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Symposium on 'Genetic polymorphisms and disease risk'

B-vitamins, genotype and disease causality*

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Despite a great deal of research effort there is still considerable uncertainty surrounding the importance of the B-vitamins in health and disease. This continuing uncertainty is partly a result of the difficulty of measuring intake, confounding in observational studies and the very large numbers required to evaluate primary prevention in randomised controlled trials. Consequently, genetic data are increasingly being used to infer nutritional effects on health and even in the formulation of nutrition policy using the approach of 'mendelian randomisation'. Genetic information has already contributed greatly to the understanding of B-vitamin metabolism and the heterogeneity of responses to intake. It has the potential to provide further nutritional insights and to assist in the elucidation of causal mechanisms, but it is important that genetic data is not viewed as an alternative to nutritional information, both are necessary when addressing nutritional problems. Similarly, the interpretation of nutrient and biomarker status in some experimental designs may require knowledge of genotype. Formal tests of gene–gene and gene–nutrient interaction are of limited value in nutritional studies and the formulation of policy. Graphical representation of diet–genotype–health data greatly assists in the elucidation of the nature of genetic effects, their interaction with nutrition and the implications for nutrition policy.

Folate: B-vitamins: Genetic polymorphism: Mendelian randomisation

B-vitamins and health

Clinical B-vitamin deficiency is relatively rare in developed countries but there are numerous reports linking B-vitamin status to health; from fertility and neural-tube defect (NTD) at the beginning of life to CVD and cancer in adulthood. The B-vitamins, and folate in particular, have been reported to be protective against early pregnancy loss[1,2], NTD[3–5] and congenital abnormalities[6] and to influence the genetic selection of embryos[7] and the risk of twin births[8,9]. In relation to the major chronic diseases, there are reports of an inverse relationship between the dietary intake of folate, or folate status, and the incidence of vascular disease, including acute myocardial infarction[10], carotid stenosis[11] carotid plaque area[12] CHD[13,14] and ischaemic stroke[15]. Folate has also been reported to be protective against a number of cancers[16], including colo-rectal cancer[17,18] and breast cancer[19–21]. A number of other benefits of folate have been reported, including effects on cognitive function[22] and bone health[23]. However, for many of these outcomes other studies have demonstrated no effect or even adverse effects, and a recent comprehensive review of evidence relating B-vitamins to disease prevention[24] has concluded that, whilst there is compelling evidence for a beneficial effect on NTD, there is no clear beneficial or detrimental effect in relation to other health outcomes.

Part of this continuing uncertainty arises because of practical experimental difficulties associated with nutritional studies. Measurement of dietary intake is difficult and observational studies are often prone to confounding with other nutrients or other health-related behaviours[25].

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Abbreviations: MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, MTR reductase; TCN2, transcobalamin; CBS, cystathionine beta-synthase; HCY, homocysteine; METH, methyl groups; ALDH, acetaldehyde dehydrogenase; ADH, alcohol dehydrogenase; VEGF, vascular endothelial growth factor; DNMT, DNA methyltransferases; HMT, histone methyl transferases; MBP, methyl-binding proteins; SAM, S-adenosylmethionine; SAH, adenosylhomocysteine; NTD, neural-tube defect; CVD, cardiovascular disease; CHD, coronary heart disease; HDL, high density lipoprotein.
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Table 1. B-vitamin-related genes and common variants

Gene	Commonly-studied polymorphisms
Methylene tetrahydrofolate reductase (chr 1)	C677T, A1298C
Methionine synthase (chr 1)	A275G
Methionine synthase reductase (chr 5)	A66G
Transcobalamin (chr 22; a plasma globulin)	C776G
Cystathionine β-synthase (chr 21)	68 bp insertion at 844
Dihydrofolate reductase (chr 5)	Few studies
Folate receptor (chr 11)	Few studies
Folate hydrolase (chr 11)	Few studies
Methylene tetrahydrofolate dehydrogenase 1 (chr 14)	Few studies
Reduced folate carrier (chr 21)	Few studies

chr, Chromosome.

A further complication is that nutrient intake is generally measured over relatively short periods close to the time of disease presentation, yet the relevant nutritional exposure may extend over very long periods, or even the entire life-course. Randomised control trials avoid problems of confounding but they typically require very large numbers to achieve sufficient statistical power to study the role of nutrition in primary prevention. Randomised controlled trials have mostly been carried out, therefore, in relation to secondary prevention which may involve a different mechanism of action.

As a result of the paucity of good nutritional data researchers are increasingly looking to the field of genetics to provide nutritional insights, suggesting that 'The use of genetic data in epidemiological investigations offers fresh hope for a discipline beleaguered by the difficulty of identifying small causal associations against a background of bias, confounding, reverse causality, and aetiological heterogeneity'[25]. Indeed, it has been proposed that genetic information should be used directly to 'inform policies for improving population health through population-level interventions'[25]. This is in addition to the often cited potential for genetic information to be used as the basis for 'personalised nutrition'. The aim of this review is to critically evaluate the use of genetic information in the study of B-vitamins, health and disease and the application of that information.

Genetics of B-vitamin metabolism

The key metabolic pathway involving the B-vitamins is the folate–methylation cycle, which is central to many aspects of metabolism, including the provision of purines and pyrimidines for DNA synthesis, methyl groups for the methylation of a wide range of compounds and the production of homocysteine which has been implicated in a number of diseases[26]. The various functions of the folate–methylation cycle suggest possible mechanisms linking B-vitamin intake to health and disease, and the genes coding for the enzymes involved in the folate–methylation cycle contain a number of well-characterised polymorphisms. The most-commonly studied of these are two variants in methylene tetrahydrofolate reductase (MTHFR; C677T and A1298C), although more recent studies have also reported variants in methionine synthase (MTR; A2756G), MTR reductase (MTRR; A66G), cystathionine β-synthase (68 bp insertion at base 844) and transcobalamin (TCN2; C776G). There are other variants in these and other B-vitamin-related genes (Table 1), but these variants are reported more rarely.

Genetic-association studies

The basis for genetic-association studies and their limitations have been reviewed elsewhere[27,28]. Essentially, in such studies a link between a polymorphism and a disease state or other trait is assumed to arise because the polymorphism has a causal role in determining the trait or that it is acting as a proxy for the functional variant that is physically close on the genome[28]. If the latter applies it is generally assumed that the functional variant occurs in the same gene as the marker polymorphism; as, for example, in the observed linkage between MTHFR A1298C and C677T and genotypes[9,29]. This logic is usually further extended to the assumption that the 'pathway' or process in which the gene is embedded is causal and that the nutritional and environmental factors that influence the pathway may influence the disease state or trait. Recently, this approach has been termed 'mendelian randomisation'[25], although the name is not informative as it simply refers to Mendel's second law of independent inheritance of characteristics which is a prerequisite for all gene–disease-association studies. The principle of mendelian randomisation is that 'if genetic variants either alter the level of or mirror the biological effects of a modifiable environmental exposure that itself alters disease risk, then these genetic variants should be related to disease risk to the extent predicted by their effect on exposure to the risk factor'[25]. In relation to the MTHFR C677T variant the 'modifiable environmental exposure' would be folate and the 'mirrored biological effect' would equate to circulating homocysteine concentrations[25]. On the basis of this logic the association between the MTHFR 677TT genotype and CVD, stroke etc. has been used to suggest that higher folate intakes would reduce the incidence of CVD[30,31]. In a similar way it has been argued for MTHFR C677T that 'a case control study of the relation between the TT genotype and the risk of NTD can be interpreted as equivalent to a randomised trial of the effect on disease risk of alteration of the availability of folate'[27]. It is implicit in these interpretations that the dietary requirement for folate is influenced by MTHFR 677 genotype. In relation to CVD the 'mirrored biological effect' is taken to be the circulating homocysteine concentration[25] but the validity of this interpretation depends on the assumed mechanism (Fig. 1). Many of the assumptions underpinning the 'mendelian randomisation' approach (confounding by linkage disequilibrium, pleiotropy, canalisation (developmental compensation) and the complexity of biological processes underlying a particular trait) have been listed elsewhere[25], but some of these terms may be difficult for non-geneticists

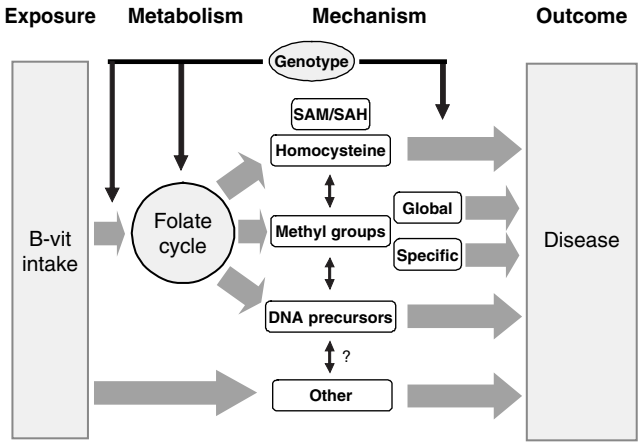

Fig. 1. Postulated causal pathways linking B-vitamin (B-vit) intake to disease. Most hypothesised mechanisms involve some aspect of the folate–methylation cycle, the activity of which is thought to be influenced by polymorphisms in a number of enzymes and transport proteins (see Table 1). SAM/SAH, *S*-adenosylmethionine and adenosylhomocysteine (folate–methylation cycle intermediates).

to understand and the list is not exhaustive. It is therefore useful to consider specific examples from the field of nutrition.

There are examples of critical developmental windows in the life-course when future health is thought to be particularly vulnerable to nutritional exposures. However, genotype is invariant throughout life and simple associations with disease provide no information on the period(s) during which the implicated environmental exposure is most critical. This uncertainty may even extend to the period before conception, as the genotype of the offspring is correlated with that of the parents. There have been numerous reports of critical windows of nutritional sensitivity occurring before birth[32,33], but they may also occur in adult life. For example, a small reported increase in breast-cancer risk associated with high folic acid intake throughout pregnancy may occur because the folic acid exposure coincides with the breast remodelling that occurs at this time[34]. If, as has been proposed, the risk of CVD is programmed before birth[32], then an association between MTHFR 677 TT genotype and CVD in adult life could arise as a result of the mutation reducing the exposure to folate *in utero*. In this example public health measures to reduce CVD risk by increasing folate intake in adult life may not be effective. This analysis is supported by the findings of two recent well-controlled large randomised controlled trials that have shown no effect, or possibly even a negative effect, of folate, vitamin B_{12} and vitamin B_6 supplementation on CVD recurrence[35,36]. These trials were of secondary prevention, which may operate by a different mechanism, but the example illustrates a potentially-important limitation to the use of mendelian randomisation in the development of nutrition policy.

A further problem is that some disease responses to nutrients such as folate follow a biphasic or more complicated response pattern (e.g. a bell-shaped association between colo-rectal cancer and folate status has been reported[37]), but genetic information is essentially binary and it is not clear how the mendelian randomisation approach might respond to this complexity. A related issue is that low and high intakes of a particular nutrient may influence different processes contributing to overall disease risk. For example, it has been hypothesised that a high folate intake might protect against the initiation of cancer, but that it may also promote the growth of pre-existing cancers[38]. In the first mechanism mendelian randomisation should identify a higher risk of cancer in individuals with the MTHFR 677 TT genotype, as this mutation has the effect of reducing the effective folate exposure for a given intake. However, the second mechanism should identify a higher risk of cancer in individuals with the MTHFR 677 CC genotype. Thus, in a disease such as cancer, with a potentially-complex relationship with folate, mendelian randomisation may pick up only the dominant response or the effects may cancel out to produce no apparent genetic association and, by inference, no predicted nutritional effect. Either way, an uncritical extrapolation of genetic findings could lead to inappropriate nutritional recommendations. Something of this complexity is actually seen in relation to colo-rectal cancer, in which the genetic link with MTHFR is unusual among disease associations in that the MTHFR homozygous TT genotype appears to be protective[18]. The logical corollary of the CVD–genotype association is that for colo-rectal cancer a decrease in folate intake would be protective, but other work[17] suggests that the effect of a low-folate diet may override the effect of genotype on colo-rectal cancer and that an increase in folate intake is advisable. This topic is the subject of ongoing research, but it illustrates the need for nutritional information when interpreting genetic data.

Information on the nutritional status of the study population is also critical to the interpretation of genotype information and its generalisation to other populations, as genotype–disease associations can depend on the nutritional status of the population[18,39]. If, for example, a genotype effect only manifests at low intakes of folate then extrapolation of this information to a population with adequate or high intakes may not be appropriate and the resulting intervention ineffective.

Genetic modulation of B-vitamin status

As a result of the difficulty of measuring nutrient intake accurately there is an increasing reliance on measures of status, or 'biomarkers' of function, in studies linking nutrition to disease. This approach extends to the assessment of nutritional deficiency and excess in populations[40], but known genetic effects on blood status and biomarkers may complicate the interpretation of such data. It is well established that B-vitamin status is affected by genotype, particularly the C677T polymorphism in MTHFR, with the T allele being associated with higher circulating concentrations of homocysteine[30,31,39] and lower circulating concentrations of plasma and erythrocyte folate[41–43]. The MTHFR A1298C mutation has also been linked to erythrocyte folate concentration, but in this case the mutant 1298CC genotype is associated with high concentrations of erythrocyte folate[29]. There are reports of other genotypes affecting B-vitamin status, e.g. MTR and vitamin B_{12}[44]. It may be deduced from this information

that B-vitamin and related biomarker status is a function of both the dietary B-vitamin intake and genotype.

Depending on the blood fraction being measured, the average folate concentration associated with the MTHFR 677 TT genotype can be anywhere between 14 and 36% lower than that in the CC genotype[41–43]. If blood folate is used to directly infer intake[45] then an average 20% effect of the TT genotype on blood folate would equate to an underestimation of folate intake in UK males[46] of about 70 μg/d. The magnitude of this genotype effect is important in relation to the typical variation in intake between individuals, and it may be questioned how much of the 'tails' of the nutrient status distribution in the population reflects true under- and overnutrition and how much reflects genotype.

The use of status to infer dietary intake is useful when studying, for example, secular changes in intake in whole populations, but the effect of genotype could complicate other uses of these data. It is well known that observational nutritional studies are prone to confounding because of covariance with other health-related behaviours or even other nutrients, but it is less-well understood that genotype may also confound purely nutritional studies when intake is inferred from blood status. Where a blood nutrient or biomarker is influenced by a genotype that also affects disease risk there is a danger that a nutrient intake–disease link may be inferred when the causal factor is actually the genotype. Depending on the nature of the genotype–health association, genetic confounding can operate to produce an apparent nutritional effect where none exists, obscure an actual nutritional effect or modulate the magnitude of an effect in either direction. The relationship between plasma and erythrocyte folate and risk of NTD[4] has been used to estimate the likely effect of changing folic acid intake on NTD incidence[47,48]. However, the MTHFR 677 TT genotype markedly increases the risk of NTD[3,6] therefore if MTHFR reduces both the concentration of blood folate for a given intake and increases the risk of NTD then the predicted effect of folate intake on NTD may be overestimated. Similarly, in relation to CVD, it has been reported that the MTHFR 677 TT genotype increases the risk of IHD, deep-vein thrombosis, pulmonary embolism and stroke[31,39,49]. In the case of NTD there has been independent verification of the effect of folate intake following fortification programmes, but care has to be taken when using blood folate status to infer an association between intake and the risk of disease when that disease is known to be influenced by MTHFR genotype, for example. This situation applies whether intake is directly calculated from measures of status[45] or whether the link with intake is implicitly assumed.

In studies in which blood status is used to infer dietary intake, or the link between dietary intake and health, it may be possible to adjust for the MTHFR-genotype effect, but the concern remains that other genotypes may have similar effects. For example, cystathionine B synthase genotype is thought to influence the concentration of homocysteine[50] and there may be other gene variants that are not know about and cannot therefore be corrected for. A further complication is that folate assays may detect different forms of folate[51], with some metabolites being more sensitive to the effect of MTHFR genotype, therefore the extent to which it is necessary to adjust for genotype may vary with the assay method used.

It may be argued that a low blood folate status resulting from poor dietary intake is the same in functional terms as that arising from genotype effects within the folate–methylation cycle[25,30,31]. However, in one case the blood status is the factor affecting the folate–methylation cycle, whilst in the other it is a product of the cycle, and the presumed biological equivalence of these states depends on the postulated mechanism linking status to disease. If folate is thought to affect health through its effect on the circulating concentration of homocysteine, then the assumption of equivalence is reasonable, but if it is operating through some other function of the cycle, or even a mechanism not related to the folate–methylation cycle, then the biological equivalence of genetic and nutritional effects has to be verified.

Causal mechanism

Knowledge of the pathway of causality is important in the interpretation of both nutritional status and genetic-association studies. A number of mechanisms have been proposed to explain the link between B-vitamins and health, most of which relate to the various functions of the folate–methylation cycle (Fig. 1). However, even in relation to the disease for which the evidence for an effect of B-vitamins is strongest (NTD) there is still uncertainty as to the mechanism by which folate is protective[26].

Epigenetic marking is probably the folate cycle-related hypothesis currently receiving most attention in the field of cancer. A common observation in many tumour types is epigenetic change consisting of altered methylation of DNA[52] and the histones associated with DNA[53]. These changes occur early in the development of the disease and the pattern of methylation correlates with cancer stage[52]. Until recently the favoured hypothesis relating B-vitamin status to vascular disease has involved homocysteine. Homocysteine has been shown to promote the biological processes that underpin atherosclerosis and thrombosis, the concentration of plasma homocysteine varies with B-vitamin exposure and the plasma concentration of homocysteine is related to the risk of vascular disease and cardiovascular mortality in the general population[30,31,54–56]. However, there is a growing body of evidence to suggest that homocysteine may not be causal[54,56–59] and increasing interest in the role of epigenetics in vascular disease. Patients with vascular disease have markedly altered DNA methylation compared with healthy controls[60], and altered global DNA methylation has also been observed in mouse and rabbit atherosclerotic lesions[61], while studies in an atherogenic mouse model have shown that altered DNA methylation precedes the development of atherosclerosis[62]. Altered methylation of the oestrogen receptor-α gene has also been demonstrated in coronary atherosclerotic plaques compared with a normal proximal aorta, with the methylation status changing with ageing[63,64]. Other mechanisms have also been proposed; for example, those of Duthie and coworkers[65] and Brockton[66].

The continuing uncertainty over B-vitamin-related causal mechanisms partly arises because of the covariance

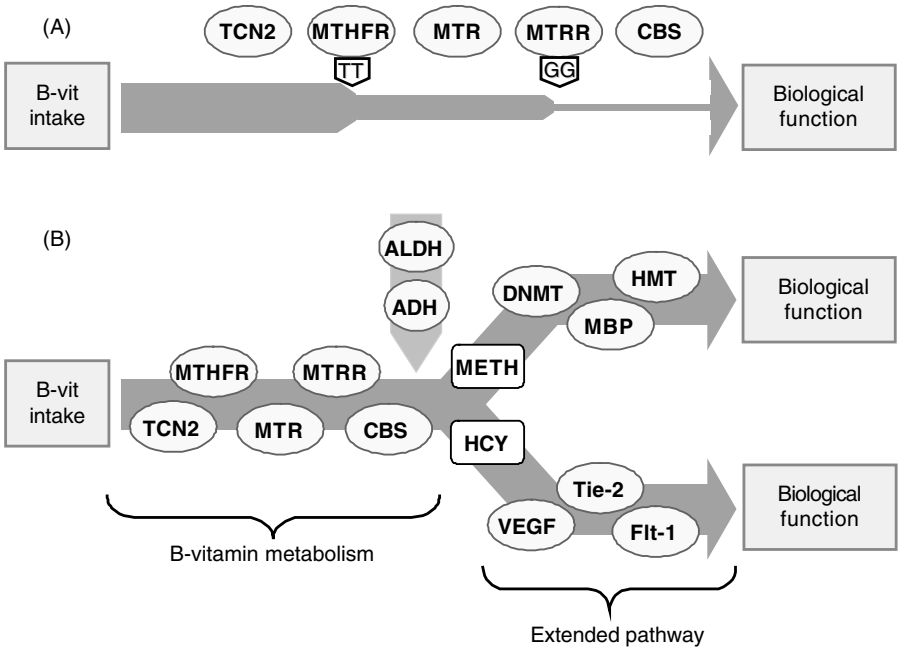

Fig. 2. (A) Idealisation of additive effects of two genetic mutations in B-vitamin-related genes (see Table 1) on biological function or disease outcome. (B) An example of the 'causal pathway' approach in which polymorphism–disease associations are studied for the full hypothesised pathway of causality linking B-vitamin intake to disease outcome. This approach goes beyond the polymorphisms directly related to B-vitamin metabolism and may include the pathways impinging on the process of interest, e.g. alcohol metabolism. TCN2, transcobalamin; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, MTR reductase; CBS, cystathionine β-synthase; HCY, homocysteine; METH, methyl groups; ALDH, acetaldehyde dehydrogenase; ADH, alcohol dehydrogenase; VEGF, vascular endothelial growth factor; DNMT, DNA methyl transferases; HMT, histone methyl transferases; MBP, methyl-binding proteins.

of metabolite concentrations and biological functions that depend on the folate–methylation cycle. Blood folate, homocysteine and MTHFR C677T genotype all co-vary, and this linkage appears also to extend to global DNA methylation[67,68], with the level of methylation being correlated with plasma homocysteine[60]. Furthermore, homocysteine is often correlated with the concentration of other folate–methylation cycle intermediates such as S-adenosylmethionine and S-adenosylhomocysteine[69]. The difficulty of interpreting this information is highlighted by the proposal that the often-reported association between homocysteine and disease may arise because homocysteine is acting as a proxy for a causal effect operating through DNA methylation, since S-adenosylhomocysteine is a potent inhibitor of the DNA methyltransferases and it changes in parallel with homocysteine concentration[69]. This Gordian knot of interactions between the key players in the competing theories makes it very difficult to establish the causal mechanism simply on the basis of observational studies correlating any of the intermediates to intake or disease. However, genetic analysis has the potential to assist in establishing mechanism.

Causal-pathway genetics

Most studies on B-vitamin genotype have focused on one or more polymorphisms in the folate cycle, but there is no reason, if the study has sufficient statistical power, why this approach cannot be extended to other genes in the full hypothesised pathway of causality linking B-vitamin intake to disease. For the two mechanisms most commonly considered in relation to vascular disease (production of homocysteine and methyl groups for DNA methylation) these possibilities can be addressed by simultaneously studying polymorphisms in the vascular factors thought to be implicated in the action of homocysteine (possible candidates include Tie-2, Flt, vascular endothelial growth factor[70,71]) and those important in methylation (e.g. DNA methyl transferases, histone methyl transferases, methyl-binding proteins[72]) in addition to the traditional folate-cycle polymorphisms. This 'causal pathway' approach can focus on purely metabolic processes or may incorporate genes controlling physiological and other higher-order functions. It can also include the pathways impinging on the process of interest. Evidence from epidemiological studies suggests that the effect of B-vitamins on disease risk may be modulated by alcohol[18,73,74] and there is evidence that polymorphisms in the alcohol dehydrogenase gene interact with alcohol consumption to influence HDL concentrations and the risk of myocardial infarction[75]. Simultaneous analysis of known polymorphisms in the pathways impinging on the process of interest (e.g. alcohol metabolism) has the potential to support or refute observational studies linking B-vitamin

intake with other exposures (Fig. 2). A further advantage of this multiple-gene approach is that, even considering only the folate-cycle genes, if more than one gene in a 'pathway' is associated with a disease the likelihood that the 'pathway' has been erroneously linked to a disease state is greatly reduced.

Multiple-gene effects

Most major human diseases with a genetic component are thought to be influenced by the partial effects of variation in many genes (polygenic), and access to low cost high-throughput genotyping has resulted in the analysis of more than one gene, and sometimes very large numbers of genes, in nutritional studies. One aim of the multiple-gene approach is to identify combinations of genotypes that act together to increase the risk of disease. For example, a number of genotype combinations have been reported to increase NTD risk: MTRR/glutamate carboxypeptidase II; MTHFR 677/cystathionine β-synthase; MTHFR 677/MTRR[76]; MTHFR 677TT/cystathionine β-synthase[3]; MTRR/MTR[77]. In relation to blood levels of homocysteine the presence of the MTRR 66 G allele has been reported to increase the effect of MTHFR 677 TT[78], whilst gene–gene interactions have also been described for many outcomes, including CVD[79,80] and Down's syndrome[81].

In some circumstances the apparent effect of multiple polymorphisms on a disease outcome can result from linkage. For example, the common MTHFR variants (C677T and A1298C) are physically very close on the genome and are highly significantly and reciprocally linked[9], therefore an outcome that is associated with the C677T variant is likely to also be linked to the A1298C variant in an opposite way, even if only one of the polymorphisms is functionally related to the outcome. Where both variants are measured it should be possible to evaluate statistically whether there are independent effects, and even where the primary effect lies[9]. An alternative approach is to analyse the effect of one variant (e.g. MTHFR A1298C) on the outcome of interest only in subjects with a particular genotype for another variant (e.g. MTHFR C677T)[29].

The combined effect of multiple polymorphisms on disease outcome is often referred to as interaction, but this term has a very specialised meaning in statistics and is usually assessed by including a multiplicative term (e.g. genotype 1 × genotype 2) in the statistical model. However, a simple additive effect of genotypes on disease risk (Fig. 2) is, for most nutritional problems, just as important in practical terms[27,82]. Whatever the nature of the combined effects of genotype, this type of analysis is often limited by the size of the study. In the UK the typical frequency of the MTHFR 677 TT mutant is 12%, with a frequency of 20% for TCN2 C776G, 28% for MTRR A66G and 3% for MTR A2756G[9]. Thus, the percentage of the population homozygous for both MTHFR 677 and TCN2 776 mutants is 2·4 (12% × 20%) and the percentage homozygous for all three mutants is 0·8 (12% × 20% × 28%). The numbers diminish rapidly with each genotype added, particularly when rare genotypes are studied (e.g. MTR 2756 GG). In some experimental designs the numbers may be increased by analysing for carriers of the mutant allele (i.e. combined heterozygotes and homozygotes rather than the just the homozygous mutants), but even this type of analysis typically requires large numbers of subjects when considering more than a few genotypes. Also, because of the very large number of possible genotype permutations, care has to be taken to avoid spurious statistical significance arising from multiple significance testing.

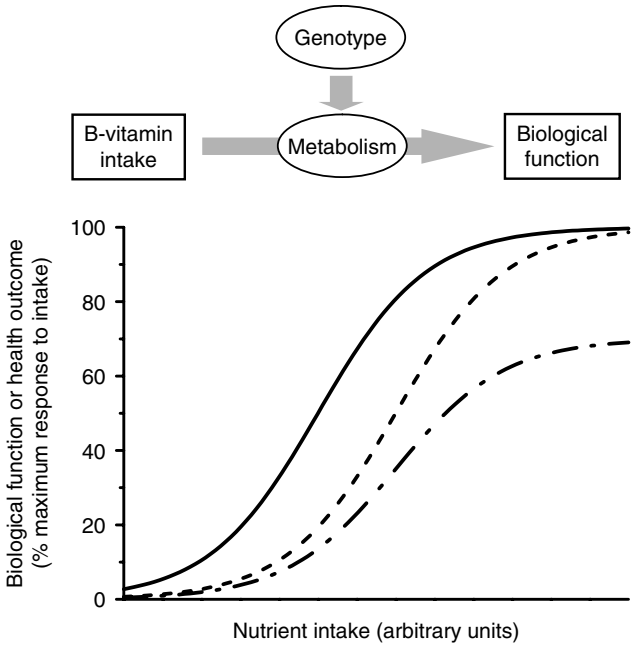

Fig. 3. Conditional effect plot relating nutrient intake to health outcome, disease state or other biological effect. The model parameters are held at a specific value for the genotypes of interest whilst allowing the others to vary. This approach is valid for most regression models, including multiple linear regression and logistic regression, with or without adjustment for covariates. The response may be binary (e.g. in case–control studies) but in this case the dependent variable is the probability of disease produced by the statistical model (usually logistic regression). Depending on the particular combination of nutrient, genotype and health outcome, the actual response may take a number of forms, and if there is an effect of genotype it may represent a delayed response to nutrient intake (- - -), a partial response (– · –), or some hybrid of these, relative to the wild type (—).

Even where functional genotype combinations are identified they may be of limited value. In rare diseases such as NTD similarly-rare genotype combinations could explain a substantial proportion of disease. However, for diseases with high prevalence such as CVD and the major cancers such genotype-combination information is likely to be of limited value, as it can only ever explain a small proportion of the disease. It is possible that there are multiple pathways to the same outcome, involving many different genotype combinations, but most studies have identified only a few. In some specific cases, e.g. where the polymorphisms may be in linkage disequilibrium (e.g. MTHFR C677T and A1298C), it is desirable to check for interaction, but for most diseases and

questions of nutritional relevance it is sufficient to assess associations independently for individual genes, providing the study is adequately powered.

As with gene–gene interactions, identification of the presence of gene–nutrient interaction may be of limited value, and the case for nutritional intervention to improve a health outcome may be valid whether or not a formal statistical interaction is detected. However, given the wide range of possible interactions (in the biological sense) between diet, genotype and health it is helpful to present the data in graphical form (Fig. 3). A common dose–response relationship in nutrition and biology in general is the sigmoid curve (Fig. 3), since there is usually some nutritional buffering within the body at very high and very low intakes. However, depending on the particular combination of nutrient, genotype and health outcome, the actual response may be linear, hyperbolic, exponential[4], bell-shaped[37] or some other form, and if there is an effect of genotype it may represent a delayed response to nutrient intake, a partial response or some hybrid of these responses. Each of these relationships represents a different type of diet–disease association and may indicate very different nutritional interventions to improve health.

Conclusions

Genetic information has already contributed greatly to the understanding of B-vitamin metabolism and the heterogeneity of responses to intake. It has the potential to provide further nutritional insights and to assist in the elucidation of causal mechanisms, but it is important that genetic data is not viewed as an alternative to nutritional information; both are necessary when addressing nutritional problems. Similarly, the interpretation of nutrient and biomarker status in some experimental designs may require knowledge of genotype. Graphical representation of diet–genotype–outcome data is particularly helpful when trying to understand the nature of the genetic effect, its interaction with nutrition and the implications for nutrition policy.

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