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Transcriptomics and functional genetic polymorphisms as biomarkers of micronutrient function: focus on selenium as an exemplar

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Micronutrients are essential for optimal human health. However, in some cases, raising intake by supplementation has not proven to be beneficial and there is even some evidence that supplementation may increase disease risk, highlighting the importance of assessing the functional status of micronutrients. Techniques such as gene microarrays and single-nucleotide polymorphism analysis have the potential to examine effects of micronutrient intake on patterns of gene expression and inter-individual variation in micronutrient metabolism. Recent genomic research related to selenium (Se) provides examples illustrating how studies of functional single-nucleotide polymorphism and gene expression patterns can reveal novel biomarkers of micronutrient function. Both *in vitro* and *in vivo* experiments show that there are functionally relevant polymorphisms in genes encoding glutathione peroxidases 1, 3 and 4, selenoprotein P, selenoprotein S and the 15 kDa selenoprotein. Disease association studies investigating these gene variants have so far been relatively small but an association of a polymorphism in the selenoprotein S gene with colorectal cancer risk has been replicated in two distinct populations. Future disease association studies should examine effects of multiple variants in combination with nutritional status. Gene microarray studies indicate that changes in Se intake alter expression of components of inflammatory, stress response and translation pathways. Our hypothesis is that Se intake and genetic factors have linked effects on stress response, inflammation and apoptotic pathways. Combining such data in a systems biology approach has the potential to identify both biomarkers of micronutrients status and sub-group populations at particular risk.

Selenium: Selenoproteins: Gene microarray: single-nucleotide polymorphism: Cancer

Micronutrients are essential for human health. In addition to deficiency-associated diseases, there is concern with regard to the disease risks associated with sub-optimal intakes as low intake of certain micronutrients has been implicated in multi-factorial diseases such as cancer, heart disease and cognitive decline, especially in the elderly when food intakes fall and micronutrient intake can become critical[1–3]. However, despite some evidence suggesting the possible benefits of increased intake of micronutrients such as folate, selenium (Se), carotenoids, vitamin B_{12} and vitamin D, for some micronutrients the benefits of supplementation have not been proven and indeed in some cases there is evidence that raising intake by supplementation may increase disease risk. For example, there is controversy over whether mandatory folate fortification of flour in the USA has been associated with increased cancer rates[4–6]. In addition, supplementation of smokers with β-carotene was found to increase cancer risk[7]. Furthermore, an earlier decrease in prostate cancer mortality after Se supplementation[8] has not been

Abbreviations: CRC, colorectal cancer; ER, endoplasmic reticulum; SECIS, selenocysteine insertion sequence; SelS, SelW, SelH, SelN, SelM, SelK, selenoproteins S, W, H, N, M, K respectively; Sep15, 15kDa selenoprotein; SePP, Selenoprotein P; SNP, single-nucleotide polymorphism; 3′-UTR, 3′-untranslated region.

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replicated in the recent SELECT study in which Se supplementation in the USA was found to increase risk of diabetes[9]. These differences and conundrums may reflect variability in baseline micronutrient status, genetic make-up in populations in conjunction with environmental risk factor exposure and lifestyle. Understanding the mechanisms by which these micronutrients influence cell function, defining the genetic factors that affect micronutrient metabolism and inter-individual variations in response to increased micronutrient intake, and developing effective biomarkers of micronutrient function can all make a major contribution to this debate.

Potential of genomic biomarkers

High-throughput genomic techniques such as gene microarrays (transcriptomics) and proteomics offer the potential to examine effects of micronutrient intake on patterns of gene and protein expression and thus give an integrated view of the response compared to measures of single parameters. These approaches allow broadening the analyses to previously unknown targets and open the way to novel hypotheses and to the discovery of new functional biomarkers. This will be illustrated, in this paper, by recent work showing how a transcriptomics approach has identified gene expression patterns affected by Se intake and some potential novel biomarkers of Se function.

The increase in information on human genetic variation is providing a basis for investigation of the role of genetics in inter-individual variations in response to micronutrients. Genetic diseases, although often relatively rare, can provide important information on micronutrient function; for example, with acrodermatitis enteropathica and Zn function, Menkes' disease and Cu metabolism[10,11]. However, study of these mutations cannot identify the role played by a nutrient in multi-factorial diseases such as cancers or the effect of long-term sub-optimal status on disease development. The sequencing of the human genome together with the subsequent HapMap project has led to the cataloguing of single-nucleotide polymorphisms (SNP) within the human genome. SNP are stable allelic variations that occur frequently throughout the DNA and so provide an extraordinarily large amount of genetic variation; their frequency can differ markedly between different ethnic groups. Critically, the majority of variants seem to have no impact on gene function and so only a small number of SNP actually have functional consequences. The effects of these functional variants on gene or protein function are usually more subtle than those of a mutation. Thus, SNP alone do not lead to a disease phenotype; however, they can alter metabolism and contribute to disease susceptibility or risk.

Examples of single-nucleotide polymorphisms affecting micronutrient responses

In the case of folate metabolism several well-characterised SNP, especially the C677T variant in methylene tetrahydrofolate reductase, are known to influence both metabolic responses to folate intake and disease susceptibility[12]. In the case of response to the dietary β-carotene the evidence for a genetic influence is more recent and less

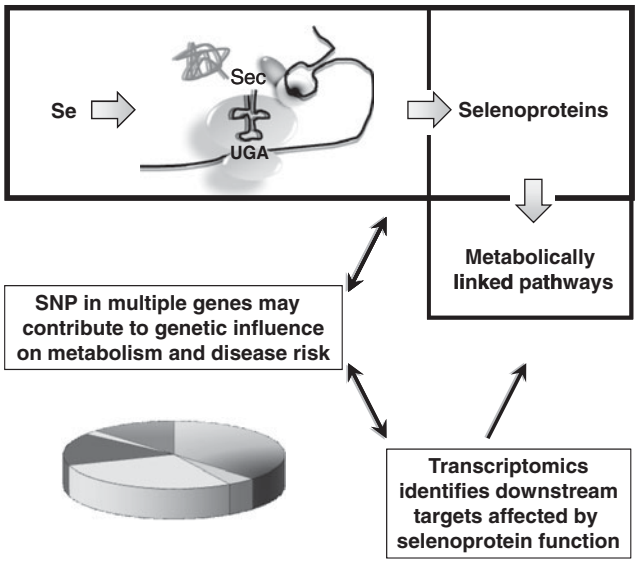

Fig. 1. Genomic approaches to selenium biology. The figure illustrates schematically the incorporation of Se into selenoproteins and their functional effects on other metabolic pathways (downstream targets). Transcriptomics can be used to identify such downstream targets. Single-nucleotide polymorphism (SNP) in genes throughout selenium metabolism may contribute individually or together to influence metabolism and risk of multi-factorial diseases.

comprehensive. Only 55–75% of absorbed β-carotene is cleaved to form retinoids and the extent of cleavage varies between individuals[13]. The basis for such variability is not known. However, recently two non-synonymous SNP (rs12934922 and rs7501331) in the gene encoding β-carotene 15,15′-monoxygenase (*BCMO1*) have been found to alter enzyme kinetics of the recombinant protein and the response of blood retinoids to a β-carotene load[14], suggesting that there may be a partly genetic basis for the inter-individual variability. Recent data from supplementation trials also suggest that SNP in selenoprotein genes influence responses to Se supplementation[15–16].

Reponses to dietary micronutrients are likely to involve several target genes/proteins within a biochemical pathway or network[12,17]. As a result, from a nutritional point of view, it is likely to be a combination of multiple SNP that gives rise to altered metabolism and probably such combinations together with micronutrient intake may determine overall physiology and disease susceptibility. An important first step is to identify those SNP that have functional consequences in terms of altering micronutrient metabolism and responses to intake.

Both studies of functional SNP and gene expression patterns from nutrigenomic experiments can lead potentially to novel biomarkers of micronutrient function. In this paper, this will be illustrated using Se as an exemplar (illustrated schematically in Fig. 1).

Nutrigenetics of selenium: mutations and functional single-nucleotide polymorphisms

In human subjects, Se is incorporated into twenty-five selenoproteins as the amino-acid selenocysteine and this is

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achieved by a unique mechanism that involves a selenocysteine-specific tRNA and recoding of the UGA codon[18]. The recoding requires a stem-loop structure (called SECIS for selenocysteine insertion sequence) in the 3′-untranslated region (3′-UTR) of selenoprotein mRNA and specific proteins such as SECIS-binding protein 2 (SBP2) and nucleolin that bind to these 3′-UTR. The selenoproteins include the glutathione peroxidases, thioredoxin reductases and selenoproteins P (SePP), W (SelW), H (SelH), N (SelN) and the 15kDa selenoprotein (Sep15). Some selenoproteins have well-characterised functions in redox control, protection from oxidative stress and thyroid hormone metabolism but the function of others is still not known or poorly understood.

An SNP or mutation may cause an amino-acid change that alters function of a selenoprotein itself or one of the components of the selenoprotein synthetic machinery function. Alternatively, it may occur in a gene regulatory region such as the promoter or, because the SECIS region within the 3′-UTR is vital for Se incorporation, in the 3′-UTR. There is now a strong body of evidence that mutations and functionally significant SNP in genes encoding components of the Se incorporation machinery or the selenoproteins have a variety of effects on cell function and responses to different stressors.

Mutations in selenoprotein-related genes

Rare mutations in genes playing a role in Se metabolism have been found to give rise to several genetic diseases. For example, mutations in the *SELN* gene are associated with congenital muscular dystrophy[19]: the mutations are found in the gene region that corresponds to the 3′-UTR and are linked to altered binding of SBP2 to the SECIS and lower levels of both mRNA and protein. In addition, mutations in SBP2 have been found to cause two distinct syndromes. One exhibits impaired thyroid function[20] and another is associated with low selenoprotein expression and a failure of spermatogenesis, muscular dystrophy and increased levels of reactive oxygen species[21].

In addition to these rare mutations, selenoprotein and Se-related genes, as with all genes, contain stable allelic variations. A recent screen of six selenoprotein genes found eleven SNP of potential functional significance out of a total of 275 identified variants[22]. This observation highlights the challenge to discriminate functional from non-functional SNP. Over the past 10 years a number of approaches, including reporter gene studies and human supplementation trials, have indicated that there are variants within several selenoprotein genes that are functionally significant.

Functional single-nucleotide polymorphisms in GPX1 *and* GPX4

The first selenoprotein gene SNP to be identified as functionally significant was a variant in the *GPX1* gene that causes a proline to leucine change at codon 198 (rs1050450) that alters enzyme activity[23]. Furthermore, *in vivo* the association between GPx1 activity and Se concentration differed between groups of different genotype suggesting that this SNP modifies the response of GPx1 activity to Se[24] The Leucine variant is relatively rare, the homozygous allele being found in only 7–11% of Caucasians and about 15% of Afro-Caribbeans.

Sequencing of the 3′-UTR region of the *GPX4* gene led to the identification of an SNP (T/C variant: rs713041) in Caucasians in a UK population[25]. Subsequently, this SNP has been found in other ethnic groups[16,26]. In the original study TT and CC individuals were found to show differences in levels of leucocyte lipoxygenase metabolites, suggesting that the SNP has functional consequences. Both *in vitro* and *in vivo* studies have since then confirmed this functionality. *In vitro*, the C variant shows a stronger capacity to support expression of a selenoprotein reporter gene than the T variant in cells transfected with a construct containing the reporter under control of either variant of *GPX4* 3′-UTR[27]. Additionally, using *in vitro* RNA-binding assays, the C variant was shown to better compete with the GPX1 3′-UTR for protein binding to the SECIS structure than the T variant[16]. Further evidence for functionality of this SNP has come from an *in vivo* study in which parameters of blood cell and plasma selenoprotein status were found to be affected differentially in healthy volunteers who were prospectively genotyped and then supplemented with sodium selenite (100 μg/d) for 6 weeks[16]. Interestingly, after withdrawal of the Se supplementation individuals who were CC showed significantly greater falls in lymphocyte GPx1 protein levels and the ratio of GPx1:GPx4 protein levels. These observations led us to hypothesise that the C variant 3′-UTR from *GPX4* competes more strongly than the T variant for one or more proteins involved in Se incorporation and as a result the SNP affects the hierarchy of selenoprotein synthesis. The mechanism could be that the SNP alters the structure of the 3′-UTR in the vicinity of the SECIS and so affects the binding of proteins involved in selenocysteine incorporation and thus selenoprotein synthesis. Computer prediction suggests that the SNP does lead to altered RNA structure within the 3′-UTR[27] but this hypothesis needs to be confirmed experimentally.

Functional single-nucleotide polymorphisms in selenoprotein P

Selenoprotein P (SePP) is the major Se transport protein in the blood[28]. Human SePP contains ten selenocysteines and accounts for approximately 65% of plasma Se. It is found in blood as at least two isoforms of approximately 50 and 60 kDa molecular weight[29]. Several SNP in *SEPP1* seem to have functional consequences. Reporter gene studies have shown that a variant in a TC repeat sequence within the promoter affects reporter activity in HepG2 cells[30]. Screening the *SEPP1* gene by DNA-HPLC techniques identified several other SNP including a G/A variant within the 3′-UTR (rs7579) and G/A variant that causes an alanine to threonine amino-acid change at codon 234 (rs3877899)[15]. Results from the SelGen Se supplementation trial showed that various parameters of Se metabolism differed between healthy individuals depending on their genotype for rs7579 and rs3877899. Both SNP affected the proportion of 50 and 60 kDa isoforms of SePP

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in plasma[31]. In addition, the genotype for these SNP also influence the response to supplementation of other selenoproteins such as lymphocyte GPx4 and GPx1, plasma GPx3 and thioredoxin reductase 1 and erythrocyte thioredoxin reductase 1[15,31]. Indeed the relationship between lymphocyte GPx4 level and SePP isoform ratio differ between individuals of different genotype for rs3877899[31]. These data illustrate how SNP in the *SEPP1* gene appear to affect Se availability for synthesis of other selenoproteins, possibly by modulating SePP capacity to transport and deliver Se.

Functional single-nucleotide polymorphisms in SELS *and other selenoprotein genes*

There is evidence that SNP in other selenoprotein genes affect the regulation of expression of the respective selenoprotein. First, Sep15 is a small protein recently implicated in cell responses to endoplasmic reticulum (ER) stress[18]. Two linked variants, a C/T substitution at position 811 (rs5845) and a G/A at position 1125 (rs5859), are present in the region of the *SEP15* gene that corresponds to the 3′-UTR[32]. Reporter gene experiments indicate that the SNP have functional consequences. Second, SelS is another selenoprotein recently found to have a function in the ER stress response[33–35]. An SNP in the *SELS* promoter at position −105 is regarded as being functionally significant as it modulates levels of inflammatory markers such as TNF-α and interleukin[33]. Third, SNP have been identified in the promoter region of the *GPX3* gene coding for the plasma glutathione peroxidase GPx3 and gene reporter experiments suggest that these affect promoter activity[36,37].

Nutrigenetics of selenium: disease association studies

On the basis of *in vitro* and *in vivo* experiments functional polymorphisms have been identified in the *GPX1*, *GPX3*, *GPX4*, *SEPP*, *SEP15* and *SELS* genes. Further information on their significance in health and disease processes is emerging from disease association studies in which allele frequencies are compared in disease and control populations.

Importance of assessing multiple genetic variants and environmental factors

Initially, disease association studies of selenoprotein SNP were carried out examining the frequency of a single SNP in relation to the disease of interest. However, selenoproteins are involved in a range of metabolic functions and such an approach does not take into account the interrelationships between the selenoprotein genes themselves or between selenoproteins and other factors from the same biochemical pathways. For example, since there is a competition/hierarchy for the use of Se in selenoprotein synthesis[17–18], an SNP in one selenoprotein gene affecting the synthesis of the corresponding protein may also alter expression of another selenoprotein in low Se supply conditions. Additionally, multiple variants in selenoproteins with related functions may cooperate to generate a different downstream response; for example, a variant in one glutathione peroxidase may modulate the effect of a genetic variant in another antioxidant protein such as a thioredoxin reductase. Furthermore, variants in non-selenoproteins may impact on the effect of an SNP in a gene coding for a selenoprotein with a related function; for example, SNP in the gene coding for the antioxidant protection enzyme manganese superoxide dismutase (*SOD2*) may impact on the biological effects of SNP in a glutathione peroxidase. Thus, the effects of a variant may be enhanced or attenuated by SNP in other genes and as a result of technological advances, it is now possible to assess the overall impact of genetic variations within multiple selenoprotein genes and associated biochemical pathways. It is also important to consider the effects of the selenoprotein variants in the context of environmental and dietary factors, particularly Se status and exposure to specific stresses.

Variants in GPX1 *and cancer risk*

A number of disease association studies have examined whether the Proline/Leucine variant in *GPX1* is associated with changes in disease risk but the majority of these were small and many have not been replicated. For example, carriage of at least one allele of the T allele (Leucine variant) has been linked to increased association with lung, breast and bladder cancer[38–40] but in the case of breast cancer this association has been replicated in only one of two subsequent studies. Importantly, these studies examined the interactions between the SNP in *GPX1* (rs1050450) and either a well-characterised functional SNP in *SOD2* (rs4880) or dietary and environmental factors; in the case of bladder cancer the effect of the SNP was apparent in combination with an SNP in *SOD2*[39] while in the case of lung cancer the SNP in *GPX1* had an impact on the association between alcohol intake and smoking with disease risk[40].

Influence of single-nucleotide polymorphisms in selenoprotein genes on colorectal cancer risk

A study of a small UK population showed the C variant of rs713041 in the *GPX4* gene to be associated with increased risk of colorectal cancer (CRC)[17]. However, this effect was not replicated in a Korean population[26] and in a study of a Czech population the T variant was found associated with disease risk[41]. Thus, at present the effect of this SNP on CRC risk is not clear; because the Se status in some of these cohorts was not known it may be that the influence of the SNP is determined by other parameters such as additional genetic factors, lifestyle factors and/or Se status.

Recently, we have used a hypothesis-driven approach to examine the influence of multiple variants in selenoprotein genes and rs4880 in *SOD2*, both individually and in combination, on risk of colorectal and prostate cancers. CRC risk was studied in two cohorts, one Korean and one Czech. Various associations between individual variants and risk were observed[26,41] and, although some associations were not replicated in the two studies, critically an

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association of rs34713741 in *SELS* was observed in the two distinct populations. The replication of the association in the two cohorts suggests that SelS plays a crucial role in colorectal function. The importance of SelS in gut function is supported by this SNP lying in close proximity to another variant that is known to be functional[33] and has been linked to gastric cancer risk[42]. Additionally, in the Czech population significant genetic interactions were observed between rs4880 (*SOD2*), rs713041 (*GPX4*) and rs960531 (*TXNRD2*), and between *SEPP1* and either *SEP15* or *GPX4*[41] suggesting that function of multiple selenoproteins in the colon affects CRC risk.

Influence of single-nucleotide polymorphisms in selenoprotein genes on prostate cancer risk

Recently studies examining genotype effects in relation to prostate cancer risk have suggested that SNP in *SEPP1* (rs7579 and rs3877899) together with other factors may influence susceptibility to prostate cancer[43,44]. One study indicated that rs7579 in *SEPP1* affected prostate cancer risk while the second suggested that risk was modulated by low Se status together with genotype for rs4880 in the *SOD2* gene and for rs3877899 in the *SEPP1*. A further study has suggested that both prostate cancer risk and survival are modified by a combination of genetic variation in the *SEP15* gene and low Se status[45].

Nutritional transcriptomics of selenium

Se availability alters the level and activity of selenoproteins and in some cases also alters mRNA levels for selenoprotein genes[18,46–47]. However, all the selenoproteins are not affected to the same extent and there is a tissue-specific selenoprotein hierarchy in which synthesis of some proteins is more sensitive to Se supply than others[46]. As a result, the pattern of selenoprotein expression changes with Se intake and therefore to assess the impact of altered Se intake it is important to measure expression of as wide a range of selenoproteins as possible. In addition, changes in selenoprotein activity lead to changes in non-selenoprotein biochemical parameters, and so assessment of the physiological responses to Se also require measurement of these downstream targets (see Fig. 1). High-throughput genomic techniques such as gene microarrays provide a new approach to identify novel targets of Se and to integrate them into key pathways and networks. Using such transcriptomics methods, the pattern of gene expression changes has been examined in response to altered Se supply in the mouse, in response to Se supplementation in human subjects and in response to altered Se supply in cell culture[48–53].

In order to assess the effects of a marginal Se deficiency on the colonic epithelium, a transcriptomics analysis was carried out on RNA isolated from the colon of mice fed either a Se-adequate or marginally Se-deficient diet for 6 weeks[48]. This marginal deficiency resulted in a lower expression of GPx1, SelH, W and M, indicating that in the colon expression of these selenoproteins at the mRNA level was particularly sensitive to dietary Se depletion. This sensitivity of GPx1 and SelW to Se depletion is compatible with earlier observations and the view that these two selenoproteins are low in the selenoprotein hierarchy[49]. The observed effects on expression of SelH and SelM suggest that these selenoproteins are also low in the hierarchy in the colon and that together with SelW they may also be sensitive biomarkers of Se function in the colon. More detailed bioinformatic analysis using a pathway approach showed that protein translation was top of the pathway list modified by Se intake and that related pathways (regulation of eiF4e and p70S6 kinase, ribosomal proteins) were also significantly affected by Se deficiency. In addition, the mammalian target of rapamycin signalling pathway, TNF-α–NF-κB pathway and proteasome degradation were also significantly affected. More detailed analysis and Real-Time PCR analysis showed that Wnt and Nrf2 pathways were also altered in expression by dietary Se intake[50]. Our current ongoing work is using functional genomic techniques such as siRNA and reporter genes driven by appropriate response elements to further investigate the responses in these pathways and the links to selenoprotein function.

We have also used a similar transcriptomics approach to evaluate the response in global gene expression profile of human lymphocytes to Se supplementation in individuals who took part in the SelGen study[51]. A gene microarray analysis showed that protein biosynthetic pathways were the most sensitive to supplementation with sodium selenite (100 μg/d) for 6 weeks[51]. Expression of Sep15 and SelK were significantly affected by the supplementation suggesting that these selenoproteins, not SelH, M and W, are most sensitive to Se supply in lymphocytes. Despite the caveat that there are likely to be species differences, comparison of the lymphocyte and colon data suggests that, the selenoproteins sensitive to Se supply differ between colon and lymphocytes. Interestingly, in contrast, the two studies revealed that protein biosynthetic pathways are sensitive to changes in Se supply in both the mouse colon and human lymphocyte, suggesting that changes in these pathways represent a response to Se that is common to several tissues. However, the mechanism by which Se influences the protein biosynthetic pathway remains unknown.

Transcriptomics analysis of breast and prostate cell lines subjected to different Se concentrations has been limited to the use of targeted arrays after treatment with high concentrations of selenite, selenomethionine or methyseleninic acid. These studies indicate alterations in the expression of apoptosis and cell cycle genes[52–53]. These changes may reflect the induction of apoptosis by high Se concentrations or different responses in cell lines derived from these particular tissues.

Selenoproteins in the colon: lessons from nutrigenetics and nutrigenomics

There is considerable interest in the metabolic role of Se and selenoproteins in the colon because of clinical data suggesting that individuals with low Se status have increased risk of CRC, whereas people with higher Se status have

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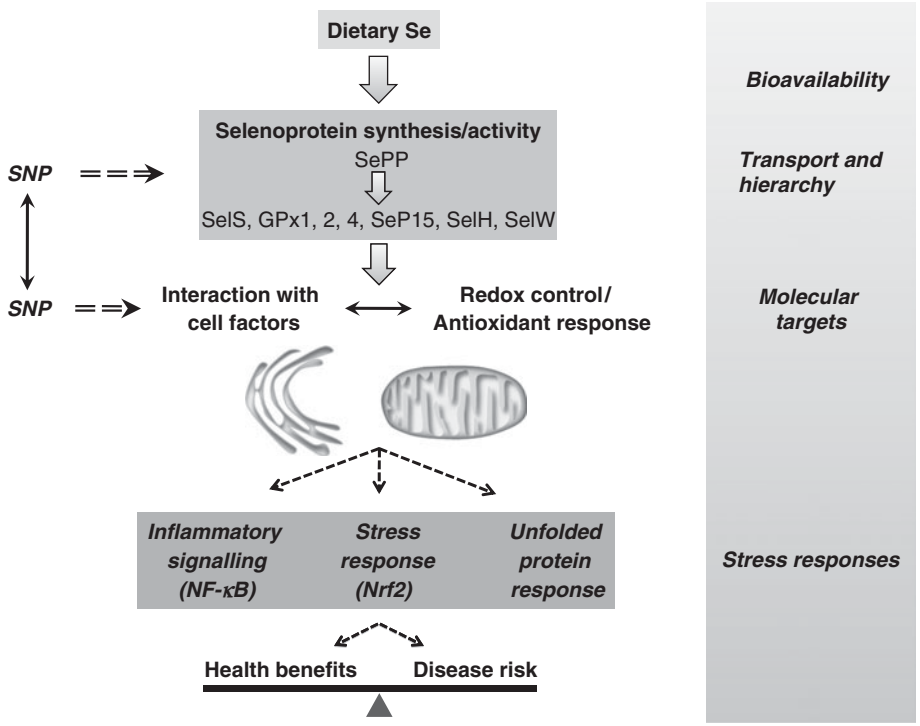

Fig. 2. Hypothetical roles of Se and selenoproteins in colonic cell function. The scheme incorporates findings from recent transcriptomics and gene association studies. Trancriptomics studies highlight GPx1, SelH and SelW as being sensitive to Se supply and single-nucleotide polymorphism (SNP) studies suggest SePP (selenoprotein P), SelS, GPx4 and Sep15 are key selenoproteins for colonic function. In addition, transcriptomics show selenoprotein activity affects stress responses and inflammatory signalling in the colon. We propose that a combination of genetic factors and Se supply regulate colonic cell function (and thus health) through selenoprotein activity and these stress response pathways.

lower risk of a recurrence of colonic tumours[54–55]. Although in one major trial in the USA Se supplementation was found to lower mortality from CRC[8], overall the epidemiological data are inconclusive with regards to Se intake and susceptibility to CRC. Additionally, Irons *et al.*[56] used transgenic mice with reduced selenoprotein synthesis due to carriage of a mutant selenocysteine transfer RNA gene, to study the effect of Se supplementation and selenoprotein function on azoxymethane-induced aberrant crypt formation. Lack of selenoprotein activity was shown to increase aberrant crypt formation and therefore risk of colonic tumours.

Understanding the role of selenoproteins in the colon may contribute considerably to clarifying the relationship between Se intake and CRC risk. As outlined in the previous sections recent genotyping studies and transcriptomics experiments are proving to be useful in highlighting selenoproteins in the colon that are either possibly important in cell function or sensitive to Se supply. Two genotyping studies in distinct populations have indicated an association between an SNP in *SELS* with CRC risk[26,41]. This replication of the association suggests that SelS has an important role in colonic function and maintaining gut health. Gene microarray studies indicate that expression of SelH, M and W expression in the colon is sensitive to Se intake while pathway analysis of these transcriptomics data shows that Se supply modulates protein synthesis, protein folding, Wnt, Nrf2 and inflammatory pathways[48,50]. These data suggest that together genetic make-up and changes in Se intake will alter a range of selenoprotein activities and associated pathways in the colon. At present it is not clear if, and how, all the effects are linked but, as illustrated in Fig. 2, our hypothesis is that Se intake and genetic factors modulate these pathways at different points in a network of linked effects and pathways. In this regard, there is evidence for links between several of these pathways: ER stress and oxidative stress[57]; inflammation[58] and apoptotic pathways[59]; Nrf2, oxidative stress and inflammation[60]. Interestingly, SelS may provide one link between such pathways because its expression is induced by ER stress[61] and regulated by NF-κB[62], ER stress and inflammatory pathways[63]. Interestingly, these pathways have been found to be dysregulated during colorectal carcinogenesis, suggesting that the capacity of Se to affect their level of activation could play a role in CRC prevention.

Understanding the role of these selenoproteins and pathways should contribute to our knowledge of the links between oxidative stress, inflammation and carcinogenesis. Future work should take a systems biology approach to address how Se intake and SNP interact to modulate the overall pattern of selenoprotein expression and associated downstream pathways such as protein synthesis, folding response, Nrf2 and inflammatory pathways.

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Conclusions

Genomics studies

Although genome-wide association studies have not identified any selenoprotein genes as cancer susceptibility loci, several relatively small association studies have now linked functional SNP in several selenoprotein genes to disease risk. These associations also provide new perspectives for mechanistic studies to uncover the role played by the corresponding selenoproteins in healthy tissues. However, larger studies combining genotyping with assessment of Se status and appropriate environmental and lifestyle factors need to be carried out and replicated in larger populations before strong arguments about these SNP and disease risk can be made.

As shown by the example of folate, it is important to consider effects of genetic variants from the viewpoint of the overall metabolic pathway[12]. In the case of Se, it can be predicted that SNP in a range of selenoprotein genes and genes encoding components of the Se incorporation machinery (e.g. SBP2, ribosomal protein L30) or Se transport may affect selenoprotein expression and associated disease risk. Therefore, in future work, genotyping for multiple variants in the whole selenoprotein metabolic pathway and their additive and interactive effects will have to be addressed to get an overall understanding of the influence of genetic factors on Se metabolism. Indeed, several studies examining links between SNP in selenoprotein genes and disease risk suggest that the disease risk is modulated by combinations of SNP. These genetic interactions could reflect the inter-dependence of selenoprotein synthesis due to the selenoprotein hierarchy in which there is a competition for available Se[18,64] and/or the complementary roles of several selenoproteins belonging to the same metabolic pathway, for example, in antioxidant protection or redox control[41] and as illustrated in Fig. 2. The replication of an association between an SNP in *SELS* with CRC risk suggests that further studies should investigate the impact of interactions between this SNP and other genetic variants both in *SELS* and other genes in the selenoprotein 'pathway'.

Transcriptomics

Transcriptomics studies illustrate the complex nature of the response to altered Se supply with a large number of pathways being affected. They have also shown that gene arrays provide a sufficiently sensitive approach to detect novel biochemical responses *in vivo* in human subjects after a relatively subtle change in Se status. Thus, they may be useful in developing novel functional biomarkers of micronutrient status. With regard to Se, data from recent genetic and microarray studies highlight the selenoproteins SelS, SelH, SelM and SelW as being either important in colonic cell function or sensitive to Se supply and as a result potential biomarkers of Se function. In addition, protein synthesis, protein folding, Wnt, Nrf2 and inflammatory pathways are sensitive to Se supply and interestingly Se supply affects protein synthesis/translation pathways in both the mouse colon and human lymphocyte suggesting that expression markers of this pathway in the lymphocyte may be a surrogate marker for Se status in the colon.

Future perspectives

The use of genomic technology has revealed that understanding the effects of micronutrients on genes and corresponding proteins, as well as the influence of genetic factors on responses to diet, requires a systems biology approach integrating micronutrient status, genetic variations and genetic interactions within metabolic pathways involving this micronutrient, downstream molecular targets and environmental stresses. Such an approach has the potential to identify novel markers of dietary exposure to micronutrients, to investigate the temporal relationship between micronutrient intake and environmental stresses in disease development and to identify sub-group populations at particular risk. This integrated overview will be facilitated by accumulation of appropriate genotyping and genomic data. The large datasets involved may require international cooperation as envisaged within the Micronutrient Genomics Project[65].

Functional SNP in genes that affect micronutrient metabolism can affect responses to increased intake. As a result, the genetic make-up of participants in supplementation trails may influence the outcome of such trials. Therefore genetics may be a factor, along with differences in environmental stress, lifestyle behaviour and Se intake, that contributes to differences in study populations that could explain why micronutrient supplementation trials, including those with Se, have been disappointing or contradictory (see, for example[4–9]). The identification of genetic differences in micronutrient metabolism may useful in improving the design of future supplementation trials.

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