Selenoproteins and human health: insights from epidemiological data

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ABSTRACT

Knowledge of the plasma selenium levels associated with optimised concentration or activity of specific selenoproteins can provide considerable insights from epidemiological data on the possible involvement of those selenoproteins in health, most notably with respect to cancer. For cohort studies, if selenoproteins such as glutathione peroxidase and selenoprotein P are relevant to cancer, one might only expect to see an effect on risk when the concentrations in the cohort range from below, to above, the level needed to optimise the activity of these enzymes. Similarly, trials would only show a beneficial effect of supplementation if selenium status were raised from below, to above, the optimal concentration for the selenoproteins likely to be implicated in cancer risk, as occurred in the NPC trial but not in SELECT. The most powerful evidence for the involvement of selenoproteins in human health comes from epidemiological studies that have related single nucleotide polymorphisms in selenoproteins to disease risk. The totality of the evidence currently implicates GPx1, GPx4, SEPS1, Sep15, SEPP1 and TXNRD1 in conditions such as cardiovascular disease, pre-eclampsia and cancer. Future studies therefore need to determine not only selenium status, but genotype, both in selenoproteins and related pathways, when investigating the relationship of selenium with disease risk.

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INTRODUCTION

Only a small proportion of studies that can properly be described as epidemiological give any information about the role of selenoproteins in human health. The biggest group consists of those studies that have identified an effect of a single nucleotide polymorphism (SNP) in a selenoprotein and related it to disease risk. The number of such studies is increasing and, funding permitting, in the future we shall be able to run substantial interventions in subjects recruited by selenoprotein genotype. Though the link is less specific, knowledge of the plasma levels associated with optimised concentration or activity of specific selenoproteins can provide considerable insights from published epidemiological data on possible involvement of those selenoproteins in health, most notably with respect to cancer. From studies currently being conducted by Burk's group in China, we should soon know what intake and plasma level of selenium (Se) are associated with maximal selenoprotein P concentration (Burk, R.F., personal communication). This will help tremendously in assessing the importance of selenoprotein P, the carrier of Se to the tissues, to human health.

Addressing specific health conditions in turn, I will present the evidence for involvement of the selenoproteins from the various strands outlined above.

CARDIOVASCULAR DISEASE

Glutathione peroxidase (GPx)

Previous epidemiological studies of Se and cardiovascular disease that did not include a substantial number of subjects of low or fairly low Se status were unable to find an effect on cardiovascular disease risk [1]. Such results point to a potential involvement of a selenoenzyme that is optimized at relatively low Se status, the most obvious candidate being the cytosolic selenoprotein, GPx1 [2,3,4].

It was therefore revealing to find a later study in a population of relatively low Se status where the risk of having a cardiovascular event was examined according to baseline GPx1 activity. Blankenberg and colleagues conducted a prospective study in a German population of 636 patients with suspected coronary artery disease, following them up for a median period of 4.7 years [4]. Mean plasma selenium was 69.5 and 74.5 μg/L at baseline in patients with and without an event, respectively. Baseline erythrocyte GPx1 activity was a strong predictor of the risk of a subsequent cardiovascular event, those in the highest, compared to the lowest quartile of GPx1 activity having a hazard ratio (HR) of 0.29 [95% confidence interval (CI) 0.15 to 0.58; P<0.001]. The inverse association between GPx1 activity and cardiovascular events remained substantially unchanged after adjustment for potential confounders showing that the relationship was independent of other cardiovascular risk factors. Unsurprisingly perhaps, higher GPx1 activity was even more protective in smokers who are subject to greater oxidative stress than non-smokers. Though it was erythrocyte GPx that was measured, the authors comment that erythrocyte GPx1 is probably a surrogate for cellular GPx1 activity in general. Such an effect was able to be seen in this population because Se status was too low for GPx1 activity to be optimized in all subjects [5].

Selenoprotein S (SEPS1)

Inflammation in the arterial wall is recognized to be an important component of the development of acute coronary syndromes [6]. The selenoprotein SEPS1 has a role in mediating inflammation through its protection of the endoplasmic reticulum (ER). SEPS1 is an ER membrane protein that participates in the processing and removal of misfolded proteins from the ER to the cytosol [7]. When the ER is functionally impaired by the build-

up of such misfolded proteins, the expression of a number of genes is induced leading to activation of the transcription factor NF-κB. Activated NF-κB then translocates to the nucleus where it activates the transcription of genes including those that encode the pro-inflammatory cytokines [7] and indeed SEPS1 itself [8]. Increased expression of SEPS1 in turn suppresses cytokine production by its ability to remove misfolded proteins from the ER. This system constitutes a SEPS1-dependent regulatory loop in the presence of inflammation [8]. Variation in the *SEPS1* gene is known to affect circulating levels of the inflammatory cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) [7].

Given the known association of SEPS1 with inflammation, the effect of genetic polymorphisms in *SEPS1* on the risk of cardiovascular disease was investigated in two independent prospective Finnish cohorts [9]. A significant association was found with increased coronary heart disease risk in females carrying the minor allele of rs8025174 in the combined analysis of both cohorts (HR 2.95; 95% CI 1.37 to 6.39). Another variant, rs7178239, increased the risk for ischemic stroke significantly in females (HR 3.35; 95% CI 1.66–6.76) and in the joint analysis of both sexes in both cohorts (HR 1.75; 95% CI 1.17 to 2.64). Suggestive associations of both variants were also seen with the known cardiovascular risk factors of BMI and waist-hip ratio [9]. These results implicate the selenoprotein SEPS1 in cardiovascular disease risk, particularly in females.

PRE-ECLAMPSIA

Selenoprotein S (SEPS1)

The pregnancy condition, pre-eclampsia, bears many similarities to cardiovascular disease and is associated with a considerably higher risk of developing vascular and metabolic

disease later in life [10]. The well-recognised endothelial dysfunction of pre-eclampsia is now believed to be part of a more generalised intravascular inflammatory reaction resulting from an excessive maternal inflammatory response to pregnancy [11]. High circulating levels of pro-inflammatory cytokines and other markers of inflammation including C-reactive protein have been reported in women with pre-eclampsia [12,13].

As with cardiovascular disease, the anti-inflammatory selenoprotein, SEPS1, is also implicated in the risk of pre-eclampsia [12]. Apart from the obvious link to inflammation, ER stress is also present in pre-eclampsia as a result of the ischaemia-reperfusion injury, a known cause of ER stress, that occurs in the pre-eclamptic placenta [12].

In a large case-control association study in a Norwegian population, Moses and colleagues [12] showed an association between a functional promoter polymorphism associated with impaired expression of *SEPS1* and pre-eclampsia. Maternal genotype and allele frequencies of the *SEPS1* g.-105G>A polymorphism were compared in 1139 pre-eclamptic and 2269 control women. Women with preeclampsia were 1.34 times more likely to have the GA or AA genotype [P = 0.0039; odds ratio (OR), 1.34, 95% CI 1.09 to 1.64] and 1.22 times more likely to carry the A allele (P = 0.023; OR 1.22, 95% CI 1.02 to 1.46). Importantly, the A allele of the *SEPS1* g.-105G>A polymorphism is associated with impaired expression of *SEPS1* and may therefore be contributing to pre-eclampsia risk.

This polymorphism is not currently in the SNP database but it is distinct from those (rs8025174 and rs7178239) mentioned above that were shown to have a relationship with cardiovascular disease.

CANCER

There has been fairly general acceptance that a Se metabolite, methyl selenol, is a proximal anti-carcinogen at supra-nutritional doses [14]. However the presence of methyl selenol is only inferred from reactions of its precursors, most notably the model compound, methylseleninic acid [15]. Furthermore for this mechanism to be effective, Se doses large enough to support the production of high, steady-state concentrations of methyl selenol are likely to be required. Despite the fact that such concentrations of methyl selenol should have been attained in the Selenium and Vitamin E Cancer Prevention Trial (SELECT) where men of good Se status (mean baseline serum Se 136 μg/L) were given a further 200 μg Se/d, there was notably no effect of Se supplementation on prostate-cancer risk [16].

Though selenoproteins (selenoenzymes) were known to reduce oxidative stress and inflammation and limit DNA damage, all of which have been implicated in cancer risk, they were at one time not thought to be important to the anti-cancer effects of Se. This was largely because their activity/concentration was already believed to be optimized in the US population that showed reduced cancer risk on supplementation with 200 μg Se/d in the Nutritional Prevention of Cancer (NPC) Trial [17]. However, we now believe that optimal expression of selenoprotein P, the carrier of Se in the plasma, would almost certainly not have been achieved in all study subjects [18], particularly not in those in the bottom tertile of plasma Se (≤ 106 μg/L) in whom Se supplementation had the greatest benefit [19]. We are now also aware that people differ substantially in their ability to increase selenoprotein expression and activity in response to additional dietary Se so that some individuals may have a higher than average requirement for Se for optimal protection against cancer [20–24].

Though the use of animal models including transgenes for unique tRNA species has helped to clarify the relative contributions of Se metabolites and individual selenoproteins to cancer risk (see Dr. Diamond's review in this special issue), strong, specific evidence of the importance of selenoproteins/selenoenzymes to cancer risk/mortality is provided by

epidemiological data from cohorts identified by functional polymorphisms in selenoprotein genes, particularly at nutritional levels of intake. Such epidemiological studies are described below and summarised in **Tables 1 and 2** [25-39].

Cytosolic glutathione peroxidase, GPx1

Glutathione peroxidases protect cells against oxidative damage by reducing hydrogen peroxide and a wide range of organic peroxides with reduced glutathione [40]. A number of recent studies have reported a link between cancer risk and polymorphisms in the *GPx1* gene at Pro198Leu (rs1050450) (see Table 1) [22,24,26,28,31,33,34]. Such a link might be explained by a genotype effect on enzyme activity [22,28]. For example, the *GPX1* Leu allele has been found to be less responsive than the Pro allele to stimulation of GPx1 enzyme activity by selenium supplementation [22].

Bladder: Possession of the *GPx1* Leu198 allele appears to confer an increased risk of bladder cancer [26]. In 213 Japanese patients, the Pro/Leu genotype was significantly associated with bladder cancer when compared with the Pro/Pro genotype (adjusted OR 2.63, 95% CI 1.45 to 4.75, p = 0.001). The effect of genotype on risk was similar for advanced prostate cancer (tumour stage Ta-1 vs T2-4, OR 2.58, 95% CI 1.07 to 6.18, P = 0.034).

An interaction exists between this gene and that for manganese superoxide dismutase (SOD2), the scavenger within the mitochondria that converts superoxide to hydrogen peroxide. A single nucleotide polymorphism Val9Ala (rs4880) (more often, and hereinafter described as Val16Ala), in the *SOD2* gene affects the secondary structure of the mitochondrial import sequence of the superoxide dismutase protein such that the Ala16 variant is imported more efficiently into the mitochondrial matrix, resulting in higher enzyme activity [41]. Individuals with at least one *SOD2*-Ala16 allele (Ala16+) therefore generate

more active superoxide dismutase, and therefore more hydrogen peroxide, than those homozygous for the Val16 variant. Hydrogen peroxide has been shown to promote the neoplastic transformation of urothelial cells [42], and to induce the matrix metalloproteinases required for tumour invasion [43,44]. As there is no catalase in mitochondria [45], the hydrogen peroxide that does not diffuse out must be removed by GPx1, with an efficiency that presumably varies by genotype, providing a rationale for the interaction between the two genes. While there was no effect on bladder-cancer risk of the *SOD2* polymorphism by itself, in *SOD2*-Ala16+ men, the risk associated with the Pro/Leu *GPx1* genotype increased by a factor of 2.4 (OR 6.31, 95% CI 1.28 to 31.24, P = 0.024).

Breast: The Leu/Leu genotype of the *GPx1* Pro198Leu polymorphism was found to be almost twice as common in DNA from breast-cancer tissue as in DNA from cancer-free individuals while the Pro/Leu genotype was underrepresented, suggesting an involvement of GPx1 in breast cancer risk [22]. However, results from five epidemiological studies that investigated the effect of this polymorphism on breast-cancer risk have been inconsistent as outlined below.

In a nested Danish case-control study of 377 cases and 377 controls, carriers of the variant Leu-allele had a 1.43-times (95% CI 1.07 to 1.92) higher risk of breast cancer compared with non-carriers [28]. By contrast, in a Canadian case-control study of 399 cases of incident, invasive breast cancer and 372 controls, no association between breast cancer and the *GPx1* Pro198Leu polymorphism was found [27]. Similarly, there was no evidence that the variant *GPx1* genotype was associated with an increased risk of breast cancer in the Long Island Breast Cancer Study Project of 1,038 cases and 1,088 controls, except in nulliparous Leu homozygotes who had increased risk (OR 2.12, 95% CI 1.01-4.48) compared with parous Pro/Pro women [29]. Interestingly, though no association was observed between the

polymorphism and breast-cancer risk in the prospective Nurses' Health Study, where 1323 women with breast cancer were compared with 1910 controls [30], an increased risk of breast cancer (OR 1.87, 95% CI 1.09-3.19) *was* observed in Leu homozygotes who were *also* homozygous for the Ala16 genotype of *SOD2* [31], a SNP allele already shown to raise the risk of bladder cancer [26].

Lung: Four studies have looked at the association between the Pro198Leu polymorphism and the risk of lung cancer [24,32,33,46]. Compared to Pro homozygotes, two studies – in Finland and Korea – found a significantly increased risk of lung cancer in Leu hetero-/homozygotes [24,32], while a small US study found a significantly-increased risk in never-smoker Leu hetero-/homozygotes (only 13 cases), though a significantly-decreased risk was found in elderly smokers [46]. However, in the fourth study, carried out in Denmark, Leu/Leu homozygosity was associated with decreased risk [33]. The authors of the Danish study were themselves surprised by their result since they had previously shown that the variant Leu-allele was associated with a significant, although moderate, 5% lower erythrocyte GPx1 enzyme activity per allele [28]. They have suggested that the apparently-protective effect of the Leu-allele of the *GPx1* polymorphism may be caused by a co-segregating functional polymorphism in another gene in the same region of the genome and not by the *GPx1* polymorphism *per se* [33] though this explanation does not sit well with the fact that the Leu allele appears to increase risk in other studies and in other types of cancer.

Prostate: In the context of the above results, it is perhaps surprising that an overall protective effect of the variant *GPx1* Leu allele was found on prostate cancer risk in 82 prostate cancer cases and 123 control individuals in FYROM [34]. It is however somewhat suspicious that while heterozygous carriers of the variant Leu allele had a significantly lower risk of prostate

cancer compared with Pro homozygotes (OR 0.38, 95% CI 0.20 to 0.75, P = 0.004), Leu homozygotes had a non-significant and lesser reduction in risk. No significant differences in erythrocyte GPx activity by genotype were found in the healthy control group of 90 subjects. In our own case-control study (2213 cases/controls) in Swedish men, we found no effect of the Pro198Leu polymorphism on prostate-cancer risk [Cooper ML, Adami HO, Grönberg H, Wiklund F, Green FR, Rayman MP, unpublished data].

***Other cancers*:** Loss of heterozygosity at *GPx1* occurs in a significant percentage of colorectal (42%) and other cancers suggesting that *GPx1* or other tightly linked genes might be involved in cancer etiology [47,48]. However in a study of 166 cases with adenocarcinoma, 974 with adenomas and 397 controls recruited from the Norwegian cohort NORCCAP, no associations were found between the *GPx* Pro198Leu polymorphism and risk of colorectal adenomas or carcinomas [49]. Similarly no associations were found between the *GPx* Pro198Leu polymorphism and risk of basal-cell carcinoma [50].

Phospholipid glutathione peroxidase, GPx4

GPx4 removes membrane-bound phospholipid hydroperoxides inhibiting lipoxygenases and affecting leukotriene biosynthesis, cell growth, apoptosis and inflammation [51,52].

Breast cancer: Though no effect of the *GPx4* C718T polymorphism was found on the risk of developing breast cancer, carriage of the T allele was found to be associated with mortality in a UK study of ten antioxidant defence genes in 4470 breast cancer cases (see Table 2) [35]. The most significant results were for two correlated SNPs in *GPx4*. The rare (T) allele of the *GPx4* C718T was associated with an increased risk of death, with a hazard ratio of 1.27 (95%

CI, 1.13 to 1.43) per rare allele carried. The frequency of the T allele is 0.46 in the UK population with 21% of women being T homozygotes. These data provide strong support for the hypothesis that a common variant of *GPx4* is associated with poor prognosis after a diagnosis of breast cancer.

Colorectal cancer: A *GPx4* C718T polymorphism (rs713041) which is known to be functional [52,53], has been shown to be linked to colorectal cancer risk in a pilot study (see Table 2) [36]. Carriage of the T allele appears to be protective in contrast to its effect on breast-cancer mortality.

15kDa selenoprotein, Sep15

The 15kDa selenoprotein (Sep15) is located in the endoplasmic reticulum (ER). It is believed to be a thioldisulfide isomerase involved in disulfide bond formation in the ER that contributes to the quality control of protein folding [54]. Evidence suggests that Sep 15 is required for apoptosis and that its loss is associated with malignancy [54].

Sep15 is expressed at high levels in normal liver and prostate but at reduced levels in the corresponding malignant organs [55]. The *Sep15* gene lies on chromosome 1p22.3 at a locus commonly deleted or mutated in human cancers [23,56] giving rise to expectations that this selenoprotein might be important to cancer risk. Two SNPs at positions 811 (C/T) and 1125 (G/A) that are in strong allelic association have been studied in the 3′-UTR of the *Sep15* gene: G/A1125 lies within a functional SECIS element [23]. The T811-A1125 variant was more effective in supporting UGA read-through than the C811-G1125 variant, but was less responsive to the addition of Se to the culture medium [21,57]. Similarly, malignant mesothelioma cells with the A1125 variant of *Sep15* were found to be less responsive to the

apoptotic and growth-inhibitory effects of Se than the G1125 variant [58]. Individuals possessing one or other of these haplotypes may therefore differ in the efficiency with which they can make Sep15 and in how well they can use dietary Se [23].

Lung cancer: A case-control study of 325 lung cancer cases and 287 controls in a population of Polish smokers of low Se status (mean plasma Se in controls 53.3 ± 14.0 μg/L), showed an effect of *Sep15* G/A1125 genotype that varied according to Se status (see Table 2 and **Figure 1**) [37]. Among individuals of lower Se status (below 50 μg/L), the risk was higher for those with the AA genotype compared to those with the GG genotype (OR 1.42, 95% CI: 0.43–4.42) whereas among those of higher Se status (above 50 μg/L), the opposite was the case (AA *vs* GG, OR 0.37, 95% CI: 0.12–1.17). Though these effects on risk are non-significant, there was a general significant association between Se concentration and lung cancer risk for the GG, GA and AA genotypes, P values for the trends in risk being 0.038, 0.035 and 0.030, respectively. The results of this study suggest that those with the AA genotype benefit most from increasing dietary Se (which does not accord with the results of the *in vitro* studies mentioned above). For those with the GA or GG genotype – 93.7% of the control population – when plasma Se rises above 70 μg/L, the odds of developing lung cancer begin to increase. This observation accords with the meta-analysis of Se and lung cancer that showed that only subjects in the lower categories of Se exposure benefit from increased Se intake [59]. This Polish study is a nice illustration of the potential complexity of interactions between genotype and Se status and confirms a role for this selenoprotein in cancer risk.

Prostate cancer: Though the frequency of the T811/A1125 haplotype is 0.25 in Caucasians and 0.57 in African Americans, who have a higher incidence of prostate cancer [21], no evidence for an effect of this polymorphism on prostate cancer risk has yet been reported. In our own case-control study (2213 cases/controls) in Swedish men of relatively low Se status,

we found no effect of the 811 (C/T) linked 1125 (G/A) polymorphism on prostate-cancer risk (see Table 2). However, in men with PSA > 100 ng/ml, the risk of prostate cancer was significantly greater in T811-A1125 homo/heterozygotes than in C811-G1125 homozygotes [Cooper ML, Adami HO, Grönberg H, Wiklund F, Green FR, Rayman MP, unpublished data].

Breast, head and neck tumours: Among African Americans (but not Caucasians), a difference in *Sep15* allele frequencies was seen in DNA from breast or head and neck tumours and that from cancer-free controls though the authors suggest that this difference is likely to be due largely to loss of heterozygosity at the *Sep15* locus [21,60].

Thioredoxin reductase 1

All living organisms have an important antioxidant defense system consisting of the selenoenzyme, thioredoxin reductase, thioredoxin and NADPH [61]. Thioredoxin reductase 1 (TXNRD1) is involved in the regulation of transcription factors, growth control, protein-DNA interaction, and in the reduction of nucleotides for DNA synthesis [62]. It is highly expressed in a number of tumour tissues [38,62].

Colorectal adenoma: TXNRD1 is abundantly expressed in the colon. In a case-control study nested within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, cases with a left-sided advanced adenoma (n = 772) and matched controls (n = 777), screen-negative for polyps, were randomly selected from participants [38]. A significant 80% reduction in advanced colorectal adenoma risk was seen for carriers of the G allele of thioredoxin reductase 1 (*TXNRD1*) IVS1-181C>G (OR 0.20, 95% CI 0.07-0.55, P trend = 0.004) (see

Table 2). A significant overall association with adenoma risk for TXNRD1 (global P 0.008) was observed. However, the authors point out that it is possible that this SNP may not indicate an association with *TXNRD1* but instead with E1A-like inhibitor of differentiation 3 (*EID3*), a small gene nested within the intron of *TXNRD1* [38,62].

Selenoprotein P

Selenoprotein P is a major selenoprotein in plasma, containing at least 40% of the total plasma Se [63]. Unlike any other selenoprotein, human selenoprotein P contains ten selenocysteine residues and it is believed to be responsible for the distribution of Se to body tissues [54]. It is also thought to be a scavenger of peroxynitrite [64].

Colorectal adenoma*:* While the human selenoprotein P gene (*SEPP1*) is abundantly expressed in normal colon mucosa, there is a significant reduction or loss of SEPP1 mRNA expression in colon cancers [65]. Since a number of SNPs have been identified in selenoprotein P, it was thought that some of those genetic variants might be associated with advanced colorectal adenoma, a colorectal-cancer precursor. In the study described in the previous section, cases with a left-sided advanced adenoma and matched controls were randomly selected from participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial [38]. Three variants in *SEPP1* (rs3797310, rs2972994, -4166), one of which was very rare (-4166), were significantly associated with advanced adenoma risk and a significant overall association with adenoma risk was observed for *SEPP1* (global P = 0.02) (see Table 2). *SEPP1* SNPs rs3877899, rs6413428 and rs12055266 were not found to have an association with advanced colorectal adenoma.

Prostate: Normally, the *SEPP1* gene is highly expressed in prostatic epithelium but it is down-regulated in a subset of human prostate tumours, mouse tumours and the androgen-dependent (LNCaP) and androgen-independent (PC-3) prostate cancer cell lines [66]. Our group investigated the effect on prostate cancer risk of one of the *SEPP1* SNPs already mentioned i.e. Ala234Thr (rs3877899), by genotyping DNA from 2,915 cases and 1,764 age-matched controls from the population-based Prostate Cancer in Sweden (CAPS) study [39]. This population had fairly low Se status i.e. mean (SD) = 76.0 (17.2) μg/L in plasma. Cases were designated aggressive or non-aggressive at diagnosis by clinical criteria.

Although the *SEPP1* Ala234 SNP allele is known to be associated with lower plasma selenoprotein P in men and this appears to reduce the concentration/activity of other antioxidant selenoproteins [67], by itself, this SNP did not affect prostate-cancer risk in our study (see Table 2). However, men homozygous for *SEPP1*-Ala234 who also carried a *SOD2*-Ala16 allele (already discussed above) had a significantly higher risk of prostate cancer (OR 1.43, 95% CI 1.17 to 1.76) and advanced prostate cancer (OR 1.60, 95% CI 1.22 to 2.09) than those who were *SOD2*-Val16 homozygotes (see Table 2) (interaction, prostate cancer P=0.05, advanced prostate cancer P=0.01). The interaction was even stronger in ever-smokers i.e. men homozygous for *SEPP1*-Ala234 who also carried a *SOD2*-Ala16 allele had almost double the risk of prostate cancer (OR 1.97, 95%CI 1.33 to 2.91; interaction P=0.001). The explanation for these results may relate to the fact that carriers of *SOD2*-Ala16 are known to produce more mitochondrial hydrogen peroxide as explained above [41] that if not removed, will promote prostate tumor-cell proliferation and migration [43,44,68,69]. In a low Se environment, male *SEPP1*-Ala234 homozygotes have lower plasma SEPP1 [67], thus limiting the transport of Se to the prostate for the production of GPx that could remove hydrogen peroxide [39]. As we have postulated that the mechanism by which these polymorphisms have their combined effect is oxidative-stress related, the greater strength of

the interaction in smokers than in the study as a whole, despite smaller numbers, might be explained by an exacerbation of oxidative stress in ever-smokers.

It is important to note that the joint effect of these polymorphisms was stronger for advanced prostate cancer than for all prostate cancer in line with other epidemiological studies where the effect of Se on localised and advanced prostate cancer was analysed separately [70-72]. As it is advanced prostate cancer that kills, this is an important finding.

Summary of the evidence on involvement of selenoproteins in cancer risk from selenoprotein polymorphisms

It is difficult to draw clear conclusions from some of the SNP evidence, as summarised in Tables 1 and 2, on the effect of selenoproteins on cancer risk. With regard to the *GPx1* Pro198Leu polymorphism, some studies, notably that on bladder cancer [26], have shown an allele effect on GPx activity while others have not. Some authors have suggested that these disparities might be explained if the change in GPx activity were to be caused by another polymorphism that co-segregates with the studied polymorphism [28]. Interestingly, though only one study showed a significant effect of carriage of the Leu allele on breast cancer risk [28], increased risk was observed in Leu homozygotes who were also homozygous for *SOD2* 16Ala [31], as was the case for bladder cancer [26], implying that the risk associated with this polymorphism is affected by other genotypes and perhaps other environmental factors as well. This is an illustration of the fact that cancer is a multifactorial complex disease and a combination of factors – not just a single polymorphism – is generally required to cause disease. It is also possible that some of the discrepancies noted above may relate to differing Se status between populations investigated. The fact that development of colorectal, breast and head and neck tumours is linked with loss of heterozygosity at *GPx1* suggests that loss of

protective GPx1 activity may increase cancer risk. Loss of heterozygosity in *Sep15* also occurs in breast or head and neck tumours, again suggesting that ability to make this selenoprotein may be important in reducing risk.

Though there are many fewer studies that have investigated associations between polymorphisms in *GPx4, Sep15, TXNRD1* and *SEPP1* and cancer, significant effects have been found for the *GPx4* C718T polymorphism on risk of death from breast cancer, for three SNPs in *SEPP1* and one in *TXNRD1* on the risk of advanced colorectal adenoma, and for the Ala234Thr *SEPP1* polymorphism in men of relatively low Se status who carry a *SOD2*-Ala16 allele on the risk of prostate cancer.

As a word of warning, however, it is possible that genetic variants in other genes at or near the loci of the selenoprotein polymorphisms under study might co-segregate, contributing to the effects observed. All of the genetic variants that might contribute to such an effect must therefore be identified before disease risk can be definitely attributed to a particular selenoprotein polymorphism. Nonetheless, on the face of it, it seems probable from epidemiological studies that have studied selenoprotein polymorphisms that the selenoproteins GPx1, GPx4, Sep15, TXNRD1 and SEPP1 are relevant to the cancer process.

Importantly, what has emerged from this analysis is that interactions between selenoprotein genes and genes in associated pathways (e.g. mitochondrial superoxide dismutase) can together influence risk, where neither might do so separately. It is a fact that genome-wide association studies (GWAS), so much favoured by funding bodies at the moment, have generally not been powered to pick up such interactions, so real effects may be missed using that fashionable approach. However, meta-analyses are now underway combining individual GWAS, and these may reveal more gene:gene interactions in the future.

Insights from case-control, prospective cohort studies and randomised controlled trials on possible involvement of selenoproteins in cancer prevention

By examining the relationship between cancer risk and Se status, it may be possible to draw some speculative inferences about the likely involvement of selenoproteins in cancer risk, given that we have some idea of the plasma Se concentrations at which certain selenoproteins are likely to be optimised. We know, for instance, that the mean plasma Se concentration needed to maximize plasma glutathione peroxidase (GPx3) activity is around 92 µg/L [5]. This is generally believed to be sufficient to optimise the activity/concentration of other selenoproteins, with the exception of selenoprotein P which is known to be saturated at a plasma level of 125 µg/L [73]. GPx3 and selenoprotein P are down-regulated in prostate cancer and their importance to prostate-cancer risk [39] and metastasis [74] has recently been demonstrated, probably on account of their antioxidative and Se-transport functions. Using epidemiological studies of prostate cancer in which plasma/serum Se have been measured as examples, let us attempt to see if their results can be explained by invoking the involvement of these selenoproteins.

For cohort studies, if these selenoproteins are involved, one might only expect to see an effect on risk of prostate cancer when the concentrations in the cohort range from below, to above, the level needed to optimise the activity of these enzymes. Thus it is hardly surprising that no effect of Se on prostate-cancer risk was found in the EPIC study where the mean plasma Se concentrations were 70.6 µg/L (95% CI: 69.7, 71.5) and 71.9 µg/L (95% CI: 71.0, 72.7) for cases and matched controls respectively [75,76]. These levels are insufficient to maximize the activity of GPx3 [5] (and that of other selenoenzymes) and are substantially below the level at which selenoprotein P is known to be saturated [73]. This would have severely limited the possibility of detecting a significant difference in prostate-cancer risk between cases and controls, assuming that these selenoproteins/selenoenzymes play an

important role [76]. The Baltimore Longitudinal Study of Aging [77] and the Physicians' Health Study [72] show significant reductions in risk at plasma Se ≥ 108 µg/L (population range 82-182 µg/L) and ≥ 120 µg/L (population range 60-190 µg/L) respectively when compared to the lowest quantile. These figures fit the theory that selenoproteins, and selenoprotein P in particular may affect prostate-cancer risk. However, the study of Nomura and colleagues of Japanese-American men in Hawaii [70] shows a higher requirement i.e. ≥ 147 µg/L, for significant reduction of risk than would fit with our theory. A possible explanation may lie in the substantially different distribution of *GPx1* genotypes in Japanese men than in men of European descent which may affect the interaction with Se status.

Results from the US Third National Health and Nutrition Examination Survey (NHANES III), suggest that increasing serum Se concentrations up to 130 µg/L are inversely associated with a reduced risk of all-cause and prostate-cancer mortality [78]. In fact, for prostate cancer the risk appears to fall quite sharply up to around 120 µg/L, after which it levels off before beginning to fall more slowly. These observations are compatible with an effect of selenoproteins, notably selenoprotein P, on prostate-cancer mortality.

Turning to trial results, in the Nutritional Prevention of Cancer (NPC) trial, a daily 200 µg Se supplement significantly reduced prostate-cancer risk only in those with initial plasma Se < 121 µg/L [79]. A later adjustment for the lower rate of PSA measurement in the Se group left only those with plasma levels < 106 µg/L still showing significant benefit from Se supplementation [79]. SELECT showed that giving 200 µg Se/d to a population of men of mean plasma Se 136 µg/L does not reduce the risk of localized prostate cancer [16]. Unfortunately SELECT results tell us nothing about the effect of Se on risk of advanced disease – only 1.1% of cases were non-localised – nor on men of lower Se status. Clearly while at least one third of NPC men did not have optimal selenoprotein P or even GPx concentration/activity, this was not true of SELECT men, all of whose selenoproteins were

likely to have been optimised. Therefore if selenoproteins (rather than methyl selenol) are important in cancer prevention, no effect would have been seen in SELECT, as was indeed the case. Such a trial conducted in Europe where Se status is substantially lower might have shown a very different outcome.

Indeed once Se intake is such as to optimise all selenoproteins (one assumes at a plasma Se concentration somewhere in the region of 120 μg/L) there appears to be no justification for giving additional Se, as it may increase disease risk. There was a non-significant increased risk of cancer in NPC participants in the highest tertile (baseline plasma Se >121.6 μg/L) and a significantly-increased risk of squamous cell carcinoma in those with baseline plasma Se in the top two tertiles [19,80]. In addition, further analysis of NPC trial data has shown an increased risk of self-reported Type-2 diabetes in those supplemented with Se, though the effect was significant only in those in the top tertile of plasma Se at baseline [81]. Furthermore, SELECT has reported a trend toward increased Type-2 diabetes incidence in participants receiving 200 μg/d Se alone [16]. Hence the advisability of supplementing individuals of already-replete status (say 120-125 μg/L or more) [73] with Se must be seriously questioned.

The above speculative analysis has largely ignored the effect of individual differences in Se requirements resulting from selenoprotein polymorphisms and other stressors [82] though this is clearly a factor to be considered. However, it does suggest that optimising the activity/concentration of at least some selenoproteins, including selenoprotein P, can reduce prostate cancer risk.

CONCLUSION

The evidence presented above goes some way to show how epidemiology can contribute to our understanding of the role of selenoproteins in health. Selenoprotein genotype-specific effects are the most enlightening but the totality of evidence currently implicates the selenoproteins GPx1, GPx4, SEPS1, Sep15, SEPP1 and TXNRD1 in conditions such as cardiovascular disease, pre-eclampsia and cancer. Ideally, studies need to determine not only Se status, but genotype, both in selenoproteins and related pathways, when investigating disease risk, since, as we have seen, these are interacting factors. The limitations of most GWAS carried out to date in detecting gene-gene interactions that affect disease risk need to be appreciated. Future interventions need to be in populations or subsets of populations that may be predicted to benefit from Se supplementation, such as those of low Se status or with a particular selenoprotein genotype [39,83]. Furthermore, scientists with a deep understanding of the likely mechanisms by which selenoproteins and Se metabolites may act, not just epidemiologists and clinical trialists, need to be intimately involved in the design of future trials, if the large sums of money required for such trials are to be spent wisely.

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Table 1. GPx1: Proline/Leucine SNP at codon 198, 3p21. Effect of 198 Leu allele on cancer risk (modified from ref [25])

Cancer	No. Subjects		SNP genotype *vs* Pro/Pro	OR (95% CI) * = significant	Location	Comments	Reference
	Cases	Controls					
Bladder	213	209	Pro/Leu +SOD2 V/A+A/Aa	**2.6 (1.5 - 4.8)*** **6.3 (1.3 - 31.2)***	Japan	Pro/Leu signif. assoc. with advanced tumor stage	26
Breast	399	372	Pro/Leu Leu/Leu	0.92 (0.68 - 1.24) 0.77 (0.46 - 1.27)	Canada	Pre-and post-menopausal	27
Breast	377	377	Pro/Leu + Leu/Leu	**1.43 (1.07 - 1.92)***	Denmark	Postmenopausal	28
Breast	1038	1088	Pro/Leu Leu/Leu	1.10 (0.92 - 1.32) 1.06 (0.79 - 1.42)	US	Long Island Breast Cancer Study	29
Breast	1229	1629	Pro/Leu Leu/Leu	0.91 (0.77 - 1.07) 1.07 (0.82 - 1.40)	US	Nurses' Health Study	30
Breast	1262	1533	Pro/Leu + SOD2 A/Aa Leu/Leu +SOD2 A/Aa	1.01 (0.83 -1.23) **1.87 (1.09 - 3.19)***	US	Nurses' Health Study	31
Lung	315	315	Pro/Leu Leu/Leu	**1.8 (1.2 - 2.8)*** **2.3 (1.3 - 3.8)***	Finland		24
Lung	200	200	Pro/Leu & Leu/Leu	2.29	Korea	Article in Korean, no CIs in abstract	32
Lung	432	798	Leu/Leu	0.60 (0.35 - 1.05)	Denmark		33
Prostate	82	123	Pro/Leu Leu/Leu	**0.38 (0.20-0.75)*** 0.61 (0.27-1.40)	FYROM	Would expect stronger effect in Leu/Leu; small study	34
Prostate	1433	780	Pro/Leu Leu/Leu	1.07 (0.89-1.29) 0.93 (0.69-1.26)	Sweden	CAPS study	Cooper et al. unpublished

[a] SOD2 A/A = SOD2-Ala16Ala; SOD2 V/A= SOD2-Val16Ala; SOD2 V/V= SOD2-Val16Val

Table 2. Effect of Selenoprotein SNPs on Cancer Risk or Mortality (modified from ref [25])

Selenoprotein SNP	Cancer	No. Subjects		Comparison	OR (95% CI)	Location	Comments	Reference
		Cases	Controls					
GPx4 C718T rs713041	Breast	569 deaths	3901	T allele *vs* CC	**1.27 (1.13-1.43)* per T allele carried**	UK	Association of T allele with mortality in 4470 breast cancer cases	35
	Colorectal	252	187	CT+ TT *vs* CC	**0.60 (0.37-0.96)***	UK	Carriage of T allele protective	36
	Prostate	1438	790	CT+TT *vs* CC	1.01 (0.91-1.32)	Sweden	CAPS study	Cooper et al. unpublished
Sep15 G1125A linked C811T	Lung	325 smokers	287 smokers	AA *vs* GG AA *vs* GG	1.42 (0.43-4.42) 0.37 (0.17-1.17)	Poland	Below 50 μg/L Se Above 50 μg/L Se	37
G1125A linked C811T	Prostate	1419 318	781 781	CG/TA+TA/TA *vs* CG/CG	1.03(0.86-1.24) **1.38 (1.05-1.83)***	Sweden	CAPS study Men with PSA>100 Incr risk if TA homo/hererozygotes	Cooper et al. unpublished
TRR TXNRD1 IVS1-181C>G rs35009941	Advanced colorectal adenoma	772	777	GC+GG *vs* CC	**0.20 (0.07-0.55)***	US	Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial Carriage of G allele protective	378
SEPP1 rs3797310 rs2972994 -4166 rs3877899	Advanced colorectal adenoma	746 750 749 752	762 764 763 766	AA *vs* GG CT *vs* CC CG *vs* CC AG *vs* GG AA *vs* GG	**1.53 (1.05-2.22)*** **0.73 (0.57-0.92)*** **P trend = 0.002*** 0.99 (0.79-1.24) 0.98 (0.61-1.56)	US	Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial	38
rs3877899 (Ala234Thr = G234A)	Prostate	2975	2149	AG *vs* GG AA *vs* GG GG + SOD2 V/A+A/A *vs* GG + SOD2 V/V[a]	0.94 (0.82-1.07) 1.09 (0.83-1.42) 1.43 (1.17-1.76)	Sweden	CAPS study	39

[a] SOD2 A/A = SOD2-Ala16Ala; SOD2 V/A= SOD2-Val16Ala; SOD2 V/V= SOD2-Val16Val

a SOD2 A/A = SOD2-Ala16Ala; SOD2 V/A= SOD2-Val16Ala; SOD2 V/V= SOD2-Val16Val

Figure Legend

Fig. 1 Joint effect of plasma Se concentration and Sep15 1125G/A polymorphism for lung cancer development. Test for trends in Sep15 genotypes: P = 0.038 for GG, P = 0.035 for GA, P = 0.030 for AA. Test for trend differences: AA *vs.* GG: P = 0.049, AA *vs.* GA: P = 0.025 (reproduced with permission from reference [37]).