Impact of the common MTHFR 677C→T polymorphism on blood pressure in adulthood and role of riboflavin in modifying the genetic risk of hypertension: evidence from the JINGO project

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Impact of the common *MTHFR* 677C→T polymorphism on blood pressure in adulthood and role of riboflavin in modifying the genetic risk of hypertension: evidence from the JINGO project

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Abstract

Background: Genome-wide and clinical studies have linked the 677C→T polymorphism in the gene encoding methylenetetrahydrofolate reductase (MTHFR) with hypertension, whilst limited evidence shows that intervention with riboflavin (i.e. the MTHFR co-factor) can lower blood pressure (BP) in hypertensive patients with the variant *MTHFR* 677TT genotype. We investigated the impact of this common polymorphism on BP throughout adulthood and hypothesised that riboflavin status would modulate the genetic risk of hypertension.

Methods: Observational data on 6076 adults of 18-102 years were drawn from the Joint Irish Nutrigenomics Organisation project, comprising the Trinity-Ulster Department of Agriculture (TUDA; volunteer sample) and the National Adult Nutrition Survey (NANS; population-based sample) cohorts. Participants were recruited from the Republic of Ireland and Northern Ireland (UK) in 2008-2012 using standardised methods.

Results: The variant *MTHFR* 677TT genotype was identified in 12% of adults. From 18-70 years, this genotype was associated with an increased risk of hypertension (i.e. systolic BP ≥140 and/or a diastolic BP ≥90mmHg): odds ratio (OR) 1.42, 95% confidence interval (CI) 1.07 to 1.90; *P*=0.016, after adjustment for antihypertensive drug use and other significant factors, namely, age, male sex, BMI, alcohol and total cholesterol. Low or deficient biomarker status of riboflavin (observed in 30.2% and 30.0% of participants, respectively) exacerbated the genetic risk of hypertension, with a 3-fold increased risk for the TT genotype in combination with deficient riboflavin status (OR 3.00, 95% CI, 1.34-6.68; P= 0.007) relative to the CC genotype combined with normal riboflavin status. Up to 65 years, we observed poorer BP control rates on antihypertensive treatment in participants with the TT genotype (30%) compared to those without this variant, CT (37%) and CC (45%) genotypes (P<0.027).

Conclusions: The *MTHFR* 677TT genotype is associated with higher BP independently of homocysteine and predisposes adults to an increased risk of hypertension and poorer BP control

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48   with antihypertensive treatment, whilst better riboflavin status is associated with a reduced

49   genetic risk. Riboflavin intervention may thus offer a personalised approach to prevent the

50   onset of hypertension in adults with the TT genotype, however, this requires confirmation in a

51   randomised trial in non-hypertensive adults.

52   **Keywords**: Hypertension; blood pressure; folate polymorphism; MTHFR; riboflavin;

53   personalised treatment; prevention.

54

Background

Hypertension is the leading risk factor contributing to all-cause death in every region in the world, estimated to affect 1.13 billion people globally and account for over 9 million deaths annually, predominantly from cardiovascular disease (CVD) [1–3]. The relationship of blood pressure (BP) with disease is age-specific and most pronounced in adults 40-69 years, where the risk of CVD is estimated to double for each 20mmHg rise in systolic BP [4]. Recent reports have highlighted the importance of targeting lifestyle and treatments strategies at the individual level in order to improve cardiovascular health [1, 5], and genome-wide association studies (GWAS) have identified specific genes linked with BP which could lead to personalised treatments for hypertension based on genetic characteristics. The earliest of such studies tested 2.5 million single nucleotide polymorphisms (SNPs) and identified eight genetic loci associated with BP, including a region near the gene encoding the folate-metabolising enzyme methylenetetrahydrofolate reductase MTHFR [6], findings confirmed by subsequent GWAS [7].

Of greater relevance to health, clinical studies have linked this gene with BP, with meta-analyses of case-control studies showing that the 677C→T polymorphism in *MTHFR* is associated with an increased risk of hypertension by 36-87% [8–10]. Previously the role of this polymorphism in CVD has been studied extensively in relation to the well-recognised phenotype, elevated homocysteine, whilst the relationship with BP is relatively under-investigated. The variant *MTHFR* 677TT genotype, which affects 10% of adults worldwide [11], is however reported to increase the risk of CVD (especially stroke) by up to 40%, albeit with a large geographical variation in the extent of excess risk, consistent with a gene-environment interaction [12–14]. In this regard only folate was previously considered, but emerging evidence suggests that riboflavin - required in the form flavin adenine dinucleotide (FAD) as a cofactor for MTHFR - may be a key modifying factor linking this polymorphism

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80   with CVD via a novel and genotype-specific effect on BP [14]. In three small randomised

81   controlled trials, we previously demonstrated lowering of systolic BP by 6 to 13 mmHg in

82   response to riboflavin when targeted at hypertensive patients with the variant *MTHFR* 677TT

83   genotype [15–17].

84   No previous study has investigated the contribution of the *MTHFR* 677C→T

85   polymorphism to BP within generally healthy adults or identified a potential prevention

86   strategy to reduce the onset of hypertension in those genetically at-risk. The aim of this study

87   was therefore to examine the impact of this polymorphism on BP throughout adulthood, and to

88   assess the role of riboflavin in modulating the genetic risk of hypertension. We hypothesised

89   that this polymorphism is associated with high BP independently of its association with

90   homocysteine, and that riboflavin status would modulate the genetic risk of hypertension.

91

92   **Methods**

93   **Design and participants**

94   Data for this investigation were drawn from two cohorts, the Trinity-Ulster Department of

95   Agriculture (TUDA) cohort study and the National Adult Nutrition Survey (NANS) of Ireland,

96   both forming part of an All-Ireland initiative under the Joint Irish Nutrigenomics Organisation

97   (JINGO) project (http://www.ucd.ie/jingo/; accessed May 2020). The TUDA study

98   (ClinicalTrials.gov Identifier: NCT02664584) comprises a cross-sectional cohort of 5186 older

99   adults (≥60 years), with the primary aim of investigating nutritional factors and gene-nutrient

100   interactions in the development of chronic diseases of ageing. Eligible participants were

101   community dwelling, non-institutionalised adults, born on the island of Ireland. Participants

102   were recruited using standardised protocols during the period of 2008 to 2012, either from GP

103   practices in the Northern and Western Trusts in Northern Ireland (UK), or from hospital

104   outpatient clinics at the Department of Medicine for the Elderly at St. James's Hospital Dublin

105   in the Republic of Ireland, as previously detailed [18]. Over a similar period (2008 to 2010),

106   detailed dietary, biomarker, health and lifestyle data were collected for the NANS cohort, a

107   nationally representative sample of Irish adults. Eligible participants were healthy adults aged

108   18-102 years, not pregnant or breast-feeding. Full sampling and methodological details for

109   NANS 2008-2010 have been described elsewhere [19]. Approval for both studies was granted

110   from the relevant ethics committees in the UK and the Republic of Ireland and all participants

111   provided written informed consent at the time of recruitment.

112

113   **Study measurements**

114   For both the TUDA and NANS cohorts, relevant health and lifestyle information was obtained

115   in face-to-face interviews conducted by trained researchers. Detailed information concerning

116   medication and vitamin supplement usage was collected. Confirmation of medication details

117   was obtained by referring to the participant's prescription; where this was unavailable during

118   the interview, the details were collected from the participant via telephone shortly after the

119   appointment. Blood samples collected at the time of the appointment were analysed for routine

120   laboratory measurements in the participating local laboratories, whereas B vitamin status

121   biomarkers were analysed centrally in specialist research laboratories at Ulster University or

122   Trinity College Dublin using standardised procedures [16]. Of particular relevance, the analysis

123   included the riboflavin biomarker, erythrocyte glutathione reductase activation coefficient

124   (EGRac), widely accepted as the gold-standard measure of riboflavin status. This coefficient

125   provides a measure of glutathione reductase enzyme saturation with its riboflavin-derived

126   cofactor and is thus a functional biomarker of riboflavin status; a low EGRac value is

127   considered to be normal, while higher values are indicative of suboptimal riboflavin status.

128   DNA samples were analysed for several SNPs, including *MTHFR* 677C→T (rs1801133), by

129   LGC Genomics (Hoddesdon, UK).

130   Trained researchers measured BP using standard operating procedures and clinical

131   guidelines, using an A&D UA-787 digital blood pressure monitor (Cardiac Services, Belfast,

132   UK) or OMRON M6 (Milton Keynes, UK), for TUDA and NANS cohorts respectively, with

133   the participant in the supine position following a 5 minute rest. In accordance with clinical

134   guidelines [20], two BP measurements were taken from the reference arm, with a 5-10 minute

135   interval between each measurement to generate a mean BP value. In the case of a >5 mmHg

136   difference, a third BP measurement was taken after 10-15 minutes and the mean of the two BP

137   measurements in closest agreement was used.

138

139   **Study outcomes**

140   The primary outcomes were systolic and diastolic BP, and occurrence of hypertension, by

141   *MTHFR* genotype and *MTHFR*-riboflavin interaction. In accordance with British and

142   European guidelines, hypertension was defined when a participant's systolic BP was $\geq$140

143   mmHg and/or their diastolic BP was $\geq$90 mmHg; as per clinical guidance, these BP categories

144   applied to all adults (>18 years) [1, 20]. An additional study outcome was BP control on

145   antihypertensive treatment by *MTHFR* genotype. Treatment was defined as taking medication

146   to lower BP, as verified by the researcher against prescription details during or following the

147   interview. Treated and controlled was defined as taking medication to lower BP and a recorded

148   systolic BP of <140 and/or diastolic BP <90 mmHg.

149

150   **Statistical analysis**

151   Analysis was limited to participants with available *MTHFR* genotype and valid BP (**Fig 1**).

152   Before statistical analysis, tests for normality were performed and variables were log-

153   transformed as appropriate. Participant characteristics were examined by *MTHFR* genotype

154   and differences between groups were analysed using one-way between-groups analysis of

155 variance (ANOVA) for continuous variables and χ^2 tests for categorical parameters. To account

156 for multiple testing, the null hypothesis was rejected for $P<0.05$ after post-hoc Bonferroni

157 correction at a family level. Logistic regression analysis was used to predict hypertension (as

158 the categorical dependent variable) using relevant independent variables, and examined the

159 association of *MTHFR* genotype with the risk of hypertension after independently adjusting for

160 established risk factors, including antihypertensive drug use (as a binary yes/no covariate

161 adjustment). Multinomial regression was performed to enable the effect of the interaction

162 between *MTHFR* genotype and biomarker status of riboflavin (i.e. deficient versus low versus

163 normal) on the risk of hypertension to be assessed; odds ratios were calculated using *MTHFR*

164 677CC genotype combined with normal riboflavin status as the reference category. Statistical

165 analysis was performed using the Statistical Package for Social Sciences (SPSS, version 21,

166 SPSS UK Ltd, Chertsey Road, Surrey, UK).

167

168 **Fig. 1** Identification of study participants from two cohorts under the Joint Irish Nutrigenomics (JINGO)
169 Initiative.
170 [*]National Adult Nutrition Survey of Ireland
171 †Trinity-Ulster and Department of Agriculture cohort study
172 [#]CC (wild type), CT (heterozygous), TT (homozygous), genotypes for the 677C→T polymorphism in
173 *MTHFR*
174

175 **Results**

176 **Study participants**

177 From an original dataset of 6360 participants (i.e. combined TUDA [$n = 5186$] and NANS [n

178 $= 1174$] cohorts), complete data for the current analysis were available for a total of 6076

179 participants (**Fig 1**). Homozygosity for the *MTHFR* 677C→T polymorphism (TT genotype)

180 was identified in 12% of the overall study sample (12.1% and 12.3 % for TUDA and NANS

181 cohorts respectively; **Additional File 1: Table S1** showing characteristics separately presented

182 for TUDA and NANS cohorts). There were no significant differences in general participant

characteristics among *MTHFR* genotype groups (**Table 1**). The expected phenotype was however evident in B vitamin biomarkers, with significantly higher plasma homocysteine and lower red blood cell folate concentrations in the TT compared to CC or CT genotypes. General participant characteristics split by study sub-cohorts (i.e. TUDA and NANS cohorts) are provided as Supplementary material (**Additional File: Table S1**).

Table 1 General participant characteristics by *MTHFR* genotype

	MTHFR Genotype[a]			
	CC (*n* = 2677)	CT (*n* = 2660)	TT (*n* = 739)	*p* value[b]
MTHFR genotype, n (%)	2677 (44)	2660 (44)	739 (12)	
Age, years	68.9 (15.1)	69.0 (15.5)	68.6 (15.6)	0.806
Sex, male	943 (35%)	961 (36%)	256 (35%)	0.678
Waist, cm	94.5 (13.9)	94.5 (14.1)	94.7 (14.7)	0.982
Height, cm	162.6 (10.2)	162.9 (10.1)	162.6 (10.0)	0.619
Weight, kg	73.7 (16.5)	73.7 (16.8)	74.2 (17.3)	0.853
Body mass index, kg/m^2	27.8 (5.2)	27.7 (5.4)	27.9 (5.2)	0.458
Current smokers, n (%)	359 (13%)	355 (13%)	89 (12%)	0.530
Alcohol Intake, units/week	8.6 (12.2)	8.8 (12.7)	8.0 (11.3)	0.402
Serum triglycerides, mmol/L	1.51 (0.84)	1.56 (0.88)	1.55 (0.78)	0.087
Serum total cholesterol, mmol/L	4.68 (1.03)	4.68 (1.06)	4.73 (1.05)	0.383
Serum HDL, mmol/L	1.51 (0.49)	1.48 (0.45)	1.49 (0.47)	0.439
Calculated LDL, mmol/L	2.50 (0.88)	2.50 (0.89)	2.54 (0.88)	0.472
Serum Creatinine, µmol/L	86.3 (27.4)	86.0 (26.4)	85.9 (27.2)	0.928
B-vitamin Biomarkers				
Red blood cell folate, nmol/L	1095 (579)[a]	1088 (626)[a]	971 (563)[b]	<0.001
Serum vitamin B12, pmol/L	295 (155)	295 (238)	296 (238)	0.194
Riboflavin status, EGRac[c]	1.35 (0.21)	1.35 (0.21)	1.34 (0.21)	0.769
Plasma homocysteine, µmol/L	14.2 (5.4)[a]	14.3 (5.4)[a]	15.7 (6.8)[b]	<0.001

Data are expressed as mean (standard deviation) or *n* (%).
[a]CC (wild type), CT (heterozygous), TT (homozygous variant), genotypes for the *MTHFR* 677C→T polymorphism.
[b]*P*-value from one-way ANOVA comparing genotype groups, following log-transformation of data for normalisation purposes, as appropriate. Different superscript letters (i.e. a, b) within a row indicate significant differences by Bonferroni post-hoc test, whilst the same letter (i.e. a, a) indicates no significant differences. Level of significance (P<0.003) adjusted for Bonferroni correction (*n* = 16).
Categorical variables assessed using chi-square analysis.

ᶜBiomarker status of riboflavin determined by the functional assay, erythrocyte glutathione reductase
199 activation coefficient (EGRac); higher EGRac values indicate lower riboflavin status.
200

Impact of *MTHFR* genotype on blood pressure and risk of hypertension

Irrespective of *MTHFR* genotype, systolic BP showed an increase with age up to approximately

80 years, whereas diastolic BP increased until about age 60 years and then declined (**Fig 2**).

Examination of BP by *MTHFR* genotype, however, showed higher BP in the TT genotype

group up to approximately 65 years compared to adults of the same age with CC or CT

genotypes, with systolic and diastolic BP in the TT genotype observed to be typical of an adult

several years older without this genetic variant. From about 65 years onwards, however, the

BP phenotype associated with this polymorphism was less evident.

Fig. 2 Systolic and diastolic blood pressure in adults 18-90 years by *MTHFR* genotype
(*n*=6070).
Data grouped by deciles of age from the youngest 10%, to the oldest 10%, of study participants. Each
line illustrates median systolic or diastolic blood pressure for adults by age: CC (green line), CT (amber
line) and TT (red line) genotypes for the *MTHFR* 677C→T polymorphism.

Among adults 18-70 years, logistic regression analysis showed that the *MTHFR* 677TT

genotype was associated with an increased risk of hypertension: odds ratio (OR) 1.42, 95%

confidence interval (CI) 1.07 to 1.90, after adjustment for antihypertensive drug use (as a

binary covariate) and other significant covariates, namely, age, male sex, BMI, alcohol, total

cholesterol and study cohort (**Table 2**), whereas homocysteine was not independently

associated with the risk of hypertension (apart from in treated adults). The OR for risk of

hypertension associated with the TT genotype remained similar whether the logistic regression

analysis was performed in all participants up to 70 years, or split into those treated or not treated

with antihypertensive drugs, albeit the relationship failed to reach statistical significance within

either treated or untreated categories (owing to the loss of statistical power as a result of a 50%

reduction in the sample size when split and considering that the variant TT genotype is

227 represented by just 12% of the overall cohort). In contrast, when this analysis was conducted

228 in the total sample (i.e. adults up to 90 years), *MTHFR* genotype was not significantly

229 associated with hypertension, whilst all other determinants of hypertension were similar to

230 those found in adults up to 70 years (not shown).

231

232 **Table 2** Factors associated with risk of hypertension in adults 18-70 years

		All (n = 2566)			On antihypertensive drugs (n = 1255)			Not on antihypertensive drugs (n = 1311)		
		OR	95% CI	P^b	OR	95% CI	P^b	OR	95% CI	P^a
Age, years		1.04	1.03-1.05	<0.001	1.01	0.98-1.04	0.568	1.05	1.03-1.06	<0.001
Sex, male		1.86	1.50- 2.32	<0.001	1.69	1.28-2.25	0.001	1.77	1.23-2.56	0.002
BMI, kg/m^2		1.06	1.04-1.08	<0.001	1.03	1.01-1.05	0.009	1.11	1.07-1.15	<0.001
Alcohol Intake, units per week		1.01	1.00-1.02	0.005	1.01	1.00-1.02	0.325	1.03	1.01-1.04	<0.001
Antihypertensive medication use[b]		2.01	1.60-2.52	<0.001						
Serum Creatinine, µmol/l		1.00	0.99-1.00	0.307	1.00	0.99-1.00	0.189	1.01	0.99-1.02	0.340
Total Cholesterol, mmol/l		1.26	1.15-1.38	<0.001	1.25	1.11-1.41	<0.001	1.23	1.07-1.41	0.004
Smoking	Past	0.98	0.80-1.19	0.826	0.97	0.75-1.26	0.834	1.02	0.74-1.41	0.888
	Current	1.03	0.79-1.33	0.845	0.92	0.64-1.31	0.630	1.10	0.75-1.62	0.618
Study Cohort[c]		1.79	1.29-2.48	<0.001	2.40	1.40-4.11	<0.001	2.09	1.31-3.32	0.002
Plasma Homocysteine, µmol/l		1.00	0.98-1.02	0.958	0.98	0.96-1.00	0.074	1.06	1.02-1.10	0.002
MTHFR genotype[d]	CT	1.18	0.98-1.43	0.082	1.35	1.05-1.73	0.018	0.98	0.73-1.32	0.889
	TT	1.42	1.07-1.90	0.016	1.40	0.95-2.06	0.093	1.37	0.88-2.11	0.161

233 CI, Confidence Interval; OR, odds ratio

234 [a]Data analysed by Logistic Regression to predict hypertension as the categorical dependent variable;

235 hypertension defined as systolic BP of ≥140 and/or a diastolic BP of ≥90mmHg [1].

236 [b]as a binary (yes/no) covariate.

237 [c]Comparing TUDA cohort with NANS cohort (reference category). See supplementary Table S1 for

238 participant characteristics presented separately for each study cohort.

239 [d]CT (heterozygous) and TT (homozygous variant) genotypes for the *MTHFR* 677C→T

240 polymorphism; reference category is the CC genotype.

241

242 Likewise no significant effect of *MTHFR* genotype on BP was observed when the total cohort

243 was analysed, but among adults 18 to 70 years, those with the TT genotype had significantly

244 higher systolic and diastolic BP after adjustment for relevant covariates including

245 antihypertensive drug use (**Table 3**). Among participants up to 70 years, 49% (n=1255) were

246 being treated with one or more antihypertensive drugs. Details of antihypertensive drug use

247 and drug combinations among treated participants are shown in **Table 4**. Almost 60% of treated

248 participants were treated with two or more medications (57%, 57% and 59% for CC, CT and

11

249   TT genotypes). For BP results among participants being treated/not treated with

250   antihypertensive drugs by MTHFR genotype, see **Additional File 1: Table S2**).

251

252   **Table 3** Blood pressure and rates of hypertension in adulthood by *MTHFR* genotype

| | *MTHFR* genotype | | | |
	CC	CT	TT	*p* value[b]
Total cohort (up to 90 years)	*n* = 2635	*n* = 2606	*n* = 719	
Age, years	68.9 (68.3, 69.5)	69.0 (68.4, 69.6)	68.6 (67.5, 69.7)	0.806
Systolic BP, mmHg	140.7 (139.8, 141.5)	141.5 (140.8, 142.4)	141.1 (139.6, 142.6)	0.373
Diastolic BP, mmHg	78.0 (77.6, 78.4)	78.5 (78.0, 78.9)	78.4 (77.6, 79.2)	0.258
Hypertension, n (%)	1373 (51%)	1411 (53%)	373 (50%)	0.302
Adults 18 to 70 years	*n* = 1124	*n* = 1138	*n* = 313	
Age, years	56.3 (55.4, 57.1)	56.4 (55.6, 57.3)	55.8 (54.2, 57.5)	0.835
Systolic BP, mmHg	135.0 (133.9, 136.0)[a]	136.1 (135.0, 137.2)[ab]	137.6 (135.5, 139.6)[b]	0.026
Diastolic BP, mmHg	79.4 (78.9, 80.0)[a]	80.0 (79.4, 80.5)[ab]	81.4 (80.3, 82.5)[b]	0.013
Hypertension, n (%)	464 (40)	514 (44)	149 (46)	0.072

253   Abbreviations: BP, blood pressure; CC (wild type), CT (heterozygous), TT (homozygous variant),
254   genotypes for the *MTHFR* 677C→T polymorphism.
255   Data are expressed as mean (95% CI) for age, as adjusted mean (95% CI) for blood pressure, and *n* (%)
256   for hypertension
257   [a]Hypertension defined as systolic BP of ≥140 and/or a diastolic BP of ≥90mmHg [1].
258   [b]Differences in blood pressure between genotype groups were assessed by one-way ANCOVA with
259   adjustment for age, sex, BMI, alcohol, total cholesterol, antihypertensive drugs use and study cohort
260   following log-transformation of data for normalisation purposes, as appropriate. Different superscript
261   letters (i.e. a, b) within a row indicate significant differences by Bonferroni post-hoc test, whilst the
262   same letter (i.e. a, a) indicates no significant differences. Categorical variables were assessed using chi-
263   square analysis.

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Table 4. Antihypertensive drug use in treated participants up to 70 years

	MTHFR genotype		
	CC (*n* = 536)	CT (*n* = 590)	TT (*n* = 154)
Drug class			
ARB	149 (28)	167 (28)	53 (34)
ACE	185 (35)	221 (37)	52 (34)
CCB	188 (35)	207 (35)	61 (40)
Diuretic	224 (42)	260 (44)	59 (39)
β-Blocker	180 (34)	187 (32)	54 (35)
α-Blocker	38 (7)	35 (6)	7 (5)
Central alpha antagonist	3 (1)	6 (1)	2 (1)
Drug combination			
1 medication	230 (43)	257 (44)	64 (41)
2 medications	200 (37)	205 (35)	52 (34)
>= 3 medications	106 (20)	128 (22)	38 (25)

Values are n (%).

Abbreviations: CC (wild type), CT (heterozygous), TT (homozygous variant), genotypes for the *MTHFR* 677C→T polymorphism; ARB, angiotensin II receptor blockers; ACE, angiotensin-converting enzyme; CCB, calcium-channel blockers

In younger and middle-aged adults (18-65 years), significantly lower Treated and Controlled rates (defined as taking antihypertensive drugs and a recorded BP within the target range i.e. systolic BP of <140 and diastolic BP <90mmHg) were observed in the TT genotype (30%; n = 24) compared to CT (37%; n = 114) or CC (45%; n = 120) genotypes (P<0.027); not shown.

MTHFR genotype and riboflavin status in relation to hypertension

The influence of riboflavin status in modifying the genetic risk of hypertension was then examined (**Fig 3**). Based on functional status and response to low-dose riboflavin from previous reports [17], participants were categorised as having normal (EGRac ≤1.26), low (EGRac 1.26-1.40) or deficient (EGRac ≥1.40) riboflavin. Low or deficient riboflavin status (observed in 30.2% and 30.0%, respectively) exacerbated the risk of hypertension associated

289   with this polymorphism, with a 3-fold increased risk (OR 3.00) for the TT genotype in

290   combination with deficient riboflavin status (95% CI, 1.34-6.68; P= 0.007) relative to the CC

291   genotype combined with normal riboflavin status as the reference category (**Fig 3**). Among

292   participants with the TT genotype, better riboflavin status was associated with a reduced risk

293   (OR 1.62 (95% CI, 0.80-3.29; P= 0.179); and normal riboflavin status with no excess genetic

294   risk of hypertension. In contrast, deficient versus low versus normal riboflavin status did not

295   alter the risk of hypertension among adults with CC or CT genotypes.

296   **Fig. 3** Influence of riboflavin status on the risk of hypertension by *MTHFR* genotype.
297   Values are odds ratios (95% confidence intervals) for risk of hypertension for CC (left panel, green),
298   CT (middle panel, amber) or TT (right panel, red) genotypes for the *MTHFR* 677C→T polymorphism.
299   Data analysed by multinomial regression adjusted for relevant covariates: age, sex, BMI, alcohol,
300   antihypertensive drug use, total cholesterol, creatinine, smoking, study cohort, plasma homocysteine,
301   red blood cell folate. Compared to the reference category (CC genotype combined with normal
302   riboflavin status), values for the TT genotype combined with deficient riboflavin status are: OR 3.00
303   (95% CI, 1.34-6.68; P= 0.007); or with low riboflavin status: OR 1.62 (0.80-3.29; P= 0.179); or with
304   normal riboflavin status: OR 0.98 (0.47-2.04; P= 0.957). Riboflavin status determined by the functional
305   biomarker, erythrocyte glutathione reductase activation coefficient (EGRac); participants categorised
306   as having normal (EGRac ≤1.26; filled circles), low (EGRac >1.26 to <1.40; open circles) or deficient
307   (EGRac ≥1.40; open squares) riboflavin status.
308

Discussion

310   Our study shows that from young adulthood to 70 years, the *MTHFR* 677TT genotype

311   predisposes an individual to a systolic BP typical of an adult several years older without this

312   genetic variant. Although this polymorphism was previously linked with BP, this is the first

313   study to examine the genetic risk of hypertension throughout adulthood, and to identify the

314   potential for riboflavin to modify the phenotype in affected adults at a younger age and before

315   the onset of hypertension. The observed effect of *MTHFR* and its modulation by riboflavin in

316   relation to hypertension risk were found to be independent of homocysteine, the typically

317   reported phenotype linking this polymorphism with CVD.

318   We observed a pattern in the current study (irrespective of *MTHFR* genotype), whereby

319   systolic BP increased into older age whereas diastolic BP increased until about 60 years and

320 then declined, as previously reported [21, 22]. The results however showed that adults with the

321 variant *MTHFR* 677TT genotype have higher systolic and diastolic BP compared to others of

322 the same age with CC or CT genotypes. The BP phenotype was not evident above 70 years,

323 presumably as a result of the confounding effect of other age-related determinants of BP. The

324 reason we focussed on the period up to 70 years, is because this is a time during which the

325 relationship of BP with disease is most pronounced, with a reported doubling in the risk of

326 CVD for each 20 mmHg rise in systolic BP [4]. The *MTHFR* 677TT genotype was associated

327 with a 42% increased risk of hypertension in adults up to 70 years, after adjustment for

328 antihypertensive drug use and other significant factors, namely, age, male sex, BMI, alcohol

329 and blood cholesterol, whereas plasma homocysteine was not independently associated with

330 hypertension risk. The extent of excess hypertension owing to this polymorphism is in good

331 agreement with previous estimates from clinical studies, with reported odds ratios in meta-

332 analyses ranging 1.36 (95% CI, 1.20-1.53) to 1.87 (1.31 to 2.68), for worldwide and Chinese

333 populations, respectively [8, 10]. Our findings however show that from young adulthood this

334 polymorphism contributes to higher BP, suggesting that affected adults could potentially

335 develop hypertension at an earlier age than those without this genetic risk.

336 Of particular relevance to cardiovascular medicine is the finding that response to routine

337 BP treatment appears to be suboptimal in adults with the *MTHFR* 677TT genotype. Overall

338 49% of participants 18-70 years in this study were under current treatment with

339 antihypertensive drugs, a rate of treatment similar to that reported for adults 20-80 years in

340 England (51%) and considerably less than in adults 20-80 years in the US (74%) or Canada

341 (80%) [21]. In the current study, in line with our previous observations [17], BP control was

342 poorer in the TT genotype, with only 30% of treated adults with the TT compared to 37% in

343 CT and 45% in CC genotypes, achieving BP control. Similarly, reported BP control rates for

344 all treated adults are 37% in England [23], and higher in North America, at 54% in the US [5]

345 and 65% in Canada [23]. Irrespective of prevailing rates of treatment or BP control however,

346 our findings suggest that within a given population, adults with the TT genotype compared to

347 others without this gene variant will be less likely to achieve target BP with routine treatment,

348 but neither the patient nor the physician will be aware of this. The economic implications of

349 suboptimal BP control are considerable, with the direct costs of hypertension estimated in 2009

350 at $370 billion annually, representing 10% of healthcare expenditures worldwide [24].

351   Uniquely this study enabled the genetic risk of hypertension owing to this polymorphism

352 to be considered in relation to riboflavin (the MTHFR cofactor). Unlike other B vitamins (e.g.

353 folate and vitamin B12), riboflavin biomarkers are rarely measured in human studies and no

354 previous cohort study to have investigated this polymorphism has considered riboflavin [25].

355 We estimated a 3-fold increased risk of hypertension when the variant TT genotype occurred

356 in combination with deficient riboflavin status (relative to the CC genotype and normal

357 riboflavin status), whereas better riboflavin status was associated with reducing the excess

358 hypertension risk, and normal riboflavin status with no genetic risk. In contrast, among adults

359 with CT or CC genotypes, riboflavin status did not influence the risk of hypertension, evidence

360 that riboflavin has a genotype-specific role in BP. The finding that riboflavin has the potential

361 to modify blood pressure in adults affected by this polymorphism is entirely consistent with

362 our earlier studies in hypertensive patients, which showed a lowering of systolic BP by 6 to 13

363 mmHg in response to riboflavin supplementation specifically in the TT genotype [15–17],

364 resulting in a marked increase in blood-pressure control from 32% to 57% (pre versus post

365 riboflavin intervention for 16 weeks), despite no change in antihypertensive treatment over the

366 intervention period [17]. Here we show the potential of riboflavin to modify BP in genetically

367 at-risk adults at an earlier age and the data suggest that the onset of hypertension could be

368 delayed through intervention with riboflavin. Ideally, such intervention would occur prior to

369 commencing antihypertensive treatment and along with lifestyle interventions as per ESC/ESH

370 guidelines for hypertension management [1], especially given that riboflavin has no known

371 adverse effects even at doses of 100-fold higher than typical dietary intakes [26]. Alternatively,

372 riboflavin could be co-administered with an antihypertensive drug as a novel combination

373 therapy targeted at patients with this genetic risk factor. The potential to prevent or treat

374 hypertension in sub-populations worldwide could be considerable, given that this genotype

375 affects 10% of people globally, ranging 4-26% in Europeans (increasing north to south), 20%

376 in Northern China, to as high as 32% in Mexico [11].

377 The impact of this polymorphism on BP throughout adulthood and the potential modifying

378 effect of riboflavin are important findings, given that this polymorphism is linked with an

379 increased risk of stroke [12–14], and recent evidence shows that living longer in better

380 cardiovascular health during mid-life is associated with lower risk of disease and mortality later

381 in life [27]. Control of BP is highly effective in reducing cardiovascular mortality [5, 23, 24],

382 with each 2 mmHg lower systolic BP associated with a 10% lower risk of stroke [4].

383 Furthermore, powerful evidence, from the SPRINT trial testing the effects of intensive versus

384 standard blood-pressure control [28] and from meta-analyses of large-scale BP lowering trials

385 [29], highlights significant benefits for cardiovascular risk especially among middle-aged

386 adults [30] of BP-lowering to values below hypertension cut-points. Because of concerns that

387 intensive treatment of BP could also pose certain risks [31], however, there have been calls for

388 newer approaches, including novel combination therapies and non-pharmacological solutions

389 [32]. Our results indicate that the most effective timeframe to target adults with this genetic

390 variant will be up to 70 years, via supplementation with riboflavin to potentially offer an

391 effective low-cost strategy to lower BP. Of note, sub-optimal riboflavin status may be more

392 widespread than is generally recognised, but is largely undocumented as riboflavin biomarkers

393 are rarely measured in human studies [25]. The UK is one of the very few countries worldwide

394 to include a riboflavin biomarker in its population-wide diet and nutrition survey and recent

395   data shows that over 50% of healthy British adults have low or deficient riboflavin status [33],

396   in close agreement with the current results in Irish adults.

397   The biological mechanism explaining MTHFR-BP relationship shown here is unknown,

398   but likely involves the potent vasodilator nitric oxide (NO) [34]. Vascular tissue concentrations

399   of 5-methyltetrahydrofolate (the product of the MTHFR reaction) were associated with NO

400   bioavailability and improved endothelial function in patients undergoing coronary artery

401   bypass graft surgery, and were found to be lower in those patients with the TT genotype [35,

402   36]. The current results, considered with our earlier trials [15–17], indicate that the biologic

403   perturbation leading to higher BP in the TT genotype is modifiable with riboflavin. Molecular

404   studies show that the decreased enzyme activity in the TT genotype is owing to loss of the

405   riboflavin (FAD) cofactor from the active site [37], but riboflavin intervention can restore

406   MTHFR activity *in vivo* [38]. Restoring MTHFR in vascular tissue could in turn lower BP

407   specifically in individuals with the TT genotype. Mechanistic studies are required, but at this

408   time the evidence does not support a direct role for homocysteine in BP. Although elevated

409   homocysteine is the characteristic phenotype linked with this polymorphism (and is responsive

410   to riboflavin in the TT genotype [38]), intervention trials to lower homocysteine have shown

411   no corresponding BP response [39], indicating that homocysteine is not causatively related to

412   hypertension. The current results suggest that this polymorphism is linked with CVD via BP

413   independently of homocysteine, and given its importance for clinical outcomes, BP may be the

414   much more relevant target to prevent CVD in those affected by the variant genotype.

415   A strength of this study is its large sample of adults 18 to 90 years stratified for the relevant

416   polymorphism using data from two cohorts sampled under a common project initiative, from

417   participating centres in Northern Ireland (UK) and the Republic of Ireland (representing two

418   distinct health systems), using standardised methodologies and centralised laboratory analysis

419   to investigate outcomes that were formulated before data collection. Furthermore, the current

420 analysis was based on an *a priori* hypothesis (linking this polymorphism and riboflavin with

421 BP) whereas other studies of genetic risk factors in relation to disease risk factors are typically

422 opportunistic studies using available data. Thus uniquely, our study provides biomarker data

423 for riboflavin, rarely measured in nutritional studies, and used here to enable the impact of

424 riboflavin on the MTHFR-BP relationship from young adulthood to be demonstrated. The

425 major limitation of this study is its cross-sectional (rather than a longitudinal) design,

426 nonetheless the study findings in relation to the genotype-specific effect of riboflavin are

427 reinforced by our earlier trials [15–17] showing significant BP-lowering in response to

428 intervention with riboflavin in CVD patients identified with the relevant genotype.

429 **Conclusion**

430 The variant *MTHFR* 677TT genotype is associated with higher BP independently of

431 homocysteine and predisposes adults to an increased risk of hypertension and poorer BP control

432 with antihypertensive treatment, whilst better riboflavin status is associated with a reduced

433 genetic risk. Supplemental riboflavin could therefore offer a stratified approach to delay the

434 onset of hypertension and/or improve blood-pressure control in adults with the TT genotype,

435 representing 10% of people globally and higher in some populations. Such an approach aligns

436 with international strategies of personalising treatments to improve cardiovascular health, but

437 the findings require confirmation in randomised trials in non-hypertensive adults.

438

439 **Abbreviations**

440 CVD, cardiovascular disease; BP, Blood Pressure; GWAS, genome-wide association studies;

441 SNPs, single nucleotide polymorphisms; EGRac, erythrocyte glutathione activation

442 coefficient; FAD, flavin adenine dinucleotide; MTHFR, methylenetetrahydrofolate reductase.

443 TUDA, Trinity-Ulster Department of Agriculture; NANS, National Adult Nutrition Survey.

444

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473 **Authors' contributions**: The authors' contributions were as follows – HM, MW and JMS

474 conceptualised and designed the study. All authors completed the acquisition, analysis and

475 interpretation of the data. HM, MW, JJS, AMM, JMS, CC, MC, MG and AF obtained study

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482 **Additional File**

483 **Additional File 1: Table S1** General study characteristics by NANS and TUDA cohorts. **Table**

484 **S2** Blood pressure and use of antihypertensive drugs in adults 18-70 years by *MTHFR*

485 genotype.

References

1. Williams B, Mancia G, Spiering W, Rosei EA, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018;39:3021–104.

2. NCD Risk Factor Collaboration. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19·1 million participants. Lancet. 2017;389:37–55.

3. Chow CK, Teo KK, Rangarajan S, Islam S, Gupta R, Avezum A, et al. Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA. 2013;310:959–68.

4. Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002;360:1903–13.

5. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart Disease and Stroke Statistics—2016 Update. Circulation. 2015;133:e38–48.

6. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet. 2009;41:666–76.

7. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011;478:103–9.

8. Yang B, Fan S, Zhi X, Li Y, Liu Y, Wang D, et al. Associations of MTHFR gene polymorphisms with hypertension and hypertension in pregnancy: a meta-analysis from 114 studies with 15411 cases and 21970 controls. PLoS One. 2014;9:e87497.

9. Yang KM, Jia J, Mao LN, Men C, Tang KT, Li YY, et al. Methylenetetrahydrofolate reductase C677T gene polymorphism and essential hypertension: a meta-analysis of 10,415 subjects. Biomed Reports. 2014;2:699–708.

10. Niu WQ, You YG, Qi Y. Strong association of methylenetetrahydrofolate reductase gene C677T polymorphism with hypertension and hypertension-in-pregnancy in Chinese: a meta-analysis. J Hum Hypertens. 2012;26:259–67.

11. Wilcken B, Bamforth F, Li Z, Zhu H, Ritvanen A, Renlund M, et al. Geographical and ethnic variation of the 677C→T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas worldwide. J Med Genet. 2003;40:619–25.

12. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: Evidence on a causal link from mendelian randomisation. Lancet. 2005;365:224–32.

13. Holmes M V, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. Lancet. 2011;378:584–94.

14. McNulty H, Strain JJ, Hughes CF, Ward M. Riboflavin, MTHFR genotype and blood pressure: a personalized approach to prevention and treatment of hypertension. Mol Aspects Med. 2017;53:2–9.

15. Horigan G, McNulty H, Ward M, Strain J, Purvis J, Scott JM. Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the 677C→T polymorphism in MTHFR. J Hypertens. 2010;28:478–86.

16. Wilson CP, Ward M, McNulty H, Strain JJ, Trouton TG, Horigan G, et al. Riboflavin

offers a targeted strategy for managing hypertension in patients with the MTHFR 677TT genotype: a 4-y follow-up. Am J Clin Nutr. 2012;95:766–72.

17. Wilson CP, McNulty H, Ward M, Strain JJ, Trouton TG, Hoeft BA, et al. Blood pressure in treated hypertensive individuals with the MTHFR 677TT genotype is responsive to intervention with riboflavin: findings of a targeted randomized trial. Hypertension. 2013;61:1302–8.

18. Porter KM, Ward M, Hughes CF, O'Kane M, Hoey L, McCann A, et al. Hyperglycemia and metformin use are associated with B-vitamin deficiency and cognitive dysfunction in older adults. J Clin Endocrinol Metab. 2019;104:4837–47.

19. Irish Universities Nutrition Alliance. National Adult Nutrition Survey: Summary Report. 2011. www.iuna.net/surveyreports. Accessed May 2020.

20. NICE. Hypertension in adults: diagnosis and management. National Institute for Health and Care Excellence (NICE Clinical Guideline CG127). London: NICE; 2011.

21. Joffres M, Falaschetti E, Gillespie C, Robitaille C, Loustalot F, Poulter N, et al. Hypertension prevalence, awareness, treatment and control in national surveys from England, the USA and Canada, and correlation with stroke and ischaemic heart disease mortality: A cross-sectional study. BMJ Open. 2013;3:e003423.

22. Ji H, Kim A, Ebinger JE, Niiranen TJ, Claggett BL, Bairey Merz CN, et al. Sex differences in blood pressure trajectories over the life course. JAMA Cardiol. 2020;5:255–62.

23. Falaschetti E, Mindell J, Knott C, Poulter N. Hypertension management in England: a serial cross-sectional study from 1994 to 2011. Lancet. 2014;383:1912–9.

24. Gaziano TA, Bitton A, Anand S, Weinstein MC. The global cost of nonoptimal blood pressure. J Hypertens. 2009;27:1472–7.

25. McNulty H, Ward M, Hoey L, Hughes CF, Pentieva K. Addressing optimal folate and related B-vitamin status through the lifecycle: health impacts and challenges. Proc Nutr Soc. 2019;78:449–62.

26. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Dietary reference values for riboflavin. EFSA J. 2017;15:e04919.

27. Corlin L, Short MI, Vasan RS, Xanthakis V. Association of the duration of ideal cardiovascular health through adulthood with cardiometabolic outcomes and mortality in the Framingham Offspring study. JAMA Cardiol. 2020;5:1–8.

28. Williamson JD, Supiano MA, Applegate WB, Berlowitz DR, Campbell RC, Chertow GM, et al. Intensive vs standard blood pressure control and cardiovascular disease outcomes in adults aged ≥75 years: a randomized clinical trial. JAMA. 2016;315:2673–82.

29. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J, et al. Blood pressure lowering for prevention of cardiovascular disease and death: A systematic review and meta-analysis. Lancet. 2016;387:957–67.

30. Vaduganathan M, Claggett BL, Juraschek SP, Solomon SD. Assessment of long-term benefit of intensive blood pressure control on residual life span: secondary analysis of the Systolic Blood Pressure Intervention Trial (SPRINT). JAMA Cardiol. 2020;5:576–581.

31. Basu S, Sussman JB, Rigdon J, Steimle L, Denton BT, Hayward RA. Benefit and harm of intensive blood pressure treatment: Derivation and validation of risk models using data from the SPRINT and ACCORD trials. PLoS Med. 2017;14:e1002410.

32. Xie X, Atkins E, Lv J, Bennett A, Neal B, Ninomiya T, et al. Effects of intensive blood pressure lowering on cardiovascular and renal outcomes: updated systematic review and meta-analysis. Lancet. 2016;387:435–43.

33. Public Health England. National Diet and Nutrition Survey Results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009 – 2011/2012). 2014.

34. McNulty H, Strain JJ, Hughes CF, Pentieva K, Ward M. Evidence of a role for one-carbon metabolism in blood pressure: can B vitamin intervention address the genetic risk of hypertension owing to a common folate polymorphism? Curr Dev Nutr. 2020;4:nzz102.

35. Antoniades C, Shirodaria C, Warrick N, Cai S, De Bono J, Lee J, et al. 5-Methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and eNOS coupling. Circulation. 2006;114:1193–201.

36. Antoniades C, Shirodaria C, Leeson P, Baarholm OA, Van-Assche T, Cunnington C, et al. MTHFR 677 C>T polymorphism reveals functional importance for 5-methyltetrahydrofolate, not homocysteine, in regulation of vascular redox state and endothelial function in human atherosclerosis. Circulation. 2009;119:2507–15.

37. Pejchal R, Campbell E, Guenther BD, Lennon BW, Matthews RG, Ludwig ML. Structural perturbations in the Ala → Val polymorphism of methylenetetrahydrofolate reductase: how binding of folates may protect against inactivation. Biochemistry. 2006;45:4808–18.

38. McNulty H, Dowey LRC, Strain JJ, Dunne A, Ward M, Molloy AM, et al. Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677C→T polymorphism. Circulation. 2006;113:74–80.

39. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. JAMA. 2004;291:565–75.