Effect of Genotype on Micronutrient Absorption and Metabolism: a Review of Iron, Copper, Iodine and Selenium, and Folates

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Received for publication: July 28, 2006

Abstract: For the majority of micronutrients, there are very little data, or none at all, on the role of genetic polymorphisms on their absorption and metabolism. In many cases, the elucidation of biochemical pathways and regulators of homeostatic mechanisms have come from studies of individuals that have mutations in certain genes. Other polymorphisms in these genes that result in a less severe phenotype may be important in determining the natural range of variation in absorption and metabolism that is commonly observed. To illustrate some of these aspects, I briefly review the increased understanding of iron metabolism that has arisen from our knowledge of the effects of mutations in several genes, the role of genetic variation in mediating the nutritional effects of iodine and selenium, and finally, the interaction between a genetic polymorphism in folate metabolism and folic acid fortification.

Key words: Micronutrients, genetic polymorphisms, iron, iodine, selenium, folates

Introduction

Recently there has been considerable interest in the role that genetic polymorphisms may play in several aspects of human nutrition, and the ill-defined terms nutrigenomics and nutrigenetics have become commonplace [1]. For some researchers, this aspect of nutrition is associated with the suggestion that diets can be personalized – that by knowing one’s genotype it may be possible to fine-tune one’s diet to ensure avoidance of chronic disease. For others, this science has value in assisting with, for example, the interpretation of epidemiological studies, in which some of the variation observed in nutrient status or requirement may be due to genetic variation at a few or several loci that determine the uptake and metabolism of various nutrients. The increase in interest and knowledge of diet-gene interactions, largely driven by the expansion of knowledge on the nature of the human genome, is at a time of rapid change in the economic and nutritional status of the population of both developed countries and so-called developing countries. As the health burden of the “western diet” is rapidly becoming apparent with an increase in...
such conditions as obesity and type 2 diabetes, there is a “westernization” of both the economies and diet of many countries in Asia and south and central America. The economic expansion of countries such as China, India, and Brazil has led to enormous disparities of both income and nutritional status within these countries. This is also likely to be combined with greater genetic susceptibility of many people within these countries to conditions such as type 2 diabetes. Thus, in many countries that were once considered “developing” there are issues concerned with both nutrient deficiency and nutrient excess, at least in terms of the major macronutrients.

The situation regarding micronutrient status (sensu latu, to include minerals, vitamins, and phytochemicals) is probably more complex. The issues of sufficient micronutrient intake to prevent deficiency diseases, such as vitamin D and rickets, have now largely (although not entirely) been displaced in many western countries. This has been replaced by the realization that the nutrient status for “optimal” health required for the prevention of chronic disease may be quite different for that to prevent deficiency. Thus, it is considered by many that the level of micronutrients within large sections of the western population is below that required for optimal health. Thus, when considering the importance (or otherwise) of genetic variation in determining micronutrient absorption and metabolism, we need to consider both that required to prevent deficiency diseases, and that required for “optimal” health, or the reduction in risk of chronic disease.

For the majority of micronutrients, there are very little data, or none at all, on the role of genetic polymorphisms on their absorption and metabolism. In many cases, the elucidation of biochemical pathways and regulators of homeostatic mechanisms have come from studies of individuals that have mutations in certain genes. Other polymorphisms in these genes that result in a less severe phenotype may be important in determining the natural range of variation in absorption and metabolism that is commonly observed. To illustrate some of these aspects, I briefly review the increased understanding of iron metabolism that has arisen from our knowledge of the effects of mutations in several genes, the role of genetic variation in mediating the nutritional effects of iodine and selenium, and finally, the interaction between a genetic polymorphism in folate metabolism and folic acid fortification.

Polymorphism in genes affecting inorganic iron uptake and transport

The interaction between diet and genetic factors can lead to both iron deficiencies and iron overload (hemochromatosis) [2]. Iron deficiency is of particular significance in early childhood and amongst menstruating women, whereas hemochromatosis is most prevalent in late-middle-aged men and post-menopausal women. Hereditary hemochromatosis leads to the accumulation of iron and eventually to the deposition of iron in vital organs. The specific genetic defects that cause this disorder have been identified and these provide an insight into the manner by which iron uptake is regulated. Other polymorphisms in these genes may not result in acute hemochromatosis, but may still influence iron uptake.

In humans, iron is strictly conserved. Each day, about 20 mg of iron is recycled from senescent red blood cells into the synthesis of new red blood cells (Figure 1A). There are no pathways in humans for iron excretion, apart from losses in epithelial cells such as skin, gastrointestinal cells, and urinary tract cells, and fluids such as blood, tears, and sweat. Thus, absorption of dietary iron must be regulated to prevent excess iron accumulation [3].

Heme iron derived from meat is an important source of iron and is highly bioavailable, although the precise route of uptake is not understood. It is thought that the metal porphyrin ring is split from the globin in the lumen, and the intact Fe porphyrin is transported across the brush border membrane. There are some suggestions that this may happen by endocytosis, and a brush border heme receptor, HCP1, has been reported [4]. Within the enterocyte, ferrous ions may be released from the heme and enter a common pool with non-heme iron [5]. Thus subsequently, the basolateral transfer of iron, and its regulation, may be the same as for both heme and non-heme iron (Figure 1B).

The pathway of absorption of non-heme iron is better understood. Within the lumen, iron is likely to be ferric ion and is poorly available. Fe$^{3+}$ ions may be chelated (e.g. with phytate or citrate) or encased as a multi-ion form such as ferritin. Fe$^{3+}$-phytate is poorly absorbed, but ferritin may be absorbed, although the precise route is yet to be discovered. Ferric reductase activity within the duodenal mucosa converts Fe$^{3+}$ to Fe$^{2+}$ ion. The cytochrome B DcytB is the most likely candidate for the ferric reductase, but the lack of phenotype with DcytB knockout mice suggests that there may be other ferric reductases [5]. Ferrous ions are transported into enterocytes via the divalent metal transporter (DMT1, also known as DCT1 or Nramp2). It seems likely that DMT1 can also transport other divalent ions such as Zn$^{2+}$, Co$^{2+}$, Mn$^{2+}$, and Cd$^{2+}$, although the physiological significance of this is not clear, and these ions may have other transport routes into enterocytes. Once within the enterocyte Fe$^{2+}$ may either be bound to ferritin or exported into the bloodstream (Figure 1B). The transport of Fe$^{2+}$ into the circulatory system is due to the coordinated expression and action of the transport protein fer-
Figure 1: A. Model for the recycling of iron and homeostatic control mediated by hepcidin. B. Enterocyte model for the uptake of heme and non-heme iron from the lumen. C. Regulation of uptake via hepcidin.
roportin 1 (also known as IREG1) and hephaestin on the basolateral surface of the enterocyte [6]. Hephaestin (a Cu-containing protein – see below), which has a high degree of homology with the serum ferroxidase ceruloplasmin, results in the oxidation of ferrous to ferric ion, which then binds to transferrin (Tf) and is transported in the serum. (Figure 1B). Uptake of Tf-bound iron into cells such as the immature erythroid cell for heme synthesis, is via the Tf receptor (TfR1) through endocytosis. The endosome is acidified resulting in release of ferric ion which is reduced by Steap3, and possibly other closely related ferric reductases, and then transported out of the endosome by DMT1 [7, 8]. The Tf-TfR1 complex is recycled to the cell membrane to begin the endocytosis cycle over again. Within macrophages, iron derived from heme is returned to the plasma via ferroportin 1 (as in enterocytes), but is oxidized probably via ceruloplasmin as opposed to hephaestin.

The regulation of this entire process is largely dependent upon the protein hepcidin [9]. Hepcidin is a 25-amino acid peptide synthesized in the liver. Hepcidin knockout mice have hemochromatosis, whereas overexpressing hep{-}cidin in mice leads to iron deficiency. Hepcidin binds to ferroportin 1, resulting in it being internalized in the cell and degraded (Figure 1C). Thus, this prevents iron being transported out of the enterocyte where it accumulates. The enterocyte only lives for a few days after which it is shed from the tips of the villi into the lumen. When iron stores are adequate or high, the liver produces hepcidin that circulates to the small intestine where it prevents the transport of iron from the enterocyte to the plasma. When iron stores are low, hepcidin production is suppressed, ferroportin proteins are expressed on the basolateral surface of the enterocyte and enable iron to be transported into the plasma. Hepcidin may have other secondary effects. For example, rising iron content with enterocytes may result in the oxidation of ferrous to ferric ion, which then binds to transferrin (Tf) and is transported in the serum. Hepcidin in mice leads to iron deficiency. Hepcidin binds to ferroportin 1, resulting in it being internalized in the cell and degraded (Figure 1C). Thus, this prevents iron being transported out of the enterocyte where it accumulates. The enterocyte only lives for a few days after which it is shed from the tips of the villi into the lumen. When iron stores are adequate or high, the liver produces hepcidin that circulates to the small intestine where it prevents the transport of iron from the enterocyte to the plasma. When iron stores are low, hepcidin production is suppressed, ferroportin proteins are expressed on the basolateral surface of the enterocyte and enable iron to be transported into the plasma. Hepcidin may have other secondary effects. For example, rising iron content with enterocytes may result in a reduction in expression of DMT1 [9].

The most common form of hereditary hemochromatosis is due to mutations in the HFE gene [2, 10]. The HFE protein is similar to MHC class I-type proteins and associates with beta2-microglobulin (beta2M); the precise role of this protein is not understood, but it seems likely it is involved with the regulation of hepcidin synthesis. The C282Y mutation is most prevalent in northwestern Europe, and it is thought that this mutation arose relatively recently, maybe between 1000–3000 years ago [11]. An older mutation, H63D, has a more global distribution. Other polymorphisms in this gene have also been described. It seems likely that the C282Y and H63D mutations reduce the synthesis of hepcidin, which in turn increases the membrane-located expression of ferroportin leading to excess iron uptake. Mutations in HFE are recessive disorders of low penetrance, and largely affect older men and post-menopausal women.

Mutations in other genes that probably affect hepcidin expression can also result in similar phenotypes. For example, those in TFR2 are much rarer than the mutations in HFE, but can result in a similar phenotype. Likewise, the infrequent mutations in HJV, causing acute juvenile hemochromatosis. Mutations can also occur in the hepcidin gene (HAMP) itself.

Mutations in the ferroportin gene can also result in hemochromatosis [6]. A particular mutation in this gene (Q248H) occurs within people of African descent at a frequency of about 20% and has been studied both with respect to the relatively high frequency (~ 10%) of iron overload amongst adult Africans in sub-Saharan Africa [12], and iron deficiency that is common amongst African children [13]. In neither case was a strong association observed. However, there is some evidence that Q248H heterozygosity may protect children from iron deficiency when they are exposed to repeated or prolonged inflammatory conditions [13].

The hypothesis that mutations in a variety of genes that result in iron overload were selected for times in which diets were low in iron are attractive, but lacking in evidence. However, it is likely that western diets are richer in heme iron than diets in prehistory, and that there is currently less infection by hookworm and other parasites that would have resulted in iron loss through bleeding into the gastrointestinal tract. Thus, it is conceivable that for most of prehistory, genes that now result in iron overload may have been favorably selected.

Hereditary conditions such as hemochromatosis may provide insights into polymorphisms and mutations that may affect iron homoeostasis. There is a public health concern that if food is fortified with iron to prevent deficiencies, then individuals with specific genotypes may absorb inappropriately high amounts of iron. However, intervention studies have provided no evidence that C282Y heterozygotes absorb more dietary iron than those who are not carrying the mutation [14, 15].

The penetrance of the hemochromatosis phenotype in individuals with these mutations is relatively low [16], and this has prompted the more systematic survey of seeking mutations in other genes that affect iron homeostasis. Lee and colleagues [17] undertook a survey of polymorphisms within 24 genes, all of which have been implicated in homeostasis, and as expected identified many polymorphisms. As with the more commonly studied polymorphisms, these may contribute to variation in iron uptake amongst people with no symptoms of iron deficiency or overload. Providing the evidence that this is the case is difficult, and there is a need to examine several polymorphisms in many genes concurrently. The use of single-nucleotide polymorphism (SNP) arrays or transcript mapping may both provide useful approaches.
Copper

Copper is required for the activity of several enzymes, including the ferroxidases ceruloplasmin and hephaestin, and cytochrome c oxidase, required for respiration and the antioxidant Cu, Zn superoxide dismutase (SOD), which represents about 1% of total cell protein. Its role in ferroxidases links it intimately to iron homeostasis [18, 19]. Copper is readily absorbed from the diet. Uptake into the mucosa cell is probably by passive diffusion. Within the enterocyte it may be bound to metallothionein or passes through the basolateral membrane into the bloodstream. Within the serum, copper is probably transported bound to either albumin or histidine, where it is delivered to tissues and the liver for further metabolism. It is transported into hepatocytes via the copper transporter, Ctrl. Once inside the hepatocyte, Cu has four different fates. Firstly, it may be bound to metallothionein, as in the enterocyte joining an intracellular copper pool. Secondly, it may be trafficked into the mitochondria via the copper chaperone Cox17 for incorporation into cytochrome c oxidase. Third, it may be bound to CCS (copper chaperone for SOD) for Cu, Zn SOD synthesis. Lastly, it may be trafficked to the trans Golgi network (TGN) by HAHI (human atox-1 homolog) which delivers it to a P-type ATPase (ATP7B), where it is incorporated into ceruloplasmin, which is transported into the serum, and is the major form of copper in the serum (>95%). Alternatively, when Cu concentration is sufficient or too high, Cu is transported from the TGN to a vesicular compartment that migrates towards the bilayer epithelium. This provides a mechanism for removal of excess Cu in the bile [20].

Mutations have been described in several of these genes, and these have been used to further elucidate the genetic and physiological basis of copper metabolism [20]. Mutations in ATP7B cause the autosomal recessive Wilson’s disease, which occurs in about 1:30,000 in most populations. Malfunction of ATP7B prevents trafficking of copper within the hepatocyte into the TGN for either ceruloplasmin synthesis or excretion via the bile. The consequence is that copper accumulates in the liver, causing oxidative damage, and serum levels of ceruloplasmin are very low. There may be leakage of Cu from the liver resulting in Cu deposits in the brain and cornea. The ATP7B gene has 21 exons, and a large number of mutations in this gene have been described. The most frequent mutation in the Caucasian population, C3207A, disrupts ATP binding, and occurs in between 40 and 60% of people with Wilson’s disease. This mutation is absent from people of Asian origin [21]. Wilson’s disease can be treated with Cu chelating agents, but can be fatal if not treated.

The related X-linked Menkes disease is caused by a mutation in ATP7A, which is functionally and structurally homologous to ATP7B but expressed not in the liver but in the TGN membranes of the placenta, gut, and brain. Copper that crosses the TGN membrane in these organs is required for incorporation into specific enzymes that may contribute, for example, to the developing brain or to supply a growing fetus. Thus, individuals who suffer from Menkes disease are deficient in many essential Cu enzymes, and die within the first few years of life.

Mutations in the ceruloplasmin gene have provided an insight into the role of this protein in regulating iron homeostasis [22]. Surprisingly, despite 95% of serum Cu being in the form of ceruloplasmin, reduced levels of this protein, either due to mutation in the ceruloplasmin gene itself or through another cause (such as Wilson’s disease), does not lead to symptoms of copper deficiency, but instead to anemia. This confirms that ceruloplasmin is not the Cu transport protein in an analogous manner to iron and ferritin, and its major role is the mobilization of iron from tissues (with the exception of enterocytes) through its ferroxidase activity. Several mutations have been documented in the 20 exons of the ceruloplasmin gene. These can result in neurological disorders due to iron accumulation in the basal ganglia, diabetes mellitus resulting from iron accumulation in the pancreas, and visual degeneration due to iron accumulation in the retina. Ceruloplasmin does not cross the blood-brain barrier, and thus while the liver is a major site of ceruloplasmin synthesis, it can also be synthesized in astrocytes. There is evidence from Xenopus, that within these tissues, ceruloplasmin may be synthesized as a glycoprophatidylinositol-linked isoform, and not excreted. Indeed there is some evidence for a rather separate brain iron cycle distinct from that of the general circulation. The precise manner by which abnormalities in ceruloplasmin levels may be associated with neurological disorders still needs to be resolved [23].

Selenium, Iodine and thyroid metabolism

Population effects of severe iodine deficiency, termed iodine deficiency disorders (IDDs), include endemic goiter, hypothyroidism, cretinism, decreased fertility rate, increased infant mortality, and mental retardation. Deficiency is an important risk factor for brain damage and motor-mental development in the fetus, the neonate, and child. 29% of the world’s population is estimated to live in areas of deficiency. This occurs primarily in mountainous regions such as the Himalayas, the European Alps, and the Andes, where iodine has been washed away by glacial and flooding. Iodine deficiency also occurs in lowland regions far from the oceans, such as central Africa.
and eastern Europe. It is likely that the extent of iodine deficiency is more widespread than is usually considered; it has been estimated that half of the population of western and central Europe suffers from iodine deficiency [24]. The severity of IDD is also dependent upon other factors – such as nutrient status of Vitamin A, iron, and selenium.

Dietary iodine is taken up readily through the gut in the form of iodide. From the circulation, it is concentrated in the thyroid gland by means of an energy-dependent sodium-iodate symporter (NIS). In the follicle cells of the thyroid gland, 4 atoms of iodine are incorporated into each molecule of thyroxine (T₄) and 3 atoms into each molecule of triiodothyronine (T₃). T₃ is 20–100 times more biologically active than T₄. These hormones are essential for neuronal development, sexual development, and growth and for regulating the metabolic rate, body heat, and energy. T₄ is the major product of thyroid secretion and is a critical signal in the plasma that mediates the thyrotropin (TSH) negative feedback loop. When there is insufficient iodine for thyroid hormone synthesis, the serum T₄ level initially falls and the pituitary gland releases TSH, which stimulates the growth and metabolic activity of thyroid follicular cells. TSH stimulates the thyroid cells to increase iodine uptake, and thyroid hormone synthesis and secretion. However, the stimulating effect of T₄ on TSH secretion is due to the activity of iodothyronine deiodinase (DIO2), which converts T₄ to the biologically active T₃. This also releases iodine into the circulation, which may either be excreted via the kidneys or reabsorbed by the thyroid gland. In addition to its role in mediating the negative feedback mechanism, DIO2 is of more widespread importance in converting T₄ to T₃ in the brain to enable proper brain development and growth. Thus, polymorphisms in two critical genes, NIS and DIO2, may be of importance for iodine uptake and metabolism.

NIS is a specialized plasma membrane glycoprotein that mediates active I⁻ transport into thyroid and other organs, such as the salivary glands, breast, and gastric mucosa. NIS consists of 15 exons and encodes a protein of 643 amino acids. About ten NIS mutations have been described to date [25], Congenital iodine transport disorder (ITD) due to one or more of these mutations is an infrequent autosomal recessive disorder. As with the majority of polymorphisms in genes determining uptake and metabolism of micronutrients, the importance of these mutations is only recognized when in a homozygous state in which they result in irreversible deleterious effects on development and acute symptoms of hypothyroidism [26].

To what extent the transport of I⁻ may be affected when these mutations are in a heterozygous state and any consequences for health have not been systematically investigated. DIO2 is a selenoenzyme and is particularly important in the brain and pituitary, as discussed above, and also in brown fat. This gene has been shown to have several polymorphisms. One of these, A274G, predicts a non-conservative Thr92Ala substitution has been associated with insulin resistance and obesity [27]. Guo and colleagues [28] investigated the potential effect of this polymorphism and two further SNPs, both within non-coding regions of the DIO2 gene, with mental retardation in an iodine-deficient area of China. They reported an association between the occurrences of the two SNPs within non-coding regions of the DIO2 gene with mental retardation, and suggested that abnormal DIO2 activity may be of particular importance in human fetal growth in regions of iodine deficiency.

Genetic mutations affecting incorporation of selenium into selenoenzymes

Severe selenium deficiency occurs in similar areas of the world to iodine deficiency, such as regions of China, alpine Europe, and central Africa. It can cause severe and fatal illness, notably Keshan disease, which is a cardiomyopathy affecting mostly young and middle-aged women. Kashin-Beck disease occurs in areas where there is combined iodine and selenium deficiency, such as in areas of Tibet, Siberia, and North Korea. Kashin-Beck disease is an osteoarthropathy of the hands and fingers, elbows, knees, and ankles in children and adolescents. It has a complex aetiology, and in addition to iodine and selenium, other abiotic and biotic factors may be involved, and the specific role of selenium is yet to be resolved [29–31]. Selenium and iodine deficiencies also occur in central Africa, and results in myxedematous cretinism, characterized by hypothyroidism from infancy resulting in short stature and mental deficiency [32–34]. The cause of this condition is thought to be due to iodine deficiency, and also dietary goitrogens, resulting in increased production of H₂O₂ in the thyroid due to stimulation by thyrotropin. Inadequate glutathione peroxidase activity due to selenium deficiency prevents removal of excess H₂O₂, leading to tissue necrosis and resulting in destruction of the gland soon after birth [35].

In contrast to these examples of selenium deficiencies, there is concern within some western European countries that the level of selenium in the diet may not be adequate for “optimal” health, and a relatively low level of selenium has been associated with increased risk of cancer and lower immune function [36]. Selenium is incorporated into about 20–30 proteins, many of which have antioxidant properties and regulate cell redox status, such as thioredoxin reductase and glu-
tathione peroxidases, while others are involved in transport of selenium to peripheral tissues through plasma selenoprotein P. As discussed above, selenium is also incorporated into iodothyronine deiodinases, such as DIO2. Mutations in rodents that abolish global selenoprotein synthesis are lethal, whereas knockout of specific selenoproteins have varying effects. For example, knockout of thioredoxin reductase is lethal in embryos [37], knockout of cytosolic glutathione peroxidase increases sensitivity to oxidative stress, and knockout of DIO2 results in abnormal neurological development [38]. Deficiencies in dietary selenium do not cause equal reductions in expression of the different selenoproteins. These differences are due in part to the differential ability of tissues and organs to retain Se under conditions of dietary limitations. For example, brain and endocrine organs preserve Se better than liver, kidney, muscles, and blood. However, even within tissues that are able to retain Se, some selenoproteins decrease at faster rates than others.

Selenium is incorporated into proteins through an unusual mechanism in which the UGA stop codon is re-cod ed to be read as a sense codon (Figure 2). This is through part of the 3’ untranslated part of the mRNA of selenoproteins, termed selenocysteine insertion sequences.
Nutrient status, food fortification and genetic polymorphism: B vitamins, folic acid and the MTHFR C677T polymorphism

The B group of vitamins, including folate, vitamin B_{12}, niacin (B_{3}), pyridoxal phosphate (B_{6}), and riboflavin (B_{2}) have historically been of major interests in the context of overt deficiency diseases such as pellagra and anemia. One of the first food fortification programs was the addition of niacin to flour to eradicate pellagra in the United States in the 1930s. More recently, the importance of the B vitamins in general and folate in particular in maintaining health and avoiding a wide range of chronic illness – including birth defects, cancer, cardiovascular disease, and dementia – has been recognized, and that apparently marginal deficiencies in the diet may have health consequences. The complex effects that folate and other B vitamins have on health is due to their effects on several processes that contribute to genomic stability and DNA repair, including the supply of nucleotides for DNA synthesis and methyl groups for epigenetic regulation, combined with the role of folate and B_{12} in the conversion of homocysteine to methionine (Figure 3). Homocysteine is a risk factor for vascular pathology – such as promotion of clot formation and blocked blood vessels, and inhibition of collagen-elastin cross-linking that can lead to connective-tissue abnormalities. Folate-deficient diets pre-conception and during pregnancy can lead to neural tube defects. This has led to both provision of advice in many countries to either consume a folate-rich diet or to take folic acid supplements, which has had no impact on the incidence of NTD, or has led to the mandatory fortification of flour with folic acid, in a similar manner to niacin, which has resulted in a reduction in the incidence of neural tube defects.

Functional polymorphisms have been described in several genes concerned with folic acid and folate metabolism. The most well studied one is the C677T polymorphism in the 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene. The MTHFR enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate (methylene-THF) to 5-methylenetetrahydrofolate, which is the carbon donor for the methylation of homocysteine to methionine. The C677T alteration converts an alanine to a valine and is associated with about a 70% reduction in activity and increased thermolability of the enzyme, and an increase in homocysteine, as the conversion to methionine is less efficient (Figures 3 and 4). As a consequence, when dietary folate levels are low, homozgyosity for MTHFR 677TT is associated with enhanced risk of neural tube defects [43], vascular disease [44, 45], late-onset Alzheimer’s [46], migraine [47], cervical intraepithelial neoplasia [48], esophageal [49], and endometrial [50] cancer. However, the same genotype is also associated with reduced risk of colon cancer [51] and acute lymphatic leukemia [52], probably due to the contribution of enhanced supply of methylene-THF to enable proper DNA synthesis and to avoid the misincorporation of dUMP into DNA.

The frequency of the MTHFR 677T allele varies substantially in different regions of the world and amongst different ethnic groups. It is low amongst sub-Saharan Africans (0.07) and Canadian Inuit (0.06) but higher amongst Caucasians, Japanese, and Chinese (0.24–0.54). There is evidence of a north-south increase in allele frequency in Europe, and a north-south decrease in frequency in China [53]. The variation in the frequency of the 677T allele raises the question of whether this is a recurrent mutation or if the allele has a common ancestral origin. Through the quantification of frequencies of nucleotide polymorphisms in introns, it seems likely that the MTHFR 677T allele has a common origin [54], and that the variation in frequency may be due to selection, with there being less selection pressure against the 677TT genotype in regions with a higher folate content to the diet.

Food fortification with folic acid in the United States has reduced the incidence of neural tube defects (NTD) [55], and has also resulted in modest reduction in other birth defects, such as cleft palate, transposition of the great
arteries, and upper limb defects [56]. In contrast, in general, dietary recommendations to take folic acid supplements or to have a high folate diet does not appear to have been successful in reducing birth defects in many countries, leading to calls for increased mandatory fortification, particularly within developing countries [57].

Fetuses that are either 677TT or 677TC are at higher risk of NTD [58,59], and are more likely to abort. However, analyses of frequency of NTD and MTHFR genotype between 1988 and 1994, and between 1994 and 1998 show striking changes in risk. During the earlier time period, there was a far greater risk of a 677TC heterozygote suffering NTD, compared to the 677CC homozygotes (risk ratio 9.8, 2.8–34.0 95%CI), but after 1994 there was no significant difference in risk between these two genotypes [60]. The sample sizes for the 677TT homozygotes were too small to obtain statistical significance, but they exhibited a similar trend. The explanation may be that beginning around 1994 there was strong encouragement by physicians for women to take folic acid supplements pre- and post-conception, and this additional folate acid reverses the abnormal folate metabolism caused by the 677T variant. In a similar manner, Muñoz-Morgan and col-

Figure 3: Folate metabolism.

Figure 4: The effect of the MTHFR C667T polymorphisms on the relative flux of folate into DNA synthesis and as a donor of methyl groups for methionine biosynthesis.
leagues [61] reported an increase in the frequency of the 677T allele in people under 20 years of age in Spain, and an increase in the frequency of the 677TT homozygous genotype from 13% in people > 20 years to 26% in people less than 20 years. This data is consistent with an increase in pregnant women taking folic acid supplements in the early 1980s – going from 10% in 1977 to 55% in 1986.

Thus, it seems probable that dietary advice when adopted and mandatory folic acid fortification of food is leading to a rapid increase in the frequency of the MTHFR 677T allele. What might the consequences of this be? There is evidence that the 677T allele increases the homocysteine level in the blood, and this has been associated with a wide range of degenerative and chronic diseases, including cardiovascular disease and Alzheimer’s disease, particularly if folate levels are less than optimal in the diet. Thus, mandatory fortification programs may well be desirable to remove the trauma and health burden of NTD, and other birth disorders, but it may result in a greater need for folate in the diet in later life, either through folate-rich foods or through supplementation.

The issues around fortification are thus both straightforward – with proven evidence for a reduction in NTD, but also fairly complex with an apparent diet-driven change in allele frequency that may have consequences for illness in the elderly. Similar factors may be important in the interplay between genotype and other micronutrients.

References


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