

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

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## 23 Abstract

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

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

35 **Results:** The variant *MTHFR* 677TT genotype was identified in 12% of adults. From 18-70  
36 years, this genotype was associated with an increased risk of hypertension (i.e. systolic BP  
37  $\geq 140$  and/or a diastolic BP  $\geq 90$ mmHg): odds ratio (OR) 1.42, 95% confidence interval (CI)  
38 1.07 to 1.90;  $P=0.016$ , after adjustment for antihypertensive drug use and other significant  
39 factors, namely, age, male sex, BMI, alcohol and total cholesterol. Low or deficient biomarker  
40 status of riboflavin (observed in 30.2% and 30.0% of participants, respectively) exacerbated  
41 the genetic risk of hypertension, with a 3-fold increased risk for the TT genotype in  
42 combination with deficient riboflavin status (OR 3.00, 95% CI, 1.34-6.68;  $P=0.007$ ) relative  
43 to the CC genotype combined with normal riboflavin status. Up to 65 years, we observed poorer  
44 BP control rates on antihypertensive treatment in participants with the TT genotype (30%)  
45 compared to those without this variant, CT (37%) and CC (45%) genotypes ( $P<0.027$ ).

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

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

## 55 **Background**

56 Hypertension is the leading risk factor contributing to all-cause death in every region in the  
57 world, estimated to affect 1.13 billion people globally and account for over 9 million deaths  
58 annually, predominantly from cardiovascular disease (CVD) [1–3]. The relationship of blood  
59 pressure (BP) with disease is age-specific and most pronounced in adults 40-69 years, where  
60 the risk of CVD is estimated to double for each 20mmHg rise in systolic BP [4]. Recent reports  
61 have highlighted the importance of targeting lifestyle and treatments strategies at the individual  
62 level in order to improve cardiovascular health [1, 5], and genome-wide association studies  
63 (GWAS) have identified specific genes linked with BP which could lead to personalised  
64 treatments for hypertension based on genetic characteristics. The earliest of such studies tested  
65 2.5 million single nucleotide polymorphisms (SNPs) and identified eight genetic loci  
66 associated with BP, including a region near the gene encoding the folate-metabolising enzyme  
67 methylenetetrahydrofolate reductase *MTHFR* [6], findings confirmed by subsequent GWAS  
68 [7].

69 Of greater relevance to health, clinical studies have linked this gene with BP, with meta-  
70 analyses of case-control studies showing that the 677C→T polymorphism in *MTHFR* is  
71 associated with an increased risk of hypertension by 36-87% [8–10]. Previously the role of this  
72 polymorphism in CVD has been studied extensively in relation to the well-recognised  
73 phenotype, elevated homocysteine, whilst the relationship with BP is relatively under-  
74 investigated. The variant *MTHFR* 677TT genotype, which affects 10% of adults worldwide  
75 [11], is however reported to increase the risk of CVD (especially stroke) by up to 40%, albeit  
76 with a large geographical variation in the extent of excess risk, consistent with a gene-  
77 environment interaction [12–14]. In this regard only folate was previously considered, but  
78 emerging evidence suggests that riboflavin - required in the form flavin adenine dinucleotide  
79 (FAD) as a cofactor for *MTHFR* - may be a key modifying factor linking this polymorphism

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 ( $\geq 60$  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  $\chi^2$  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 <sup>‡</sup>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



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

188  
 189

**Table 1** General participant characteristics by *MTHFR* genotype

	<i>MTHFR</i> Genotype <sup>a</sup>			<i>p</i> value <sup>b</sup>
	CC ( <i>n</i> = 2677)	CT ( <i>n</i> = 2660)	TT ( <i>n</i> = 739)	
<i>MTHFR</i> genotype, <i>n</i> (%)	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 <sup>2</sup>	27.8 (5.2)	27.7 (5.4)	27.9 (5.2)	0.458
Current smokers, <i>n</i> (%)	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>B-vitamin Biomarkers</b>				
Red blood cell folate, nmol/L	1095 (579) <sup>a</sup>	1088 (626) <sup>a</sup>	971 (563) <sup>b</sup>	<0.001
Serum vitamin B12, pmol/L	295 (155)	295 (238)	296 (238)	0.194
Riboflavin status, EGRac <sup>c</sup>	1.35 (0.21)	1.35 (0.21)	1.34 (0.21)	0.769
Plasma homocysteine, µmol/L	14.2 (5.4) <sup>a</sup>	14.3 (5.4) <sup>a</sup>	15.7 (6.8) <sup>b</sup>	<0.001

190 Data are expressed as mean (standard deviation) or *n* (%).

191 <sup>a</sup>CC (wild type), CT (heterozygous), TT (homozygous variant), genotypes for the *MTHFR* 677C→T  
 192 polymorphism.

193 <sup>b</sup>*P*-value from one-way ANOVA comparing genotype groups, following log-transformation of data for  
 194 normalisation purposes, as appropriate. Different superscript letters (i.e. a, b) within a row indicate  
 195 significant differences by Bonferroni post-hoc test, whilst the same letter (i.e. a, a) indicates no  
 196 significant differences. Level of significance (*P*<0.003) adjusted for Bonferroni correction (*n* = 16).  
 197 Categorical variables assessed using chi-square analysis.

198 <sup>c</sup>Biomarker status of riboflavin determined by the functional assay, erythrocyte glutathione reductase  
199 activation coefficient (EGRac); higher EGRac values indicate lower riboflavin status.  
200

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

202 Irrespective of *MTHFR* genotype, systolic BP showed an increase with age up to approximately  
203 80 years, whereas diastolic BP increased until about age 60 years and then declined (**Fig 2**).  
204 Examination of BP by *MTHFR* genotype, however, showed higher BP in the TT genotype  
205 group up to approximately 65 years compared to adults of the same age with CC or CT  
206 genotypes, with systolic and diastolic BP in the TT genotype observed to be typical of an adult  
207 several years older without this genetic variant. From about 65 years onwards, however, the  
208 BP phenotype associated with this polymorphism was less evident.

209  
210 **Fig. 2** Systolic and diastolic blood pressure in adults 18-90 years by *MTHFR* genotype  
211 ( $n=6070$ ).

212 Data grouped by deciles of age from the youngest 10%, to the oldest 10%, of study participants. Each  
213 line illustrates median systolic or diastolic blood pressure for adults by age: CC (green line), CT (amber  
214 line) and TT (red line) genotypes for the *MTHFR* 677C→T polymorphism.  
215

216 Among adults 18-70 years, logistic regression analysis showed that the *MTHFR* 677TT  
217 genotype was associated with an increased risk of hypertension: odds ratio (OR) 1.42, 95%  
218 confidence interval (CI) 1.07 to 1.90, after adjustment for antihypertensive drug use (as a  
219 binary covariate) and other significant covariates, namely, age, male sex, BMI, alcohol, total  
220 cholesterol and study cohort (**Table 2**), whereas homocysteine was not independently  
221 associated with the risk of hypertension (apart from in treated adults). The OR for risk of  
222 hypertension associated with the TT genotype remained similar whether the logistic regression  
223 analysis was performed in all participants up to 70 years, or split into those treated or not treated  
224 with antihypertensive drugs, albeit the relationship failed to reach statistical significance within  
225 either treated or untreated categories (owing to the loss of statistical power as a result of a 50%  
226 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 <sup>b</sup>	OR	95% CI	P <sup>b</sup>	OR	95% CI	P <sup>a</sup>	
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 <sup>2</sup>	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 <sup>b</sup>	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 <sup>c</sup>	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	
<i>MTHFR</i> genotype <sup>d</sup>	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 <sup>a</sup>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 <sup>b</sup>as a binary (yes/no) covariate.

237 <sup>c</sup>Comparing TUDA cohort with NANS cohort (reference category). See supplementary Table S1 for  
 238 participant characteristics presented separately for each study cohort.

239 <sup>d</sup>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

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

	<i>MTHFR</i> genotype			<i>p</i> value <sup>b</sup>
	CC	CT	TT	
Total cohort (up to 90 years)	<i>n</i> = 2635	<i>n</i> = 2606	<i>n</i> = 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, <i>n</i> (%)	1373 (51%)	1411 (53%)	373 (50%)	0.302
Adults 18 to 70 years	<i>n</i> = 1124	<i>n</i> = 1138	<i>n</i> = 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) <sup>a</sup>	136.1 (135.0, 137.2) <sup>ab</sup>	137.6 (135.5, 139.6) <sup>b</sup>	0.026
Diastolic BP, mmHg	79.4 (78.9, 80.0) <sup>a</sup>	80.0 (79.4, 80.5) <sup>ab</sup>	81.4 (80.3, 82.5) <sup>b</sup>	0.013
Hypertension, <i>n</i> (%)	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 <sup>a</sup>Hypertension defined as systolic BP of ≥140 and/or a diastolic BP of ≥90mmHg [1].

258 <sup>b</sup>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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272 **Table 4.** Antihypertensive drug use in treated participants up to 70 years

	<i>MTHFR</i> 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)

273 Values are n (%).

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

277

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

282

### 283 ***MTHFR* genotype and riboflavin status in relation to hypertension**

284 The influence of riboflavin status in modifying the genetic risk of hypertension was then  
 285 examined (**Fig 3**). Based on functional status and response to low-dose riboflavin from  
 286 previous reports [17], participants were categorised as having normal (EGRac ≤1.26), low  
 287 (EGRac 1.26-1.40) or deficient (EGRac ≥1.40) riboflavin. Low or deficient riboflavin status  
 288 (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  $\leq$ 1.26; filled circles), low (EGRac  $>$ 1.26 to  $<$ 1.40; open circles) or deficient  
307 (EGRac  $\geq$ 1.40; open squares) riboflavin status.  
308

### 309 **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

445 **Declarations**

446 **Ethics approval and consent to participate:**

447 **TUDA:** Ethical approval was obtained from the Office for Research Ethics Committees  
448 Northern Ireland (reference number 08/NIR03/113), the Research Ethics Committee in St  
449 James's Hospital, and the Adelaide and Meath Hospital, Dublin. All participants provided  
450 written informed consent at the time of recruitment.

451 **NANS:** The study was approved by the Clinical Research Ethics Committee of the Cork  
452 Teaching Hospitals, University College Cork, and the Human Ethics Research Committee of  
453 University College Dublin. All eligible and willing participants gave their written consent  
454 according to the Helsinki declaration.

455 **Consent for publication:** Not applicable.

456 **Availability of data and materials:** Data from this study are held in full compliance with  
457 Ulster University's Research Governance and Ethics Policy for Human Research (2018)  
458 <https://internal.ulster.ac.uk/research/office/rofficeeg.php>, which in turn is fully aligned  
459 with the UK's Data Protection Act 2018. The data underlying the results presented in  
460 the study are available from Mr Nick Curry, Head of Research Governance at Ulster  
461 University at [n.curry@ulster.ac.uk](mailto:n.curry@ulster.ac.uk).

462 **Competing interests:** There is a patent granted in Europe and pending elsewhere by Ward,  
463 McNulty, Strain, Horigan and Scott, on the use of riboflavin in the treatment of hypertension;  
464 the other authors have no conflicts of interest to declare.

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466 and the Marine and Health Research Board (under the Food Institutional Research Measure,  
467 FIRM, initiative) and from the Northern Ireland Department for Employment and Learning  
468 (under its Strengthening the All-Island Research Base initiative). The work combines two  
469 studies completed as part of an All-Ireland initiative under the Joint Irish Nutrigenomics

470 Organisation, JINGO. The funders had no role in the study design, in the collection, analysis  
471 or interpretation of the data, in the writing of the report, or in the decision to submit for  
472 publication.

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  
476 funding. HM, MW, CFH, JJS, RR, CC, AMM, GH, KM, MOK, MJG, AF, JW, BAM, AMcC,  
477 LK, and JMS were responsible for the methodology. HM, MW, JJS, CC, AMM, AF and JMS  
478 provided study supervision. MW and HM drafted the original version of the manuscript. All  
479 authors critically revised drafts of the manuscript and approved the final version.

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481 research and was responsible for the original study concept.

#### 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.

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