Proceedings of the Nutrition Society (2016), **75**, 405–414 doi:10.1017/S0029665116000197
 First published online 12 May 2016

24th Irish Postgraduate Conference hosted by the Ulster University and held at Ramada Hotel, Portrush on 12–13 February 2015

Irish Section Postgraduate Meeting

Riboflavin status, MTHFR genotype and blood pressure: current evidence and implications for personalised nutrition

E. McAuley, H. McNulty, C. Hughes, J. J. Strain and M. Ward*
Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, BT52 1SA, UK

Clinical deficiency of the B-vitamin riboflavin (vitamin B_2) is largely confined to developing countries; however accumulating evidence indicates that suboptimal riboflavin status is a widespread problem across the developed world. Few international data are available on riboflavin status as measured by the functional biomarker, erythrocyte glutathione reductase activation coefficient, considered to be the gold standard index. One important role of riboflavin in the form of flavin dinucleotide is as a co-factor for the folate-metabolising enzyme methylenetetrahydrofolate reductase (MTHFR). Homozygosity for the common C677T polymorphism in MTHFR, affecting over 10 % of the UK and Irish populations and up to 32 % of other populations worldwide, has been associated with an increased risk of CVD, and more recently with hypertension. This review will explore available studies reporting riboflavin status worldwide, the interaction of riboflavin with the *MTHFR* C677T polymorphism and the potential role of riboflavin in personalised nutrition. Evidence is accumulating for a novel role of riboflavin as an important modulator of blood pressure (BP) specifically in individuals with the *MTHFR* 677TT genotype, with results from a number of recent randomised controlled trials demonstrating that riboflavin supplementation can significantly reduce systolic BP by 5–13 mmHg in these genetically at risk adults. Studies are however required to investigate the BP-lowering effect of riboflavin in different populations and in response to doses higher than 1·6 mg/d. Furthermore, work focusing on the translation of this research to health professionals and patients is also required.

Riboflavin: Methylenetetrahydrofolate reductase: MTHFR C677T: Blood pressure: Personalised nutrition

Riboflavin (vitamin B_2) is a water-soluble B-vitamin defined chemically as 7,8-dimethyl-10-1′-D-ribityl isoalloxazine. It acts as a precursor for FMN and FAD[1]. Clinical riboflavin deficiency is not generally considered to be a problem in the developed world but in recent years evidence has shown that sub-optimal status may be more widespread than generally perceived based on studies reporting the functional biomarker, erythrocyte glutathione reductase activation coefficient (EGRac) generally considered as the gold standard index of status. Few international data are however available based on EGRac and reports on riboflavin status are more commonly based solely on dietary intake data. Although riboflavin is required for numerous metabolic reactions its role (in the form of FAD) as a cofactor for the folate-metabolising enzyme, methylenetetrahydrofolate reductase (MTHFR) has recently received particular attention. Homozygosity (*MTHFR* 677TT genotype) for a common polymorphism in MTHFR, affecting over 10 % of the UK and Irish populations and up to 32 % of other populations worldwide[2], has been associated with an increased risk of CVD[3] and more recently with hypertension[4]. Emerging evidence from intervention trials supports a novel role for riboflavin supplementation in protecting against hypertension specifically in individuals with the *MTHFR* 677TT genotype[5–7]. This genotype-specific effect of riboflavin potentially offers a personalised approach for the prevention and treatment of

Abbreviations: BP, blood pressure; EGRac, erythrocyte glutathione reductase activation coefficient; GP, general practitioners; MTHFR, methylenetetrahydrofolate reductase; NO, nitric oxide.

***Corresponding author**: Professor M. Ward, email mw.ward@ulster.ac.uk

hypertension. This review will explore available studies reporting riboflavin status in populations worldwide and consider its potential role in human health, with a particular emphasis on the interaction of riboflavin with the *MTHFR* C677T polymorphism. In this regard, the potential role of riboflavin in personalised nutrition and its translation to the management of patients with hypertension will also be discussed.

Riboflavin and health

Roles of riboflavin in human health

Riboflavin is a water-soluble B-vitamin, which acts as a precursor for the coenzymes FMN and FAD, the metabolically active vitamin forms[1]. These coenzymes (FAD and FMN) participate in intermediary metabolism and catalyse numerous oxidation–reduction reactions, playing a fundamental role in the metabolism of energy, certain drugs and toxins and in antioxidant protection[8,9]. Furthermore, riboflavin interacts with a number of other nutrients, including metabolically linked B-vitamins and iron. Early animal studies have linked riboflavin deficiency with impaired iron absorption, increased intestinal loss of iron, and/or impaired iron utilisation for the synthesis of Hb[10]. More recently, supplementation with riboflavin has been shown to enhance circulating Hb levels in human subjects[11] furthermore, correcting riboflavin deficiency in individuals who were both riboflavin and iron deficient improved the response of iron deficiency anaemia to iron therapy[12]. Irrefutable evidence has shown the metabolism of other B-vitamins is dependent on riboflavin coenzymes. Riboflavin is involved in vitamin B_6 metabolism; the enzyme pyridoxine-phosphate oxidase requires FMN for the conversion of pyridoxine phosphate to its coenzyme form pyridoxal-5 phosphate[13]. Historical evidence from animal studies reported that pyridoxine-phosphate oxidase activity is sensitive to changes in dietary riboflavin intake and thus riboflavin deficiency may alter pyridoxal 5′ phosphate activity[14]. Research from our centre conducted a number of years ago confirmed the interrelationship between riboflavin and vitamin B_6 in human subjects and showed that riboflavin supplementation of older adults not only improved biomarker status of riboflavin, but also enhanced blood pyridoxal-5 phosphate (vitamin B_6) concentrations[15]. Niacin synthesis is also reliant on the FAD-dependent enzyme kynurenine mono-oxygenase, which is required for the synthesis of the coenzymes NAD and nicotinamide adenine dinucleotide phosphate from tryptophan. Riboflavin deficiency decreases the conversion of tryptophan to NAD and nicotinamide adenine dinucleotide phosphate resulting in niacin deficiency[8]. Work from our centre and others have demonstrated the key role riboflavin plays in C_1 metabolism via its role as a co-factor for the MTHFR enzyme.

Riboflavin absorption and transport

Dietary riboflavin occurs mainly in the form of FAD and smaller amounts occur as FMN or as free riboflavin[16]. Unlike free riboflavin, FAD and FMN must be hydrolysed in the intestinal lumen to yield free riboflavin prior to absorption. Animal studies have shown that the uptake of dietary riboflavin from the intestine is increased in riboflavin deficiency[17] and urinary excretion was found to increase linearly with increasing dietary intakes in individuals with optimal status[18]. The transport of flavins in blood is by loose binding to albumin and tight binding to a number of immunoglobulins in serum particularly IgA, IgG and IgM[19]. A number of physiological factors have been reported to influence the rate of intestinal absorption of riboflavin. Diets high in psyllium gum decrease the rate of intestinal absorption, whereas bile salts increase absorption[20]. Alcohol is reported to interfere with the digestion of food flavins into riboflavin and the intestinal absorption of riboflavin[21]. Notably, concentrations of riboflavin synthesised by bacterial metabolism in the human colon may be more than 6-fold higher than dietary intakes[22].

Table 1. Food sources of riboflavin in the UK (NDNS)[27]

Sources	mg/average serving	mg/100 g
Milk	0·45	0·90
Yoghurt	0·35	0·44
Eggs	0·26	0·52
Fortified breakfast cereal	0·22	0·74
Spinach	0·21	0·24
Chicken	0·15	0·15
Cheese	0·11	3·67
Bread (White)	0·09	0·36

NDNS, National Diet and Nutrition Survey (2008–2009 to 2011–2012).

Riboflavin requirements and sources

Worldwide dietary recommendations for riboflavin range from 1·1 to 1·6 mg/d for adults, an increment of 0·3 mg/d is recommended during pregnancy to cover the increased tissue synthesis for fetal and maternal development and an additional 0·4–0·5 mg/d during lactation[23–25]. Clinical signs of deficiency in human subjects appear at intakes <0·5–0·6 mg/d and urinary excretion of riboflavin is seen at intake levels of approximately 1 mg/d[1]. Riboflavin is found in a wide variety of food but yeast extract and offal products especially those based on liver are the only rich sources, containing more than 2 mg/100 g[26]. The latest National Diet and Nutrition Survey reported that milk and milk products, meat, and fortified breakfast cereal make the greatest contribution to riboflavin intake in a British diet[27] (Table 1). Unlike any European country, the USA has a mandatory riboflavin enrichment policy to replace the riboflavin lost from starch during milling (0·40 mg for wheat starch)[28]. Similarly, a mandatory enrichment of starch with riboflavin (4 mg/kg for starch) is in place in Canada. No toxic effects have been reported in relation to intakes of riboflavin at doses higher than dietary recommendations[29–32], the absorption of riboflavin appears to be limited to approximately 30 mg at any one time[13] with apparently little or no absorption at higher doses[33].

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

Furthermore, the Institute of Medicine in the USA did not establish a tolerable upper intake level for riboflavin when the RDA was revised in 1998[1]. A harmless side-effect of high-dose riboflavin intake is the increased intensity of urine colour to bright yellow.

Riboflavin deficiency

Isolated clinical riboflavin deficiency does not have unique or characteristic physical features. The classical symptoms, angular stomatitis, cheilosis and glossitis, are not specific to riboflavin deficiency and may be due to other vitamin deficiencies. Furthermore, after 3–8 months of inadequate riboflavin intake other symptoms are reported to appear including magenta tongue, seborrheic dermatitis, vascularisation of the cornea and normochromic normocytic anaemia[1,8]. Other than dietary inadequacy certain endocrine abnormalities i.e. adrenal and thyroid hormone insufficiency[20,34] and certain drugs can inhibit the conversion of riboflavin into its active coenzyme derivatives[35,36].

Newborn infants are at increased risk of riboflavin deficiency when maternal status is poor during pregnancy[37] or as a result of phototherapy treatment for hyperbilirubinaemia[38]. Pregnant women with riboflavin deficiency have been reported to be 4·7 times more likely to develop preeclampsia compared to those with adequate riboflavin status[39]. It has been suggested this may be associated with mitochondrial function, oxidative stress and blood vessel dilation[39]. Furthermore, a common polymorphism (C677T) in the gene encoding the FAD-dependent enzyme MTHFR has been associated with an increased risk of preclampsia[40]. A number of studies have reported that riboflavin requirements are higher with increased physical activity levels[41,42], excessive alcohol consumption and smoking[21,43].

Assessment of biomarker status of riboflavin

Riboflavin status can be measured in a range of biological samples, including urine, plasma and erythrocytes. The method for the estimation of riboflavin status, which is regarded as the gold standard is EGRac, a functional assay that measures the activity of glutathione reductase before and after *in vitro* reactivation with its prosthetic group FAD[44]. EGRac is calculated as a ratio of FAD stimulated to unstimulated enzyme activity, with higher values reflective of lower riboflavin status. However currently there is no consensus as to the appropriate EGRac cut-off values to indicate low/high status, with studies reporting deficiency ranges from >1·2, >1·3 or >1·4[11,45,46]. Recent changes made to the assay methodology resulted in the acceptance of a cut-off ≥1·3 although others have suggested that this cut-off value should be further increased[46]. A systematic review by Hoey *et al.*[44] identified EGRac to be an effective biomarker of riboflavin status at a population level with severe deficient-to-normal riboflavin status. This conclusion was drawn from randomised controlled trials and found EGRac to be sensitive to changes in supplementation periods of at least 4 weeks with doses ranging from 1·0 to 5·0 mg. The EGRac assay reflects long-term riboflavin status; however a number of conditions are known to affect the performance of the assay, including deficiency of glucose-6-phosphate dehydrogenase, β-thalassemia, hypothyroidism and hyperthyroidism[47].

Riboflavin status can also be assessed by urinary excretion although this is influenced by age, physical activity, body temperature, treatment with certain drugs and negative nitrogen balance[48]. Riboflavin excretion is reduced to 40 μg/24 h during deficiency compared with 120 μg/24 h when optimal status is achieved[49]. A number of studies have used biological samples particularly plasma and erythrocytes to measure riboflavin status directly but the results are inconsistent[50,51]. FMN is generally regarded as a more useful marker of status than FAD, which appears to be relatively unresponsive to riboflavin intakes[52].

Riboflavin status: the global picture

In the developing world riboflavin deficiency is commonly acknowledged; less well recognised however is the evidence emerging to suggest that sub-optimal riboflavin status may also be more wide-spread in developed countries than previously considered. The majority of population-based studies report dietary intake data only for riboflavin and relatively few have included a biomarker of riboflavin status.

A number of European studies have identified low dietary intake of riboflavin[27,53,54]. Furthermore, Troesch *et al.*[55], reported the percentage of men and women with dietary riboflavin intakes below the recommended nutrient intake using national dietary surveys; intakes were lowest in the Netherlands (25–50 % of men and >50–70 % of women) followed by the UK (5–25 % of men and >25–50 % of women), then Germany (5–25 % of men and women) and the USA (<5 % of men and 5–25 % of women). A systematic review including data from adults ≥65 years (*n* 28 000) in Europe, North America, Australia and New Zealand concluded that 41 % of males and 31 % of females had dietary intake values below the estimated average requirement, with riboflavin identified as one of six nutrients considered to be a possible public health concern[56]. Similarly in Asia, a number of large population-based studies and national dietary surveys have reported inadequate riboflavin intakes[57–59]. It has been suggested that the Chinese population tend to excrete very little riboflavin and thus their requirement may be lower than that of other populations[60]; however, this requires further investigation.

Far fewer studies have reported EGRac values and when they are reported they are typically limited to certain age and ethnic groups and lack standardised EGRac cut-off thresholds making comparisons between population groups difficult. Using a cut-off EGRac value of ≥1·40, one study of 311 children in Botswana reported riboflavin deficiency in up to 40 %[61]. In elderly free living adults in Guatemala (*n* 433) the prevalence of

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

riboflavin deficiency was reported to range from 50 to 75 % using a cut-off EGRac value of >1·3, and status was found to be strongly correlated with milk consumption[62]. Consistent with this evidence, a larger study conducted in an elderly Taiwanese population (*n* 2379) reported that one in four had marginal riboflavin deficiency based on EGRac >1·2[63]. More recently, biomarker status of riboflavin was investigated in women of child bearing age in Cambodia; 89–92 % of women were reported to be deficient or have suboptimal status based on an EGRac value ≥1·3. For comparison purposes in the latter study, a small convenient sample of women in urban Vancouver Canada (*n* 49; two-thirds European descent and one-third Chinese), were investigated and somewhat surprisingly 70 % were reported to have suboptimal or deficient riboflavin status[64].

The UK is one of the very few countries worldwide to report biomarker data for riboflavin in a population-based survey. The most recent National Diet and Nutrition Survey indicates that all age/sex groups had a mean EGRac >1·30, with the poorest status reported in 11–18 year olds (EGRac values of 1·47 reported for boys and 1·53 for girls). However, 21 % of girls reported dietary riboflavin intakes below the lower reference nutrient intake[27]. The high prevalence of biochemical riboflavin deficiency in the UK population is not fully understood as dietary intakes for riboflavin are sufficient, with the exception of 11–18 year olds with mean intakes of 1·97 mg/d for men and 1·50 mg/d for women reported.

Based on the available evidence sub-optimal riboflavin status appears to be common in many populations. The significance of these findings is not clear; however, it is possible that marginal riboflavin status in the absence of clinical deficiency may have adverse functional effects and long-term consequences for health. A standardised method of EGRac assessment is required and consideration of the current threshold (EGRac ≥1·3) needs to be reinvestigated to better reflect functional impairment. The intake and requirements of populations requires further investigation based on robust biomarker data.

Riboflavin and C_1 metabolism

The B-vitamins folate, vitamins B_{12} and B_6 and riboflavin are fundamental for C_1 metabolism, the metabolic process involving the transfer and utilisation of C_1 units in a network of biochemical pathways required for DNA and RNA biosynthesis, amino acid metabolism and methylation reactions. Riboflavin in its co-factor form FAD is critical for the folate metabolising enzyme MTHFR, required for the irreversible conversion of 5, 10-methylenetetrahydrofolate to the predominant circulating and cellular form of folate, 5-methyltetrahydrofolate, which then serves as a methyl donor for the remethylation of homocysteine to methionine. A common variant in the *MTHFR* gene is the 677C → T polymorphism, which involves a point mutation, in which cytosine (C), localised at nucleotide 677 of the gene, is replaced by thymidine (T), in turn producing an alanine to valine substitution in the enzyme. This results in a thermolabile form of MTHFR with approximately 30 % decreased enzyme activity and elevated homocysteine concentration *in vivo*[65]. The prevalence of the *MTHFR* 677TT genotype is reported to be 10 % worldwide, but this varies in different geographical regions and ethnic groups; ranging from 4 to 26 % in Europe, 4 to 18 % in USA, 20 % in Northern China to as high as 36 % in Mexico[2].

In vitro evidence suggests that the reduced activity of the variant enzyme is the result of an increased propensity to dissociate from its FAD cofactor[66,67]. Early animal studies showed that MTHFR enzyme activity is lower in the livers of riboflavin deficient rats than in controls[68]. These findings were confirmed by Bates and Fuller[69] who reported a dose-dependent relationship between riboflavin status and MTHFR activity. More recently in human studies, riboflavin supplementation was shown to lower plasma homocysteine specifically in individuals with the *MTHFR* 677TT genotype[70], suggesting that the variant enzyme can be stabilised by optimising riboflavin status. A number of studies have identified riboflavin as an important determinant of homocysteine among individuals with the TT genotype, which is independent of folate status[50,71,72]. This evidence confirms the modulating role of riboflavin in determining homocysteine concentration in individuals with the TT genotype.

Riboflavin, C_1 metabolism and CVD risk

In addition to its role as the main genetic determinant of plasma homocysteine concentration, the C677T polymorphism in *MTHFR* has been independently associated with a higher risk of CVD, certain cancers, neural tube defects and most recently with hypertension. Of particular interest, extensive evidence has led to a number of meta-analyses reporting a strong association between this polymorphism and CVD, particularly stroke[73–76]. It has been estimated that individuals with the *MTHFR* 677TT polymorphism have a 14–21 % increased risk of CHD[75,77,78]. Of note, these meta-analyses have identified important geographical influences on the extent of excess CVD risk due to this polymorphism, strongly suggesting that environmental factors may have a modulating effect on the phenotype and thus CVD risk.

Novel role of MTHFR genotype and blood pressure

Globally, hypertension accounts for 16·5 % of deaths each year (9·4 million); an estimated 45 % of deaths due to heart disease and 51 % of deaths due to stroke are a result of hypertension[79]. High blood pressure (BP), even within the normal range substantially increases the risk of CVD and death, while a lowering of systolic BP by as little as 2 mmHg can decrease CVD risk by as much as 10 %[80]. Hypertension is a polygenic disease that occurs as a result of a complex interaction of diverse environmental conditions and genetic factors. Risk factors include high dietary sodium intake,

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

Table 2. Meta-analyses of association of C677T polymorphism in MTHFR with hypertension*

Author	Sample size (*n*)	Populations	Odds ratio (95% CI)†
Qian *et al.*[116]	2814 cases 3099 controls	Caucasian Chinese	1·24 (1·02, 1·50)
Niu *et al.*[117]	1520 cases 1334 controls	Chinese	1·87 (1·31, 2·68)
Yang *et al.*[4]	6584 cases 6760 controls	Worldwide	1·36 (1·20, 1·53)
Wu *et al.*[118]	5207 cases 5383 controls	Worldwide	1·62 (1·32, 1·99)
Yang *et al.*[119]	5418 cases 4997 controls	Worldwide	1·59 (1·32, 1·92)

MTHFR, methylenetetrahydrofolate.
* A number of these meta-analyses have considered hypertension in pregnancy, only results for hypertension have been considered in this review.
† Odds ratio refers to MTHFR CC *v*. TT genotypes.

excess weight, excessive alcohol consumption and lack of physical activity[81]. Notably, twin studies have reported the heritability of BP variation to be as much as 50 %[82,83]. In the past decade, genome-wide association studies have identified several genetic loci associated with BP variation in European populations, including one near the gene encoding the folate metabolising enzyme MTHFR[84–86]. This finding was also replicated in non-European cohorts including Chinese, Japanese, Indian and US populations[87–90].

Generally consistent with these findings from genome-wide association studies, there is a growing body of evidence from observational studies to support a specific association between the 677C → T polymorphism in the *MTHFR* gene and BP variation[91–94]. As the evidence has accumulated numerous meta-analysis were conducted, all reporting significant associations of the *MTHFR* 677C → T polymorphism with hypertension (Table 2). Although there is strong evidence linking this polymorphism with hypertension, a number of observational studies have reported conflicting or inconclusive results. Many of the studies that failed to detect significant associations used small sample sizes or suffered possible selection bias[93,95–99]. Some studies have reported a gender specific association; one large population study in >3000 Japanese individuals reported that the *MTHFR* 677C → T polymorphism was associated with a 42 % increased risk of hypertension in women but not in men[96]. In contrast, in a Spanish cohort, the TT genotype was found to be a significant predictor of hypertension in men but not in women, however, only 26 % of the sample were females[100].

Recent work conducted at our centre has considered the BP-lowering effect of riboflavin supplementation in individuals with the *MTHFR* 677TT genotype. The first of these trials was conducted in premature CVD patients (mean age 54 years) and demonstrated that riboflavin (1·6 mg/d for 16 weeks) decreased systolic BP (−13·2 (SD 15·0) mmHg; $P \leqq 0{\cdot}02$) and diastolic BP (−7·5 (SD 12·0) mmHg; $P = 0{\cdot}02$) specifically in individuals with the TT genotype, while no BP response was observed in those with CC or CT genotype[5]. These findings were later confirmed in a 4-year follow-up cross-over design study, which demonstrated in the same cohort (mean age 59 years) that riboflavin (at the same dose and duration of intervention) significantly lowered systolic BP (−9·2 (SD 12·8) mmHg; $P = 0{\cdot}001$) and diastolic (−6·0 (SD 9·9) mmHg; $P = 0{\cdot}003$) BP specifically in the TT genotype group[6]. These findings were subsequently confirmed in hypertensive patients without overt CVD aged 70 years (5·6 (SD 2·6) mm Hg lowering in systolic BP; $P = 0{\cdot}033$)[7]. The extent of response to riboflavin supplementation observed in these trials appears to lessen with increasing age. Based on the available evidence[5–7] and in agreement with preliminary findings from a large population-based study[101], it appears that age is a significant factor in relation to the BP phenotype and its responsiveness to riboflavin and should be considered in future studies. To date, a low-dose supplementation level (1·6 mg/d) of riboflavin has been used and the effect of higher doses is not known. Thus, it remains possible that greater BP-lowering could be achieved with a larger dose of riboflavin. Of note, in all of the aforementioned trials the BP-lowering effect of riboflavin was shown to be independent of the number and type of antihypertensive drugs being currently administered.

Mechanism of MTHFR C677T polymorphism, riboflavin and blood pressure

The exact mechanism by which the *MTHFR* C677T polymorphism affects BP (and riboflavin modulates the relationship) has not been clearly identified; however, there are a number of plausible explanations which could explain these effects. In two separate studies involving patients undergoing coronary artery bypass graft surgery, it was identified that those with the *MTHFR* 677TT genotype had reduced vascular concentrations of 5-methyltetrahydrofolate which in turn were associated with deregulation of nitric oxide (NO); a potent vasodilator known to play a key role in BP[102,103]. This group and others have not considered the role of riboflavin; however, riboflavin supplementation, could in theory stabilise the variant MTHFR enzyme and restore 5-methyltetrahydrofolate concentrations in vascular cells, thereby improving NO bioavailability, which could in turn improve endothelial function and lower BP in individuals with the TT genotype. It is also possible that this novel gene–nutrient interaction may be a result of an imbalance of non-methylated folate derivatives in the endothelial cells in individuals with the TT genotype which in turn could reduce endothelial NO synthase coupling. In individuals with the TT genotype an accumulation of formylated tetrahydrofolates has been detected in erythrocytes, while only 5-methyltetrahydrofolate was found in the erythrocytes of individuals with the CC genotype[104]. It has been suggested that an accumulation of 10-formyl tetrahydrofolate in endothelial cells may affect folate metabolism and in turn affect endothelial NO synthase activity[105]. Therefore, riboflavin may enhance endothelial NO synthase activity by correcting the imbalance in methylated *v*. non-methylated tetrahydrofolate in

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

those with the TT genotype or by increasing 5-methyltetrahydrofolate and thus decreasing BP irrespective of BP-lowering drugs[105]. Further work is required to investigate mechanisms linking this polymorphism with BP and the potential for riboflavin to provide a targeted option to treat elevated BP in this genotype group.

Implications for the use of riboflavin as a personalised blood pressure management option

The concept that nutrient recommendations require differentiation for specific subgroups of the population is not a new concept and was described as far back as the 1970s[106]. Many definitions for personalised nutrition exist; however recently, Ronteltap *et al.*[107] defined personalised nutrition at three levels, where one level builds on the foundations of another and level 3 is regarded as the ultimate personalisation, with advice based on the individual's diet, phenotypic parameters and genetic profile. Given that evidence is accumulating to support the role of riboflavin in modulating BP in individuals with the *MTHFR* 677TT genotype, translating this research, both to health professionals responsible for the management of BP and those genetically at risk, should be considered. However, limited evidence is available regarding the attitude of health professionals towards riboflavin as a targeted treatment option for BP management.

In recent years, there is increasing public interest in genetic testing in the health field, in a study investigating attitudes to genetic testing among 2000 individuals, 81 % of respondents believed that knowing their genetic risk could lead to better control of their lives[108]. A number of studies have reported that individuals identified as having a higher disease risk through genetic testing may be more motivated to change dietary habits[109–111]. Nevertheless a number of concerns towards genetic testing have been reported including cost, privacy, misuse of genetic information and fear that results could influence insurance companies and job opportunities[107,108,110,112]. Despite these criticisms many believe that genetic testing has the potential to motivate consumers to adopt changes that aim to prevent the onset and development of diseases[113,114]. Currently however, personal genetic testing is only easily obtained by Direct-to-Consumer genetic testing kits without interpretation by a healthcare professional[115]. Furthermore, very few diet–gene–health relationships have been tested for causality in human intervention studies[109]. This has resulted in concerns regarding the analytical validity and clinical utility of the genetic testing for general consumer purposes.

Potential role of the health professional in personalised medicine

Given the important role of general practitioners (GP) in BP management their attitude towards riboflavin as a treatment option for hypertension in individuals with the *MTHFR* 677TT genotype is important for the translation of this novel role for riboflavin. A number of studies have highlighted the role of GP in genetic testing of their patients[120,121]; however, few have considered the attitude of GP towards targeted treatment options for diseases and the potential role of GP in the delivery of personalised medicine. One study of Canadian oncologists, cardiologists and family physicians (*n* 363) reported that although the majority of respondents agreed that personalised medicine could influence treatment plans and improve outcomes, a number of barriers were perceived, including lack of clinical guidelines, limited provider knowledge and the lack of evidence-based clinical information. These Canadian physicians recognised that they lacked the education, information and support they needed to practice personalised medicine effectively and that they required national strategies, resources and training[121]. Thus, although many health care professionals recognise the potential of nutrigenomics in the prevention and treatment of diseases, many feel pessimistic about incorporating this new concept into their practice as they do not believe it provides sufficient information to adequately advise patients[122,123]. A number of challenges have been identified in the delivery of this information to the patient and it is clear that increasing genomics education in the training of health care professionals is required. Such intervention was previously found to improve both self-reported and assessed genomics knowledge among medical students[124]. Further work is clearly needed to investigate attitudes, particularly of GP, towards riboflavin as a treatment option for hypertension in patients identified with the *MTHFR* 677TT genotype.

Conclusion and future work

There is emerging evidence that sub-optimal riboflavin status is a problem not confined to developing countries, but also evident in the developed world. Apart from the widely recognised roles of riboflavin in human health, a novel and important role of riboflavin in modulating BP specifically in individuals with the *MTHFR* 677TT genotype is emerging. Optimal riboflavin status may therefore be particularly important in maintaining health for the 10 % of individuals worldwide (and up to 30 % in some populations) who share this genetic characteristic and are thus at increased risk of developing hypertension. Riboflavin potentially offers a personalised approach to the prevention and treatment of hypertension in these genetically at risk individuals. Further studies are however required to further investigate the BP-lowering effect of riboflavin in different populations and in response to doses higher than 1·6 mg/d. Furthermore, work focusing on the translation of this research to health professionals and patients is also required.

Acknowledgements

E. McA.'s PhD studentship was funded by the Northern Ireland Department for Employment and Learning who had no role in the design, analysis or writing of this paper. DSM Nutritional Products Ltd partly supported project costs associated with E. McA.'s PhD.

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

Financial Support

This work was supported by funding from the Northern Ireland Department for Employment and Learning which funded the PhD studentship for E. McA. The Northern Ireland Department for Employment and Learning had no role in the design, analysis or writing of this paper.

Conflicts of Interest

There is a patent granted in Europe and pending elsewhere by M. W., H. McN. and J. J. S. and on the use of riboflavin in the treatment of hypertension.

Authorship

E. McA. drafted the manuscript. M. W., H. McN., C.H. and J. J. S. critically revised the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

References

1. Food and Nutrition Board, Institute of Medicine (1998) Riboflavin. In *Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B_6, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline*, pp. 87–122 [RM Pitkin, editor]. Washington, DC: National Academy Press.
2. Wilcken B, Bamforth F, Li Z *et al.* (2003) 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* **40**, 619–625.
3. Klerk M, Verhoef P, Clarke R *et al.* (2002) MTHFR 677C→T polymorphism and risk of coronary heart disease – a meta-analysis. *JAMA* **288**, 2023–2031.
4. Yang B, Fan S, Zhi X *et al.* (2014) 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* **9**, e87497.
5. Horigan G, McNulty H, Ward M *et al.* (2010) Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the 677C→T polymorphism in MTHFR. *J Hypertens* **28**, 478–486.
6. Wilson CP, Ward M, McNulty H *et al.* (2012) Riboflavin offers a targeted strategy for managing hypertension in patients with the *MTHFR* 677TT genotype: a 4-y follow-up. *Am J Clin Nutr* **95**, 766–772.
7. Wilson CP, McNulty H, Ward M *et al.* (2013) Blood pressure in treated hypertensive individuals with the *MTHFR* 677TT genotype is responsive to intervention with riboflavin: findings of a targeted randomized trial. *Hypertension* **61**, 1302–1308.
8. McCormick DB (2006) Riboflavin. In *Modern Nutrition in Health and Disease*, 10th ed., pp. 391–399 [ME Shils, editor]. New York: Williams & Wilkins.
9. Powers HJ (1999) Current knowledge concerning optimum nutritional status of riboflavin, niacin and pyridoxine. *Proc Nutr Soc* **58**, 435–440.
10. Powers HJ, Weaver LT, Austin S *et al.* (1993) A proposed intestinal mechanism for the effect of riboflavin deficiency on iron loss in the rat. *Br J Nutr* **69**, 553–561.
11. Powers HJ, Hill MH, Mushtaq S *et al.* (2011) Correcting a marginal riboflavin deficiency improves hematologic status in young women in the United Kingdom (RIBOFEM). *Am J Clin Nutr* **93**, 1274–1284.
12. Powers HJ (1995) Riboflavin-iron interactions with particular emphasis on the gastrointestinal tract. *Proc Nutr Soc* **54**, 509–517.
13. McCormick DB (1989) Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol Rev* **69**, 1170–1198.
14. Rasmussen KM, Barsa PM & McCormick DB (1979) Pyridoxamine (pyridoxine) 5′-phosphate oxidase activity in rat tissues during development of riboflavin or pyridoxine deficiency. *Proc Soc Exp Biol Med* **161**, 527–530.
15. Madigan SM, Tracey F, McNulty H *et al.* (1998) Dietary intake and status of riboflavin and vitamin B_6 and biochemical response to riboflavin supplementation in free living elderly people. *Am J Clin Nutr* **68**, 389–395.
16. Powers HJ (2003) Riboflavin (vitamin B-2) and health. *Am J Clin Nutr* **93**, 1274–1284.
17. Said HM & Mohammadkhani R (1993) uptake of riboflavin across the brush border membrane of rat intestine: regulation by dietary vitamin levels. *Gastroenterology* **105**, 1294.
18. Sauberlich HE, Skala JH & Dowdy RP (1974) *Laboratory Tests for the Assessment of Nutritional Status*. Boca Raton, FL: CRC Press.
19. McCormick DB (1990) Riboflavin. In *Present Knowledge in Nutrition*, 6th ed., pp. 146. Washington, DC: International Life Sciences Institute.
20. Rivlin RS (1991) Disorders of vitamin metabolism: deficiencies, metabolic abnormalities and excesses. In *Cecil Textbook for Medicine*, 19th ed., pp. 1170 [JH Wyngaarden, LH Jr Smith, JC Bennett and F Plum, editors]. Philadelphia: WB Saunders.
21. Pinto JT, Huang JP & Rivlin RS (1987) Mechanisms underlying the different effects of ethanol upon the bioavailability of riboflavin and flavin adenine dinucleotide. *J Clinc Invest* **78**, 1343.
22. Yuasa H, Hirobe M, Tomei S *et al.* (2000) Carrier-mediated transport of riboflavin in the rat colon. *Biopharm Drug Dispos* **21**, 77–82.
23. Scientific Committee for Food (1993). *Nutrient and Energy Intakes for European Community. (Opinion expressed on 11 December 1992). Thirty-first Series of Reports of the Scientific Committee for Food*. Luxembourg: Commission of the European Communities.
24. Department of Health (1991) *Dietary Reference Values for Food, Energy and Nutrients for the United Kingdom*. London: HMSO.
25. Food and Nutrition Board, Institute of Medicine (1998) *Dietary Reference Intakes for Thiamine, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline*. Washington, DC: National Academy Press; 1998.
26. Scientific Committee for Food (2000). *Nutrient and Energy Intakes for European Community. (Opinion Expressed on 22 November 2000). Thirty-third Series of Reports of the Scientific Committee for Food*. Brussels: Commission of the European Communities.
27. Bates B, Lennox A, Bates C *et al.* (2014) *National Diet and Nutrition Survey: Results from Years 1–4 (combined) of the Rolling Programme (2008/2009–2011/12)*. London: Public Health England and the Food Standards Agency.
28. Allen L, de Benoist B, Dary O *et al.* (2006) *Guidelines on Food Fortification with Micronutrients*. Geneva,

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

Switzerland: World Health Organisation and Food and Agriculture Organisation of the United Nations.
29. McCormick DB (1994) Riboflavin. In *Modern Nutrition in Health and Disease*, 8th ed., pp. 366 [ME Shils, JE Olsen and MM Shike, editors]. Philadelphia.
30. Food and Nutrition Board. Institute of Medicine (2000) Riboflavin. In *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, biotin and Choline*, pp. 87 [RM Pitkin, editor]. Washington, DC: National Academy Press.
31. Rivlin RS (1979) Effect of nutrient toxicities (excess) in animals and man: riboflavin. In *Handbook of Nutrient and Foods*, p. 25. [M Recheigl, editor]. Boca Raton: CRC Press.
32. Cooperman JM & Lopez R (1984) Riboflavin. In *Handbook of Vitamins: Nutritional, Biochemical and Clinical Aspects*, p. 299 [J Machlin, editor]. NY: Marcel Dekker.
33. Zempleni J, Galloway JR & McCormick DB (1996) Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* **63**, 54–66.
34. Cimino JA *et al.* (1987) Riboflavin metabolism in the hypothyroid human adult. *Proc. Soc Exp Biol Med* **184**, 121.
35. Apeland T, Mansoor MA, Pentieva K *et al.* (2003) Fasting and post methionine load concentrations of homocysteine, vitamin B2 and vitamin B6 in patients on antiepileptic drugs. *Clinical Chemistry* **49**, 1005–1008.
36. Dutta P, Pinto JT & Rivlin RS (1985) Antimalarial effects of riboflavin deficiency. *Lancet* **2**, 1040.
37. Bates CJ, Prentice AM, Paul AA *et al.* (1982) Riboflavin status in infants born in rural Gambia, and the effects of a weaning food supplement. *Trans R Soc Trop Med Hyg* **76**, 253–258.
38. Tan KL, Chow MT & Karim SMM (1978) Effect of phototherapy on neonatal riboflavin status. *J Pediatr* **78**, 780–781.
39. Wacker J, Fruhauf J, Schulz M *et al.* (2000) Riboflavin deficiency and preeclampsia. *Obstet Gynecol* **96**, 38–44.
40. Wu XM, Yang KX, Tang XD *et al.* (2015) Folate metabolism gene polymorphisms MTHFR C677T and A1298C and risk for preeclampsia: a meta-analysis. *J Assist Reprod Genet* **32**, 797–805.
41. Belko AZ, Obarzanek E, Roach R *et al.* (1984) Effects of aerobic exercise and weight loss on riboflavin requirements of moderately obese, marginally deficient young women. *Am J Clin Nutr* **40**, 553–561.
42. Soares MJ, Satyanarayana K, Bamji MS *et al.* (1993) the effect of exercise on the riboflavin status of adult men. *Br J Nutr* **69**, 541–551.
43. Benton D, Haller J & Fordy J (1997) The vitamin status of young British adults. *Int J Vitam Nutr Res* **67**, 34–40.
44. Hoey L, McNulty H & Strain JJ (2009) Studies of biomarker responses to intervention with riboflavin: a systemic review. *Am J Clin Nutr* **89**, 1960S–1980S.
45. Pan W-H, Chang Y-P, Yeh W-T *et al.* (2012) Co-occurrence of anemia, marginal vitamin B6, and folate status and depressive symptoms in older adults. *J Geriatr Psychiatry Neurol* **25**, 170–178.
46. Hill MHE, Bradley A, Mushtaq S *et al.* (2009) Effects of methodological variation on assessment of riboflavin status using the erythrocyte glutathione reductase activation coefficient assay. *Br J Nutr* **102**, 273–278.
47. Clements JE & Anderson BB (1980) Glutathione reductase activity and pyridoxine (pyridoxamine) phosphate oxidase activity in the red cell. *Biochim Biophys Acta* **632**, 159–163.
48. Chastain JL & McCormick DB (1987) Flavin catabolites: identification and quantitation in human urine. *Am J Clin Nutr* **46**, 830.
49. Horwitt MK, Harvey CC, Hills OW *et al.* (1950) Correlation of urinary excretion of riboflavin with dietary intake and symptoms of ariboflavinosis. *J Nutr* **42**, 247–264.
50. Hustad S, Ueland P, Vollset S *et al.* (2000) Riboflavin as a determinant of plasma total homocysteine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. *Clin Chem* **46**, 1065–1071.
51. Jacques PF, Kalmbach R, Bagley PJ *et al.* (2002) The relationship between riboflavin and plasma total homocysteine in the Framingham offspring cohort is influenced by folate status and the C677T transition in the methylenetetrahydrofolate reductase gene. *J Nutr* **132**, 283–288.
52. Hustad S, McKinley MC, McNulty H *et al.* (2002) Riboflavin, flavin mononucleotide, and flavin adenine dinucleotide in human plasma and erythrocytes at baseline and after low-dose riboflavin supplementation. *Clin Chem* **48**, 1571–1577.
53. Preziosai P, Galan P, Deheeger M *et al.* (1999) breakfast type, daily nutrient intakes and vitamin and mineral status of French children, adolescents, and adults. *J Am Coll Nutr* **18**, 171–178.
54. Matrix J, Aranda P, Sanchez C *et al.* (2003) Assessment of thiamin (vitamin B_1) and riboflavin (vitamin B_2) status in an adult Mediterranean population. *Br J Nutr* **90**, 661–666.
55. Troesch B, Hoeft B, McBurney M *et al.* (2012) Dietary surveys indicate vitamin intakes below recommendations are common in representative Western countries. *Br J Nutr* **108**, 692–698.
56. ter Borg S, Verlaan S, Hemsworth J *et al.* (2015) Micronutrient intakes and potential inadequacies of community-dwelling older adults: a systematic review. *Br J Nutr* **113**, 1195–1206.
57. Shi Z, Zhen S, Wittert GA *et al.* (2014) Inadequate riboflavin intake and anemia risk in a Chinese population: five-year follow up of the Jiangsu Nutrition Study. *PLoS ONE* **9**, e88862.
58. Ministry of Health and Welfare Korea Centers for Disease Control and Prevention (2013) *Korea Health Statistics 2010–2012: Korea National Health and Nutrition Examination Survey (KNHANES V-3)*. Osong, Korea: Korea Centers for Disease Control and Prevention.
59. Choi JY, Kim YN & Cho YO (2015) Evaluation of riboflavin intakes and status of 20–64 year old adults in South Korea. *Nutrients* **7**, 253–264.
60. Brun TA, Campbell TC, Boreham J *et al.* (1990) Urinary riboflavin excretion after a load test in rural China as a measure of possible riboflavin deficiency. *Eur J Clin Nutr* **44**, 195–206.
61. Abrams SA, Mushi A, Hilmers DC *et al.* (2003) A multinutrient-fortified beverage enhances the nutritional status of children in Botswana. *J Nutr* **133**, 1834–1840.
62. Boisvert WA, Castaneda C, Mendoza I *et al.* (1993) Prevalence of riboflavin deficiency among Guatemalan elderly people and its relationship to milk intake. *Am J Clin Nutr* **58**, 85.
63. Yang FL, Liao PC, Chen YY *et al.* (2005) Prevalence of thiamin and riboflavin deficiency among the elderly in Taiwan. *Asia Pac J Clin Nutr* **14**, 238–243.
64. Whitfield K, McCann A, Karakochuk C *et al.* (2015) High rates of riboflavin deficiency in women of childbearing age in Cambodia and Canada. *J Nutr* **145**, 628–633.
65. Frosst P, Blom HJ, Milos R *et al.* (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* **10**, 111–113.

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

66. Guenther BD, Sheppard CA, Tran P *et al.* (1999) The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocystaemia. *Nat Struct Biol* **6**, 359–365.
67. Yamada K, Chen Z, Rozen R *et al.* (2001) Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. *Proc Natl Acad Sci USA* **98**, 14853–14858.
68. Narisawa K, Tamura T, Tanno K *et al.* (1968) Tetrahydro-folate-dependent enzyme activities of the rat liver in riboflavin deficiency. *Tohoku J Exp Med* **94**, 417–430.
69. Bates CJ & Fuller NJ (1986) The effect of riboflavin deficiency on methylenetetrahyfrofolate reductase (NADPH) (EC 1·5·1·20) and folate metabolism in the rat. *Br J Nutr* **55**, 455–464.
70. McNulty H, Dowey le RC, Strain JJ, *et al.* (2006) Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677C→T polymorphism. *Circulation* **113**, 74–80.
71. McNulty H, McKinley MC, Wilson B *et al.* (2002) Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. *Am J Clin Nutr* **76**, 436–441.
72. Garcia-Minguillan CJ, Fernandez-Ballart JD, Ceruelo S *et al.* (2014) Riboflavin status the effects of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) polymorphisms on homocysteine. *Genes Nutr* **9**, 435.
73. Wald DS, Law M & Morris JK (2002) Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *Br Med J* **325**, 1202.
74. Holmes MV, Newcombe P, Hubacek JA *et al.* (2011) 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* **378**, 584–594.
75. Lewis SJ, Ebrahim S & Davey Smith G (2005) Meta-analysis of MTHFR 677C→T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? *BMJ* **331**, 1053.
76. Klerk M, Verhoef P, Clarke R *et al.* (2002) MTHFR 677C→T polymorphism and risk of coronary heart disease – a meta-analysis. *JAMA* **288**, 2023–2031.
77. Wald DS, Morris JK & Wald NJ (2011) Reconciling the evidence on serum homocysteine and ischemic heart disease: a meta-analysis. *PLoS ONE* **6**, e16473.
78. Clarke R, Bennett DA, Parish S *et al.* (2012) Homocysteine and coronary heart disease: meta-analysis of MTHFR case-control studies, avoiding publication bias. *PLoS Med* **9**, e1001177.
79. Lim SS, Vos T & Flaxman AD (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2224–2260.
80. Lewington S, Clarke R, Qizilbash N *et al.* (2002) 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* **360**, 1903–1913.
81. Whelton PK, He J, Appel LJ *et al.* (2002) Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *JAMA* **288**, 1882–1888.
82. Luft FC (2001) Twins in cardiovascular genetic research. *Hypertension* **37**, 350–356.
83. Mongeau JG (1987) Heredity and blood-pressure in humans – an overview. *Pediatr Nephrol* **1**, 69–75.
84. Tomaszewski M, Debiec R, Braund PS *et al.* (2010) Genetic architecture of ambulatory blood pressure in the general population insights from cardiovascular gene-centric array. *Hypertension* **56**, 1069–1076.
85. Levy D, Ehret GB, Rice K *et al.* (2009) Genome-wide association study of blood pressure and hypertension. *Nat Genet* **41**, 677–687.
86. Newton-Cheh C, Johnson T, Gateva V *et al.* (2009) Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* **41**, 666–676.
87. Takeuchi F, Isono M, Katsuya T *et al.* (2010) Blood pressure and hypertension are associated with 7 Loci in the Japanese population. *Circulation* **121**, 2302–2309.
88. Markan S, Sachdeva M, Sehrawat BS *et al.* (2007) MTHFR 677 CT/MTHFR 1298 CC genotypes are associated with increased risk of hypertension in Indians. *Mol Cell Biochem* **302**, 125–131.
89. Liu C, Li H, Qi Q *et al.* (2011) Common variants in or near FGF5, CYP17A1 and MTHFR genes are associated with blood pressure and hypertension in Chinese Hans. *J Hypertens* **29**, 70–75.
90. Flister MJ, Tsaih SW, O'Meara CC *et al.* (2013) Identifying multiple causative genes at a single GWAS locus. *Genome Res* **23**, 1996–2002.
91. Heux S, Morin F, Lea RA *et al.* (2004) The methylentetrahydrofolate reductase gene variant (C677T) as a risk factor for essential hypertension in Caucasians. *Hypertens Res* **27**, 663–667.
92. Jiang S, Hsu YH, Xu X *et al.* (2004) The C677T polymorphism of the methylenetetrahydrofolate reductase gene is associated with the level of decrease on diastolic blood pressure in essential hypertension patients treated by angiotensin-converting enzyme inhibitor. *Thromb Res* **113**, 361–369.
93. Nishio H, Lee H, Fujii M *et al.* (1996) A common mutation in methylenetetrahydrofolate reductase gene among the Japanese population Japanese. *J Hum Genet* **41**, 247–251.
94. Bayramoglu A, Kucuk M, Guler HI *et al.* (2015) Is there any genetic predisposition of MMP-9 gene C1562T and MTHFR gene C677T polymorphisms with essential hypertension? *Cytotechnology* **67**, 115–122.
95. Benes P, Kankova K, Muzik J *et al.* (2001) Methylenetetrahydrolfolate reductase polymorphism, type II diabetes mellitus, coronary artery disease, and essential hypertension in the Czech population. *Mol Genet Metab* **73**, 188–195.
96. Inamoto N, Katsuya T, Kokubo Y *et al.* (2003) Association of methlenetetrahydrolfolate reductase gene polymorphism with carortid atherosclerosis depending on smoking status in Japanese general population. *Stroke* **34**, 1628–1633.
97. Guillen M, Corella O, Portoles JI *et al.* (2001) Prevalence of the methylenetetrahydrofolate reductase 677C>T mutation in the Mediterranean Spanish population. Association with cardiovascular risk factors. *Eur J Epidemiol* **17**, 255–261.
98. Nakata Y, Katsuya T, Takami S *et al.* (1998) Methylenetetrahydrofolate reductase gene polymorphism – relation to blood pressure and cerebrovascular disease. *Am J Hypertens* **11**, 1019–1023.
99. Fowdar YJ, Lason MV, Szvetko AL *et al.* (2012) Investigation of homocysteine-pathway-related variants in essential hypertension. *Int J Hypertens* **2012**, 190923.

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press

100. Rodriguez-Esparragon F, Hernandez-Perera O, Rodriguez-Perez JC *et al.* (2003) The effect of methylenetetrahydrofolate reductase C677T common variant on hypertensive risk is not solely explained by increased plasma homocysteine values. *Clin Exp Hypertens* **25**, 209–220.
101. Reilly R, McNulty H, Ward M *et al.* (2013) The MTHFR 677TT genotype and its interaction with related B-vitamins in hypertension. *Ann Nutr Metab* **63**, 160.
102. Antoniades C, Shirodaria C, Warrick N *et al.* (2006) 5-Methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and eNOS coupling. *Circulation* **114**, 193–201.
103. Antoniades C, Shirodaria C, Leeson P *et al.* (2009) 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* **119**, 2507–2515.
104. Bagley PJ & Selhub J (1998) A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. *Proc Natl Acad Sci USA* **95**, 13217–13220.
105. Strain JJ, Hughes CF, McNulty H *et al.* (2015) Riboflavin lowers blood pressure: a review of a novel gene-nutrient interaction. *Nutr Food Sci Res* **2**, 3–6.
106. Kaput J (2008) Nutrigenomics research for personalised nutrition and medicine. *Curr Opin Lipid* **19**, 1–11.
107. Ronteltap A, van Trijp H, Berezowska A *et al.* (2013) Nutrigenomics-based personalised nutritonal advice: in search of a business model? *Genes Nutr* **8**, 153–163.
108. Makeeva OA, Markova VV, Roses AD *et al.* (2010) An epidemiologic-based survey of public attitudes towards predictive genetic testing in Russia. *Person Med* **7**, 291–300.
109. Joost H, Gibney M, Cashman K *et al.* (2007) Personalised nutrition: status and perspectives. *Br J Nutr* **98**, 26–31.
110. Cherkas LF, Harris JM, Levinson E *et al.* (2010) A survey of UK public interest in internet-based personal genome testing. *PLoS ONE* **5**, e13473.
111. Stewart-Knox B, Bunting B, Gilpin S *et al.* (2008) Attitudes toward genetic testing and personalised nutrition in a representative sample of European consumers. *Br J Nutr* **101**, 982–989.
112. Aro AR, Hakonen A, Hietala M *et al.* (1997) Acceptance of genetic testing in a general population: age, education and gender differences. *Patient Educ Couns* **32**, 41–49.
113. Bloss CS, Schork NJ & Topol EJ (2011) Effect of direct-to-consumer genome wide profiling to assess disease risk. *N Engl J Med* **364**, 524–534.
114. McBride CM, Koehly LM, Sanderson SC *et al.* (2010) The behavioral response to personalised genetic information: will genetic risk profiles motivate individuals and families to choose more healthful behavours? *Patient Educ Couns* **31**, 89–103.
115. Norrgard K (2008) DTC genetic testing for diabetes, breast cancer, heart disease and paternity. *Nat Educ* **1**, 1.
116. Qian X, Zhiagang L, Miao T *et al.* (2007) A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. *Eur J Hum Genet* **15**, 1239–1245.
117. Niu WQ, You YG, Qi Y *et al.* (2012) Strong association of methylenetetrahydrofolate reductase gene C677T polymorphism with hypertension and hypertension-in-pregnancy in Chinese: a meta-analysis. *J Hum Hypertens* **26**, 259–267.
118. Wu YL, Hu CY, Lu SS *et al.* (2014) Associations between methylenetetrahydrofolate reductase (MTHFR) C677T/A1298C polymorphisms and essential hypertension: a systematic review and meta-analysis. *Metabolism* **63**, 1503–1511.
119. Yang KM, Jia J, Mao L *et al.* (2014) Methylenetetrahydrofolate reductase C677T gene polymorphism and essential hypertension: a meta-analysis of 10,415 subjects. *Biomed Rep* **2**, 699–708.
120. Bouwman L, te Molder H & Hiddink G (2008) Patients evidence and genes: an exploration of GPs' perspectives on gene-based personalised nutrition advice. *Fam Pract* **25**, 1116–1122.
121. Bonter K, Desjardins C, Currier N *et al.* (2011) Personalised medicine in Canada: a survey of adoption and practice in oncology, cardiology and family practice. *BMJ Open* **1**, e000110.
122. Weir M, Morin K, Ries N *et al.* (2010) Canadian health care professionals' knowledge, attitudes and perceptions of nutritional genomics. *Br J Nutr* **104**, 1112–1119.
123. Pirzadeh-Miller S, Bellcross C, Robinson C *et al.* (2011) Direct-to-consumer genetic testing: helpful, harmful, or pure entertainment? *Commun Oncol* **8**, 263–268.
124. Cherkas LF, Harris JM, Levinson E *et al.* (2010) A survey of UK public interest in internet-based personal genome testing. *PLoS ONE* **5**, e13473.

https://doi.org/10.1017/S0029665116000197 Published online by Cambridge University Press