MTHFR who are predisposed to hypertension.

The analysis indicates for the first time that gender appears to be a significant determinant of both systolic and diastolic BP in individuals with the TT genotype, similar to the population generally. The results indicate that in those with the genetic variant males are more predisposed to hypertension with significantly higher systolic and diastolic BP compared to females. In addition just over a third of TT males are achieving goal BP (≤140/90 mmHg) (NICE Guidelines 2011) compared to almost two thirds of females. Other studies in the population generally have shown a significant difference in male and female blood pressure readings, with a less noticeable difference in post-menopausal women suggesting that oestrogen may have a protective role (Staessen et al. 1990; Wiinberg et al. 1995; Reckelhoff 2001). In this study the majority of females were at a post-menopausal stage, yet the differences were still well defined potentially highlighting a novel gender genotype association with hypertension.

Contrary to what we might have predicted a non-significant trend indicated increasing age was potentially associated with a decreased risk of hypertension. The participants in this study were relatively well age matched; if each age category had been equally represented potentially there may have been more defined differences. In the general population an increased age is associated with an increased risk of hypertension (Cohen et al. 2012) but in those with the TT genotype this trend was not observed, hypertension was more pronounced in younger individuals. This trend was observed in a study by Horigan et al. (2010) who sampled premature cardiovascular disease patients with a relatively young cohort (mean age of TT group 54.0 years) yet hypertension was pronounced within this group. The cohort was also 68% male. Therefore the results may indicate that hypertension
may be more marked in younger individuals within the TT genotype. Therefore genotype related hypertension may be affected less by confounding factors compared to hypertension associated with ageing.

Increasing BMI was not found to be a determinant as the results showed a non-significant decrease in hypertension risk as BMI increases. A study by Vasku et al. (2002) studied a population with 3 polymorphisms associated with hypertension (ATG, M235T and T174M) the results showed a significant increase in the risk of hypertension in those with a BMI above 25kg/m$^2$. The study did not sample a population with the MTHFR polymorphism but allows an insight into a population where polymorphisms are present.

Results from the general population indicate an increased BMI is associated with an increased hypertension risk (de Simone et al. 2006). The results from this study may be due to the majority of participants having a normal BMI therefore the overweight and the obese category were not as well represented making it more difficult to make comparisons.

Some differences by category of BMI were observed when the cohort was split by gender. The highest systolic blood pressure for males was in the obese category, yet the lowest systolic blood pressure for females was in the obese category suggesting a possible difference in how obesity interacts with the polymorphism between genders.

The results have indicated that certain medication types may be more effective at targeting BP in females. Calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors and alpha blockers indicated significantly lower systolic blood pressures in females taking these medications compared to males. It is unknown whether this was the singular effect of this medication or the combination effect of medications. ACE inhibitors were the third most commonly used medication with an increased response in females. A study by Jiang et al. (2004) which analysed the effectiveness of ACE inhibitors within a population with the MTHFR polymorphism showed that TT individuals had a significantly increased
response to ACE inhibitors compared to CC and CT genotypes, with females showing a further reduction in systolic BP of 2.2mmHg.

The results of this study indicated that alpha blockers were the least commonly prescribed medication, yet 100% of TT females using them were achieving goal BP. This effect may have been observed as the current hypertension NICE Guidelines (2011) suggest alpha blockers as a fourth line treatment for persistent hypertension, it is likely when they are using alpha blockers they will also be on other antihypertensive medications, and therefore this result is most likely due to the combination effect of medications. No other studies have looked at the medication effect in the TT genotype, therefore the results from this study showing TT females have an increased BP response to certain medications is novel data and further research is required. There are a number of studies which have looked at medication response in the general population. A collaborative study of the general population by Wolf-Maier et al. (2004) showed that females who were being treated with anti-hypertension medication were more likely to be reaching goal BP than males across a number of countries.

In terms of BP control, more females reached the target of ≤140/90 mmHg (NICE Guidelines 2011) than males when being treated with antihypertensive medication. It is unknown whether the effects of the TT genotype cause this difference in control; no study has assessed gender response to medications in the TT genotype specifically. In a recent study by Wilson et al. (2013) almost 70% of the TT individuals who were being treated for hypertension were not reaching goal BP, the cohort was 67% male. The high level of uncontrolled hypertension could have been driven by male gender within this TT group. The data from Wilson et al. (2013) was included in the database for this study therefore results may show similar trends. These findings highlight that there appear to be gender differences in terms of BP control in this TT group, certain medications may be more or
less effective for male or female gender; further research is required on an intervention basis to determine the specific medication response within the TT population.

The results indicate that homocysteine and B-vitamins did not seem to predict BP in this group but a non-significant trend was observed, were an increased homocysteine concentration and decreased folate status appeared to be associated with an increased risk of systolic hypertension in this group. A high red cell folate was associated with a decreased risk of systolic hypertension, with a high serum folate showing an even further decrease in the associated risk, the results were non-significant. There is currently no strong evidence to support a role for folate status and hypertension in TT individuals, although this important research question is currently being investigated in an ongoing study at NICHE.

There is the potential for folate to have an association as the results of a recent meta-analysis which tested normal populations showed a decrease in systolic blood pressure of 2.03 mmHg after folic acid supplementation, the significant result of pooled data. The effect was not apparent in diastolic blood pressure (McRae et al. 2009). This study did not genotype individuals therefore it is unknown if the effects would be more defined in a TT population as the current result may have shown a dilution effect. The folate status of TT individuals was shown to be significantly comprised compared to the CC group yet this result was not observed for riboflavin status, an observation that has previously been reported by Horigan et al. (2010). This therefore highlights folate may have an important role similar to riboflavin in determining hypertension at baseline in TT individuals.

The results for riboflavin indicated that a higher riboflavin status was associated with an increased of systolic hypertension, contrary to what was expected. A study carried out at NICHE by Horigan et al. (2010) showed TT individuals with the lower baseline status of riboflavin had an increased systolic BP of 8.8 mmHg and diastolic BP of 4.0 mmHg.
compared to TT individuals with a higher riboflavin status. Previous work at NICHE has shown the significant effect of riboflavin in BP lowering in TT individuals firstly by Horigan et al. (2010), then in a 4 year follow up study by Wilson et al. (2012) followed by a further study by Wilson et al. (2013)

Alcohol consumption did not predict BP in this cohort, there was a non-significant trend observed that it appears to be associated with a decreased risk of systolic hypertension. The data collected relating to alcohol intakes did not give reference to the amount consumed therefore it cannot be determined in terms of the quantity effect. A study has shown a moderate intake can have a protective effect towards hypertension but excessive intakes are negatively linked and exacerbate the condition. This study sampled the general population therefore it can only be assumed the same effect would be observed in TT populations (Halanych et al. 2010). Comparisons between individuals who smoked and those who didn’t were not made as the cohort only consisted of a small unrepresentative number of smokers (n 4).

The strengths of this study were that the sample population consisted of a large TT population; TT individuals are difficult to recruit as there is only 10% worldwide prevalence (Wilcken et al. 2003). A further strength is that the biochemical markers which were measured are well established and provide accurate results. The limitations of this study were that although the cohort represented a large number of TT individuals, the sample population solely consisted of the homogenous genotype. Therefore the study was limited because it could not compare the baseline results of the TT group to other genotype populations such as CC or CT. A further limitation was that the biochemical data was not completed for the entire data set, therefore biochemical results presented were for a sub-set of the data. A limitation for medication analysis is that there were a number of potential
medication combinations; therefore results could not be interpreted as the effect of a single medication.

Future work to explore determinants of BP in individuals with this polymorphism should involve a larger subset of participants with other genotypes, to allow comparisons to be made within and between genotypes. An observational study with TT, CC and CT genotype would allow determinants to be established. Furthermore research into the different classes of anti-hypertensive medications and their effect on blood pressure within each genotype group may establish the type or combination which may be more effective for each genotype group.

**Conclusion**

In conclusion to the study, gender has been highlighted as a significant determinant of BP in hypertensive individuals with the TT population, with female gender being associated with having a reduced risk of hypertension. Increasing age and BMI did not appear to be significant determinants within this cohort. Gender differences were also apparent in response to medications although further work in this area is required. No conclusion can be drawn regarding the role of B-vitamins and this should be further investigated in a larger sample with all three MTHFR genotypes included.

**Acknowledgements**

Thank you to for her continued support and guidance throughout the project, her advice has been invaluable. Thank you to PhD student for access to her database and for her work and support throughout. Also thank you to for her assistance with project work when completing duties for intervention study. Finally thank you to all the participants who participated in the study.
Appendix 1

Questionnaire for 09/NIR01/68 and REC/11/0081
Participant Identification Number: ____________________

GENERAL HEALTH & LIFESTYLE QUESTIONNAIRE

1. What age are you? ____________

2. What is your current weight?
   _____ Stone _____ pounds OR _____ Kilograms

3. What height are you?
   _____ Ft _____ inches OR _____ Metres

4. Do you smoke? 
   Yes ☐ No ☐

5. Do you drink alcohol? 
   Yes ☐ No ☐

6. Do you have diabetes? 
   Yes ☐ No ☐
   If you ticked 'Yes' please indicate if:
   Insulin dependent (Type 1) diabetes ☐
   OR
   Non-insulin dependent (Type 2) diabetes ☐

7. Have you ever had a heart attack? Yes ☐ No ☐

8. Have you ever had a stroke? Yes ☐ No ☐

9. Have you been diagnosed with high blood pressure? Yes ☐ No ☐
   If you ticked 'Yes' please indicate in what year this was: ____________

Version 1.2

1/04/2009
Diet & Supplements

1. Do you eat breakfast cereals? Yes □ No □
   If you ticked 'Yes', please write below the name of the breakfast cereal you would most frequently eat:
   Brand and Name: ________________________________
   How often would you eat this breakfast cereal:
   □ Twice per day or more
   □ Once per day
   □ Several times per week
   □ Less often

2. Do you consume milk? Yes □ No □
   If you ticked 'Yes':
   (A) Do you take it as a drink i.e. a glass full: Yes □ No □
   If you ticked 'Yes' How often would you take milk as a drink?
   □ Twice per day or more
   □ Once per day
   □ Several times per week
   □ Less often
   (B) Do you take milk in your tea/coffee: Yes □ No □
   If you ticked 'Yes', how many cups of tea/coffee would you usually consume per day? _________

3. Do you eat –
   Cheese? Yes □ No □
   If you ticked 'Yes', how often:
   □ Twice per day or more
   □ Once per day
   □ Several times per week
   □ Less often
   Yoghurt? Yes □ No □
   If you ticked 'Yes', how often:
   □ Twice per day or more
   □ Once per day
   □ Several times per week
   □ Less often
4. Do you take any vitamin supplements (e.g. vitamins in tablet form, cod liver oil, etc)?

Yes □          No □

If you ticked 'Yes', please write down the following information about supplement(s) that you are taking:

**Supplement 1 Name and Dosage:** ____________________________

How often do you typically take this supplement? ______________

How long have you been taking supplement 1 for? ______________

**Supplement 2 Name and Dosage:** ____________________________

How often do you typically take this supplement? ______________

How long have you been taking supplement 2 for? ______________

**Supplement 3 Name and Dosage:** ____________________________

How often do you typically take this supplement? ______________

How long have you been taking supplement 3 for? ______________

*Many thanks for taking the time to complete this questionnaire*
Appendix 2

Data from Figure 2 in Tabular Form
<table>
<thead>
<tr>
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<th>Systolic Blood Pressure (mmHg)</th>
<th>SD</th>
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<td><strong>BMI Categories</strong></td>
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<tr>
<td>Normal (n 48)</td>
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</tr>
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<td>Overweight (n 38)</td>
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<td>76-90 (n 20)</td>
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<td>61-75 (n 55)</td>
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</tr>
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<td>76-90 (n 19)</td>
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References


