



ORIGINAL ARTICLE

Genome-wide association study of selenium concentrations

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Abstract

Selenium (Se) is an essential trace element in human nutrition, but its role in certain health conditions, particularly among Se sufficient populations, is controversial. A genome-wide association study (GWAS) of blood Se concentrations previously identified a locus at 5q14 near *BHMT*. We performed a GW meta-analysis of toenail Se concentrations, which reflect a longer duration of exposure than blood Se concentrations, including 4162 European descendants from four US cohorts. Toenail Se was measured using neutron activation analysis. We identified a GW-significant locus at 5q14 ($P < 1 \times 10^{-16}$), the same locus identified in the published GWAS of blood Se based on independent cohorts. The lead single-nucleotide polymorphism (SNP) explained ~1% of the variance in toenail Se concentrations. Using GW-summary statistics from both toenail and blood Se, we observed statistical evidence of polygenic overlap ($P < 0.001$) and meta-analysis of results from studies of either trait ($n = 9639$) yielded a second GW-significant locus at 21q22.3, harboring *CBS* ($P < 4 \times 10^{-8}$). Proteins encoded by genes at 5q14 and 21q22.3 function in homocysteine (Hcy) metabolism, and index SNPs for each have previously been associated with betaine and Hcy levels in GWAS. Our findings show evidence of a genetic link between Se and Hcy pathways, both involved in cardiometabolic disease.

† The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors and that the last two authors should be regarded as joint Last Authors.

Received: August 18, 2014. Revised: September 23, 2014. Accepted: October 20, 2014.

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Introduction

Selenium (Se) is an essential trace element in human nutrition; its deficiency has been associated with a variety of health disorders and has been the subject of intensive investigation (1,2). However, the potential benefits of Se's anticancer effects have recently been tempered by its potential adverse cardiometabolic effects, necessitating further mechanistic selenoprotein research as well as studies on dietary or supplemental Se intake for optimal health (1,3–5).

Biomarkers of Se exposure such as blood and nail Se concentrations vary substantially and are influenced by diet, geographical region, supplements and smoking status (2). Characterizing genetic variation in response to Se exposure may be especially useful in identifying individuals most susceptible to the adverse effects of Se, who might be advised to avoid Se supplementation. A recent genome-wide association study (GWAS) of blood Se concentrations (6) reported a GW-significant locus at 5q14, harboring *BHMT*, *BHMT2* and *DMGDH* (6). Although blood and toenail Se concentrations are validated biomarkers of Se exposure, the latter may reflect a longer average duration of exposure and perhaps a more stable trait amenable to GWAS (7,8). We thus aimed to perform the first GWAS of toenail Se concentrations and to compare our findings with those reported for blood Se, enabling insight to the genetic architecture underlying similar traits which purportedly capture different measures of exposure duration.

Results

We conducted a GW meta-analysis of toenail Se concentration using data from a total of 4162 men and women sourced from

four independent population-based studies: Coronary Artery Risk Development in Young Adults (CARDIA), Johnston County Osteoarthritis Project (JoCo), Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) (Supplementary Material, Table S1 and Fig. S1). We observed little evidence for genomic inflation ($\lambda = 1.02$, Supplementary Material, Fig. S2). Several single-nucleotide polymorphisms (SNPs) in low LD (CEU: $r^2 > 0.30$) reached GW-significance ($P < 5 \times 10^{-8}$) and mapped to 5q14.1; the same locus reported by Evans *et al.* (6) in a GWAS of blood Se (Table 1). Our lead SNP (rs17823744) explained ~1% of the variance in toenail Se concentrations in the NHS and HPFS. None of our nominally significant loci for toenail Se were associated with blood Se.

In gene-based analysis of Se metabolism and selenoprotein candidate genes, only thioredoxin reductase 1 (*TXNRD1*) was significantly associated with toenail Se levels (Table 2, gene-level $P < 7 \times 10^{-3}$). The top SNP for this gene also met sub-GW significance (Table 1). No candidate genes in these pathways were significantly associated with blood Se levels.

GW associations of toenail Se were significantly enriched for genes involved in DARPP32 events (Reactome), the β -catenin phosphorylation cascade (Reactome), G1 phase cell cycle regulation (Reactome), PP2A-mediated dephosphorylation (Reactome) and the ERK inactivation pathway (Reactome; FDR < 0.05). GW associations of blood Se did not yield significant enrichment for specific biological pathways.

We observed no significant overlap of associated effects in the two data sets when applying a genome-wide test for pleiotropy ($P = 0.86$). However, in tests for genetic overlap between GW significant ($P1 \leq 5 \times 10^{-8}$), suggestive ($P1 \leq 1 \times 10^{-5}$) and nominal ($P1 \leq 0.05$) blood Se SNPs (data set1) and toenail Se SNPs (data

Table 1. Top loci associated with toenail Se concentrations and their corresponding associations with blood Se ($n = 5477$) in an independent and previously reported GWAS^a

Chr	Position (Hg18)	Index SNP ^b	EA/NEA	Toenail Se ($n = 4162$)				Evans <i>et al.</i> blood Se ($n = 5477$)			Closest gene(s) ^b , eQTL ^d
				EAF	$\beta^c \pm SE$	P-value	I^2	Effect	P-value	I^2	
1	82 993 022	rs17498581	T/C	0.10	0.024 ± 0.006	9.34 × 10 ⁻⁶	42	+	0.99	28	<i>LPHN2</i> , <i>TLL7</i>
1	241 584 774	rs2802728	T/C	0.90	0.026 ± 0.006	6.28 × 10 ⁻⁶	0	-	0.09	0	<i>SDCCAG8</i>
2	143 476 161	rs352889	A/G	0.39	0.016 ± 0.004	4.16 × 10 ⁻⁶	22	+	0.74	0	<i>KYNU</i> , <i>ARHGAP15</i>
5	28 41 906	rs7702905	A/G	0.28	-0.017 ± 0.004	8.09 × 10 ⁻⁶	33	+	0.56	0	<i>IRX2</i> , <i>C5orf38</i>
5	78 372 981	rs248381	A/G	0.50	-0.025 ± 0.003	3.01 × 10⁻¹³	26	-	1.07 × 10⁻²⁵	0	<i>DMGDH</i> , <i>ARSB</i> , <i>BHMT2</i>
5	78 380 732	rs17823744	A/G	0.88	-0.045 ± 0.005	9.91 × 10⁻¹⁷	0	-	1.62 × 10⁻²¹	0	<i>DMGDH</i> , <i>ARSB</i> , <i>BHMT2</i>
5	78 447 080	rs7700970	T/C	0.30	0.030 ± 0.004	2.21 × 10⁻¹¹	26	+	2.88 × 10⁻¹⁸	71	<i>BHMT</i> , <i>BHMT2</i> , <i>JMY</i>
5	78 452 172	rs567754	T/C	0.33	-0.018 ± 0.004	4.80 × 10 ⁻⁶	41	-	3.22 × 10⁻¹⁶	26	<i>BHMT</i> , <i>BHMT2</i> , <u><i>JMY</i></u> ↑
5	78 780 798	rs6859667	T/C	0.96	-0.045 ± 0.008	1.24 × 10 ⁻⁷	19	-	3.75 × 10 ⁻⁶	0	<i>HOMER1</i> , <i>JMY</i>
5	79 178 950	rs10079417	T/C	0.23	0.018 ± 0.004	6.10 × 10 ⁻⁶	18	+	0.30	0	<i>CMYA5</i> ,
5	133 474 474	rs756699	T/C	0.86	0.022 ± 0.005	9.61 × 10 ⁻⁶	0	+	0.78	31	<i>VDAC1</i> , <u><i>TCF7</i></u> ↑
8	21 01 183	rs17685410	A/G	0.30	0.018 ± 0.004	1.60 × 10 ⁻⁶	38	+	0.06	0	<u><i>MYOM2</i></u> ↓, <i>CSMD1</i> , <i>KBTD11</i> ↓, <u><i>ARHGEF10</i></u> ↓
11	102 715 194	rs313426	A/G	0.31	-0.018 ± 0.004	2.24 × 10 ⁻⁶	0	+	0.23	0	<i>DYNC2H1</i> , <i>DCUN1D5</i> , <i>PDGFD</i>
12	52 561 533	rs1596370	A/G	0.21	-0.021 ± 0.004	8.34 × 10 ⁻⁷	0	-	0.21	11	<i>CALCOCO1</i> , <i>HOXC13</i>
12	103 169 535	rs7975161	T/C	0.16	-0.023 ± 0.005	1.18 × 10 ⁻⁶	0	+	0.84	0	<i>TXNRD1</i> ↑, <i>NFYB</i>
18	21 225 855	rs6508367	T/C	0.96	-0.040 ± 0.009	7.50 × 10 ⁻⁶	23	-	0.28	0	<i>ZNF521</i> , <i>SS18</i>
19	19 690 101	rs2163813	T/G	0.74	-0.018 ± 0.004	4.56 × 10 ⁻⁶	58	-	0.42	0	<i>ZNF14</i> , <i>ZNF101</i> , <i>ATP13A1</i> ↑

EA, effect allele; NEA, non-effect allele; EAF, effect allele frequency; eQTL, expression quantitative trait loci.

^aShown are top SNPs ($P < 5 \times 10^{-6}$) associated with toenail Se concentrations pruned for linkage disequilibrium ($r^2 < 0.3$). P-values in bold face achieve genome-wide significance ($P < 5 \times 10^{-8}$).

^bGenic SNPs and their corresponding genes are in bold face.

^cEffect sizes represent change in natural logarithm transformed toenail Se $\mu\text{g/g}$ per effect allele. Only direction of effect is presented for blood Se since Evans *et al.* modeled residuals of a linear regression, thus limiting interpretation and comparison with effect sizes presented for toenail Se.

^dExpression QTLs for index SNP or perfect proxy (CEU: $r^2 = 1$) derived from blood (9) are underlined. Arrows indicate increased and decreased expression, respectively, relative to effect allele.

Table 2. Associations between Se function and metabolism candidate genes and Se concentrations^a

Chr	Gene	Toenail Se			Blood Se		
		Gene level P-value	Best SNP	Best SNP P-value	Gene level P-value	Best SNP	Best SNP P-value
1	DIO1	0.04	rs2272928	5.76×10^{-3}	0.41	rs4926616	0.11
1	SEP15	0.22	rs1407131	0.05	0.17	rs7537393	0.014
3	GPX1	0.15	rs3811699	0.06	0.05	rs1865741	0.02
5	SEPP1	6.99×10^{-3}	rs2329999	1.30×10^{-3}	0.37	rs230814	0.10
5	GPX3	0.04	rs3805433	3.96×10^{-3}	0.01	rs12517537	6.68×10^{-4}
12	TXNRD1	1.00×10^{-5}	rs7975161	1.18×10^{-6}	0.08	rs17035401	0.02
14	GPX2	0.31	rs12147448	0.12	0.21	rs7160073	0.03
14	DIO2	0.18	rs2267872	0.05	0.12	rs224995	0.03
14	DIO3	0.17	rs2895917	0.02	0.34	rs2895917	0.10
15	SELS	0.39	rs6598440	0.035	0.58	rs2277593	0.14
19	GPX4	0.65	rs757232	0.14	0.46	rs2074445	0.10
22	TXNRD2	0.84	rs13057441	0.11	0.29	rs2238786	0.05

^aShown are results for candidate genes achieving at least nominal significance ($P \leq 0.05$) in gene-based testing. P-values in bold face are those achieving gene-level significance ($P < 0.0008$).

Table 3. Top loci from GW meta-analysis of toenail and blood Se concentrations^a

Chr	Position (Hg18)	Index SNP	EA/NEA	EAF	n	Z-score ^b	P-value	I ²	Closest gene(s) ^c , eQTL ^d
5	78 313 985	rs672413	A/G	0.32	9639	7.53	5.21E-14	0	ARSB ↓, LHFPL2, DMGDH
5	78 327 716	rs705415	T/C	0.14	8054	-6.23	4.64E-10	52	DMGDH, ARSB
5	78 336 153	rs3797535	T/C	0.08	9639	7.94	2.05E-15	0	DMGDH, ARSB, BHMT2
5	78 340 070	rs11951068	A/G	0.07	9639	6.72	1.86E-11	0	DMGDH, ARSB, BHMT2
5	78 352 232	rs921943	T/C	0.29	9639	13.14	1.90E-39	39	DMGDH, ARSB, BHMT2
5	78 421 601	rs10944	T/G	0.49	9639	12.65	1.13E-36	44	BHMT2, BHMT
5	78 452 172	rs567754	T/C	0.34	9639	-9.11	8.38E-20	49	BHMT, BHMT2, JMY
5	78 460 944	rs558133	A/C	0.69	9639	-6.55	5.60E-11	0	BHMT, BHMT2, JMY
5	78 780 798	rs6859667	T/C	0.96	9639	-6.92	4.40E-12	9	HOMER1, JMY
21	43 351 566	rs6586282	T/C	0.17	9639	-5.89	3.96E-09	15	CBS , PKNOX1 ↓, U2AF1, STAG3OS1
21	43 356 005	rs1789953	T/C	0.14	9639	5.52	3.40E-08	0	CBS ↓, PKNOX1 , U2AF1, WDR41
21	43 360 033	rs234709	T/C	0.45	9639	-5.84	5.23E-09	0	CBS ↓, PKNOX1 , U2AF1

EA, effect allele; NEA, non-effect allele; EAF, effect allele frequency; eQTL, expression quantitative trait loci.

^aShown are genome-wide significant SNPs ($P < 5 \times 10^{-8}$) associated with Se concentrations pruned for linkage disequilibrium ($r^2 < 0.3$).

^bEffect sizes represent change in Z-score per effect allele.

^cGenic SNPs and their corresponding genes are in bold face.

^dExpression QTLs for index SNP or perfect proxy (CEU: $r^2 = 1$) derived from blood (9) are underlined. Arrows indicate increased and decreased expression, respectively, relative to effect allele.

set2) with $P2 \leq 0.05$, the number of SNPs associated in both data sets was significantly more than expected ($P < 6.7 \times 10^{-6}$ for GW significant, suggestive and nominal data set1 SNPs). Moreover, we observed a significant concordance of genetic risk (i.e. direction of effects) between the two data sets ($P < 0.001$).

Given evidence of polygenic overlap across phenotypes, we conducted a sample size weighted meta-analysis across all studies of toenail and blood Se and identified a second GW-significant locus at 21q22.3; harboring CBS ($P < 4 \times 10^{-8}$, Table 3, Supplementary Material, Figs S3 and S4); and enrichment for genes involved in the biological process of hearing (Panther, FDR < 0.03). The latter is related to Gene Ontology's biological process: Sensory Perception of Sound (GO:0007605); which was not a significant pathway (FDR = 0.79). CBS is plausibly linked to DMGDH-BHMT-BHMT2 but is also a confirmed locus for homocysteine (Hcy) status (10,11). To gain insight to potential confounding or pleiotropy, we examined whether any of our other top Se loci were associated with Hcy status in the Women's Genome Health Study (11). With the exception of CBS, none of our top Se loci (Tables 1 and 3) was associated with Hcy levels after the Bonferonni

correction ($P \geq 0.02$). We also conducted the complementary experiment: we tested whether any additional Hcy loci were associated with Se. After the Bonferonni correction, the variant in CPS1 associated with higher Hcy levels (rs742239, A) was significantly associated with higher Se in the combined analysis of toenail ($P = 0.16$) and blood ($P = 0.0004$) Se levels (combined $P = 0.0003$). Besides gene-based associations between CBS and toenail ($P = 0.001$) and blood Se ($P = 0.0008$), only nominally significant associations were observed for other candidates of the methionine (Met) metabolism and trans-sulfuration (TS) pathways: DNMT1 ($P = 0.02$ for toenail Se), CSAD ($P = 0.04$ for toenail Se) and MAT2B ($P = 0.049$ for blood Se).

Discussion

Se is an essential mineral, but its role in certain health conditions, particularly among Se sufficient populations, is controversial warranting further research on dietary or supplemental Se intake for optimal health. Information on modifiable and common genetic factors impacting exposure to Se may advance this

Box 1. Normal plasma Hcy concentrations are maintained by converting Hcy to cysteine by action of the enzyme cystathionine β synthase (CBS) or by methylating Hcy to form methionine in reactions that can be accomplished by one of three separate enzymatic pathways: (i) methionine synthase (MS) methylates Hcy by using 5-methyltetrahydrofolate (5-MeTHF) as the methyl donor and vitamin B-12 as a cofactor; (ii) betaine homocysteine S-methyltransferase (BHMT) methylates Hcy by using betaine as the methyl donor; and (3) BHMT2 methylates Hcy by using S-methylmethionine (SMM) as the methyl donor (Fig. 1). Dimethylglycine dehydrogenase (DMGDH) catalyzes the oxidative demethylation of dimethylglycine, the product of BHMT-mediated methylation, to form sarcosine. The methionine formed from Hcy serves as the precursor of S-adenosylmethionine (SAM), the primary methyl donor for numerous methylations. When it donates its methyl group, SAM is converted to S-adenosylhomocysteine (SAH), and SAH can be recycled to Hcy (17,18). As shown in Figure 1 and further discussed in the text, perturbations of these pathways may impact selenium (Se) elimination and utilization.

research. The current GWAS of toenail Se concentrations confirms an association with the 5q14 locus previously reported in a recent GWAS of blood Se concentration (6), but combining results for toenail and blood Se concentrations, we reveal a second locus mapping to 21q22.3. Our findings demonstrate genetic links between long-term Se exposure and the Hcy metabolic pathways.

Se exists in the diet in the form of selenomethionine (SeMet), selenocysteine (Sec), selenate or selenite. Hydrogen selenide (H_2Se), a vital metabolite for selenoprotein synthesis and Se excretion, is generated from Sec by selenocysteine β -lyase or from dietary selenite via its reduction with glutathione (3,12). Approximately 53 and 39% of Se present in plasma and serum is in the form of Sec residues in selenoprotein P (SePP) and glutathione peroxidase 3 (GPx3), respectively, while the remaining Se is incorporated non-specifically in albumin and other proteins as SeMet (13,14). Because SePP and GPx3 are fully expressed in Se adequate individuals, variation in plasma Se levels is almost exclusively limited to variation in the non-specific component (14). Serum and plasma Se measures are sensitive to recent Se exposure (14,15), which may partly explain the null findings of a recent GWAS of serum Se (16). Blood presumably reflects Se exposure over ~120 days, corresponding to the lifespan of its erythrocyte content, while toenail Se reflects exposure over a period of 26–52 weeks (15). The GWAS of blood Se conducted by Evans *et al.* (6) as well as the current GWAS of toenail Se both report significant SNPs mapping to an intronic region of *DMGDH*. A second SNP (rs7700970) in this region and in low LD (CEU: $r^2 = 0.22$) to our index SNP in *DMGDH* (rs17823744) also reached GW significance and mapped to *BHMT*. In conditional analysis, Evans *et al.* confirmed an independent effect of a second SNP in *BHMT* (rs506500), which was also nominally significant in our GWAS of toenail Se ($P = 7.59 \times 10^{-5}$). Leveraging the evidence of shared genetics across these two measures of Se exposure, we revealed a second GW-significant locus mapping to *CBS*, which Evans *et al.* (6) initially described as highly suggestive. Whether these loci reflect variation in SeMet, Sec or another minor Se constituent is unclear. Nevertheless, our finding suggests that blood and

toenails are stable measures of Se exposure amenable to GWAS and share a similar genetic architecture, further corroborating the utility of these biomarkers in traditional epidemiological studies of Se exposure.

Proteins encoded by *DMGDH*, *BHMT*, *BHMT2* and *CBS* all function in Hcy metabolism, which is composed of two intersecting pathways, the Met metabolism and TS pathways (Box 1) (17). Perturbations of these pathways are usually discussed with reference to their impact on Hcy levels and modification by vitamin B status. Polymorphisms in *CBS* associated with higher Se levels have previously been associated with higher Hcy levels in GWAS (10,11). The latter suggests these variants likely reduce *CBS* activity, since *CBS* is the regulatory enzyme in the TS pathway, which is the only known activity for Hcy elimination (19). An impaired TS pathway resulting in elevated Hcy levels may alter the Met cycle and production of S-adenosylmethionine (SAM), a primary methyl donor for numerous methylation reactions, including those required for Se excretion (Fig. 1) (12,20,21). Alternatively, the role of glutathione in metabolism of selenite may contribute to variation in Se levels when TS is altered (22). Finally, SeMet and its metabolites are reportedly substrates for all steps of the TS pathway *in vitro* (23), but the *in vivo* importance of this pathway to SeMet utilization remains to be demonstrated (24).

Among GW-significant SNPs mapping to *BHMT-BHMT2-DMGDH*, variants linked with higher blood/toenail Se have been previously associated with higher liver betaine-homocysteine methyltransferase (*BHMT*) enzyme activity and protein level (25) and lower plasma betaine levels (26,27). Unlike *BHMT*, *BHMT-2* cannot use betaine (28), thus we hypothesize that the causal gene in this region is *BHMT*. Given three fungible sources of methyl groups (Fig. 1), it is unlikely that variants in *BHMT* alter Se levels through a mechanism downstream of Met generation but rather through a mechanism specific to *BHMT*. Se can react with cysteine-rich regions present within the catalytic domain of enzymes such as protein kinase C where it may form selenenyl-sulfide bonds with cysteines resulting in lower enzyme activities (29). As *BHMT* is a zinc-dependent cysteine-rich enzyme, an altered thiol redox status could influence its activity in much the same way as for protein kinase C (30). How this potential interaction between Se and *BHMT* might impact utilization or accumulation of Se is unclear.

Strengths of the current study include the use of four independent population studies with toenail Se measured in the same laboratory by the same technique, allowing for a sufficiently sized sample with relatively uniform measures of Se for analysis. However, a few limitations of the study warrant consideration. Corresponding Hcy measures were available for too few of the participants and thus did not allow us to explore in detail potential pathways shared by Hcy and Se. Within Se-sufficient populations, such as those of the current study, whether higher Se levels associated with variation at 5q14 or 21q22.3 predispose to favorable or unfavorable (i.e. toxic) effects remains an open question. Although blood and toenail Se concentrations are accepted markers of human Se status, Se concentrations themselves do not reflect its functional significance (31). Rather, the nutritional functions of Se are achieved by SePP, GPx3 and 23 other selenoproteins (31). In gene-based analysis, toenail Se levels were significantly associated with *TXNRD1* and nominally significant with selenoprotein P, plasma, 1 (*SEPP1*). None of the candidate genes was significantly associated with blood Se levels. While our associations with *TXNRD1* and *SEPP1* await confirmation, our most significant associations at 5q14 and 21q22.3 for both toenail and blood Se concentrations suggest markers of long-term Se exposure are more strongly influenced by pathways

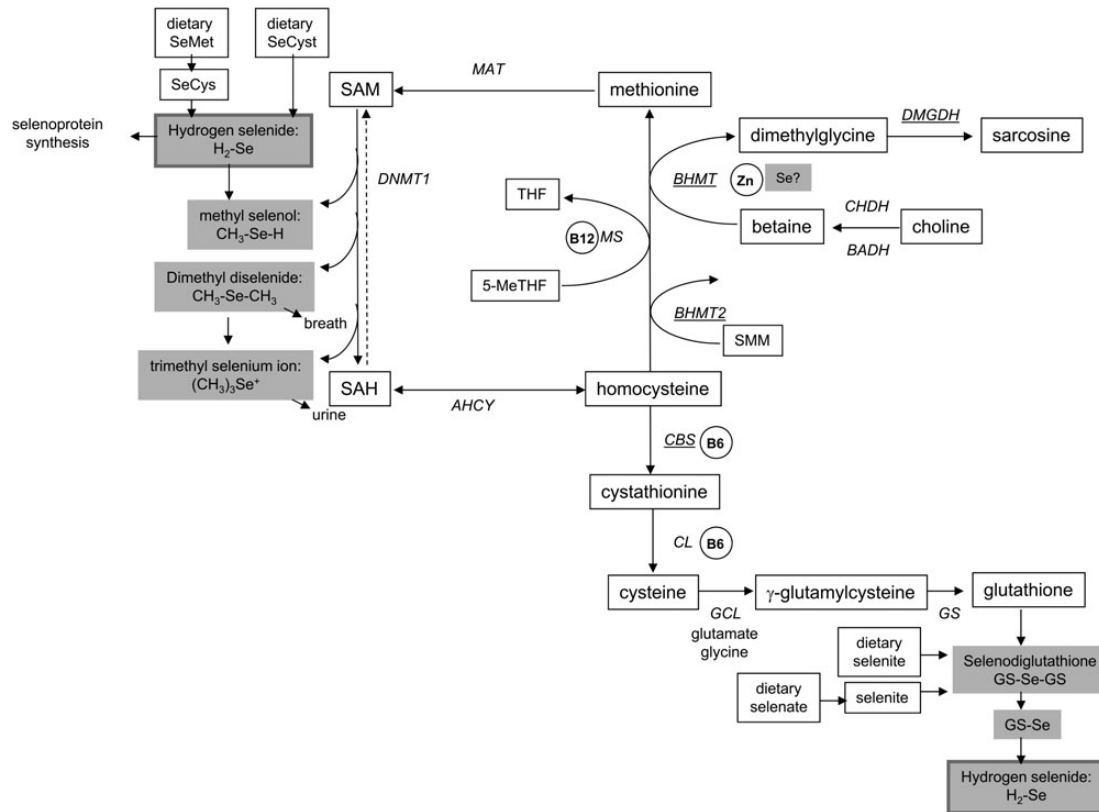


Figure 1. Hcy metabolism.

independent of those related to the mineral's biological function. Clearer interpretation of our findings may require follow-up in populations with a wider distribution of Se exposure.

Our findings are important in the context of increasing interest in the relationship between Se and Hcy status as well as the roles each potentially plays in cardiometabolic disease. Elevated Hcy levels have consistently been associated with cardiovascular disease risk in addition to other clinical conditions (32). Associations between measures of Se exposure and cardiometabolic outcomes are contradictory (1,2). Cross-section epidemiological studies report inverse relationships between serum Se levels and Hcy (33), which contrasts with the positive relationship observed in animals studies (30,34,35). Human Se supplementation trials observed no effect of Se on Hcy levels (36,37). The discrepancy in results between the human and animal studies may be due to the extreme level of Se deficiency attained in the animal studies, type of Se administered in experiments or cross-species differences in relative importance of each pathway (30,34,38,39). Regarding the latter, we note that unlike genetic variation in the folate-dependent pathway, BHMT variation does not appear to impact Hcy levels (10,11,40–42) and together suggests a less dominant role of the BHMT/2 remethylation pathway in human Hcy balance, a theory which contrasts that proposed for mice (39). Nevertheless, our genetic findings suggest the interconnection between Hcy, B vitamins, choline, betaine and Met might extend to include Se.

In summary, we confirm two loci associated with long-term measures of Se exposure. Both encode proteins involved in the Met and TS pathways surrounding Hcy metabolism. Future molecular studies of these pathways or health outcomes implicated by these pathways might consider Se in addition to the traditional set of co-factors. Likewise, future studies of dietary or

supplemental Se might account for genetic variation in Se response and monitor change in both Se function and Hcy metabolism.

Materials and Methods

Study populations

Coronary Artery Risk Development in Young Adults (CARDIA)

The CARDIA study is a prospective multicenter study with 5115 adults Caucasian and African-American participants of the age group 18–30 years, recruited from four centers. The recruitment was done from the total community in Birmingham, AL, from selected census tracts in Chicago, IL, and Minneapolis, MN; and from the Kaiser Permanente health plan membership in Oakland, CA. Details of the CARDIA study design have been previously published (43). Eight examinations have been completed since initiation of the study in 1985–1986, respectively, in the years 0, 2, 5, 7, 10, 15, 20 and 25. Written informed consent was obtained from participants at each examination and all study protocols were approved by the institutional review boards of the participating institutions.

Johnston County Osteoarthritis Project (JoCo)

Begun in 1990, JoCo is an ongoing, population-based, prospective cohort in Johnston County, NC, with follow-up examinations approximately every 5–7 years. Participants were enrolled following household enumeration and represented civilian, non-institutionalized African-Americans and Caucasians ≥ 45 years who were residents of one of six townships in Johnston County for at least 1 year and who were physically and mentally capable of completing the study protocols. Although this was a study

initially designed to assess osteoarthritis, there was no pre-selection based on specific diseases, and data about multiple medical conditions have been collected at each time point. At baseline and each follow-up, participants completed two in-home or clinic interviews and a clinical examination. Venipuncture for DNA collection was instituted at the first follow-up (1999–2003) and cohort enrichment (2003–2004). Details of the study's methods have been previously reported (44).

Nurses' Health Study (NHS)

The NHS was established in 1976 when 121 700 female registered nurses aged 30–55 years and residing in 11 large US states completed a mailed questionnaire on medical history and lifestyle characteristics (45). Every 2 years, follow-up questionnaires have been sent to update information on exposures and newly diagnosed diseases and every 2–4 years, diet is assessed using a validated semi-quantitative FFQ. Blood was collected from 32 826 NHS members between 1989 and 1990. Women contributing to the current analysis were those previously selected for one of four independent GWAS in nested case–control studies of the NHS cohort, initially designed for outcomes of type 2 diabetes (T2D) (46), coronary heart disease (CHD) (47) and breast cancer (BrCa) (48). Details pertaining to study design have been reported elsewhere (46–48).

Health Professionals Follow-up Study (HPFS)

The HPFS was initiated in 1986 when 51 529 male health professionals between 40 and 75 years of age and residing in the USA completed an FFQ and a questionnaire on lifestyle and medical history. The participants have been followed with repeated questionnaires on lifestyle and health every 2 years and FFQs every 4 years. Between 1993 and 1996, a blood sample was requested from all active participants in the HPFS and collected from 18 225 men (49). Men contributing to the current analysis were those previously selected for one of two nested case–control studies of the HPFS cohort, initially designed for outcomes of T2D (46) and CHD (47), as previously described (46,47).

This study was conducted according to the principles expressed in the Declaration of Helsinki. All participants in the contributing studies gave written informed consent including consent for genetic analyses. Local institutional review boards approved study protocols. The JoCo protocol was additionally approved by the review board at the Centers for Disease Control and Prevention.

Assessment of toenail Se concentrations

Detailed procedures for toenail collections and processing in each cohort have been reported previously (50–52). Toenail clippings from all 10 toes were provided by NHS and HPFS participants in 1982–1983 and 1986–1987, respectively. Toenail collections for CARDIA took place in 1986. and Toenail clippings from all 10 toes were provided by NHS, HPFS and CARDIA participants in 1982–1983, 1986–1987 and 1987, respectively (50). Toenail collection for JoCo was instituted during the first and second follow-up evaluations. Toenail Se concentrations were measured using neutron activation analysis at the University of Missouri Research Reactor between 2009 and 2011 for NHS and HPFS, 2006 and 2009 for CARDIA and 2004–2005 for JoCo. Details of analytical methods and information regarding validation of these measures have previously been reported (15,53,54). Samples of nail clippings from all toes were combined, providing a time-integrated measure of exposure over approximately the prior year owing to the elimination half-life of toenail Se, the growth

rate of toenails and the differential length of time (distance) from cuticle synthesis to toenail clipping comparing the smallest with largest toes. Potential laboratory drift was controlled by both standard comparison procedures for neutron activation analysis and repeated analysis of representative sample subsets. Intra-assay coefficients of variation for Se were 2.45% for CARDIA, 3% for JoCo and 2.4% for NHS and HPFS. We excluded individuals with toenail Se concentrations $>2.0 \mu\text{g/g}$, which could reflect exogenous contamination or considerable excess ingestion of Se supplements (15,53,54).

Genotyping, quality control and imputation

DNA was extracted from blood samples using conventional methods. Genotyping was completed using the Affymetrix Genome-Wide Human (Affy) 6.0 array (NHS-T2D, HPFS-T2D, NHS-CHD, HPFS-CHD, CARDIA), Illumina Human-Hap550 array (NHS-BrCa) or Illumina 1M Duo array (JoCo). Details of genotyping process and quality control (QC) for each study population have been described previously (46–48,55,56). In the CARDIA study, genotyping was completed for 1720 individuals of European ancestry with a sample call rate $\geq 98\%$. Of these, 1585 had Se data and were included in these analyses. A total of 578 568 SNPs passed QC (MAF $\geq 2\%$, call rate $\geq 95\%$, HWE $\geq 10^{-4}$) and were used for imputation. At a minimum, DNA samples that did not meet a 90% completion threshold, and SNPs with low call rates ($<95\%$), were dropped. EIGENSTRAT (57) was used to carry-out ancestry analyses and any putative non-European samples (either the HapMap YRI or CHB + JPT samples) were excluded for subsequent analyses. EIGENSTRAT was also used to generate informative principal components for population substructure.

Each study used MACH (58) or BEAGLE (59) to impute up to ~2.5 million autosomal SNPs with NCBI build 36 of Phase II HapMap CEU data (release 22) as the reference panel. Genotypes were imputed for SNPs not present in/on arrays or for those where genotyping had failed to meet the QC criteria. Imputation results are summarized as an 'allele dosage' (a fractional value between 0 and 2), defined as the expected number of copies of the minor allele at that SNP.

Genome-wide meta-analysis of toenail Se concentrations

Each study used ProbABEL (60) or PLINK (61) to perform GWA-testing for natural log-transformed Se concentrations across ~2.5 million SNPs, based on linear regression under an additive genetic model. Analyses were stratified by gender and adjusted for age, smoking status [never/former, current ($<15 \text{ cig/day}$), current ($\geq 15 \text{ cig/day}$)], geographical area of residence (NHS, HPFS, JoCo) or study site (CARDIA) and top eigenvectors (number varies by data set). Prior to meta-analysis, we removed SNPs with a minor allele frequency <0.02 or low imputation quality scores, defined as BEAGLE's allelic r^2 or MACH's $r^2 < 0.30$.

Meta-analysis was conducted using a fixed effects model and inverse-variance weighting as implemented in METAL (see URLs in Supplementary Material). The software also calculates the genomic control parameter and adjusts each study's standard errors. Heterogeneity across studies was investigated using the I^2 statistic and Q-test (62). The genomic inflation factor λ for each study as well as the meta-analysis was estimated from the median χ^2 statistic. SNP–Se associations ($n = 2\,378\,986$) based on results from at least 50% of the maximum sample size were used to generate Manhattan, quantile–quantile and regional association plots and for *post hoc* analysis (see below). SNPs imputed by all studies ($n = 1\,167\,113$) were excluded from further

presentation or discussion. The latter QC step did not remove potentially novel loci but rather only SNPs in LD with those reported in Tables 1 and 3 and as shown in Supplementary Material, Figures S1 and S3. We considered P -values of $<5 \times 10^{-8}$ to indicate GW significance (63).

Previously published GWAS of blood Se by Evans et al. (6)

We compared our results with the publically available summary statistics of a previously reported GWAS of blood Se (6). Briefly, participants were recruited in Australia from twins and their families ($n = 2603$) and in the UK from pregnant women ($n = 2874$). Erythrocyte Se (Australian samples) or whole blood Se (UK samples) were measured using inductively coupled plasma mass spectrometry. Genotyping was performed with Illumina chips and >2.5 million SNPs were imputed from HapMap data. Summary level data were provided from each contributing study. We filtered and re-meta-analyzed the study-level results as described above for the GWAS of toenails; which was similar to the approach taken in the original analyses (6).

Candidate gene-based, pathway and pleiotropy analyses

We examined all SNPs in 59 genes spanning Se function, Se metabolism, Met and TS pathways for associations with toenail and blood Se concentrations using VEGAS (64). We used Meta-Analysis Gene-set Enrichment of variant Associations (MAGENTA, version 2.4) (65) to test whether GW meta-analysis results for toenail and blood Se were enriched for members of specific biological pathways. Finally, we implemented SECA: SNP effect concordance analysis using GWAS summary results of toenail and blood Se levels to examine polygenic risk shared across these two closely related traits. See Supplementary Methods for details pertaining to these analyses.

Cross-trait GW meta-analysis

In light of results from our GWAS of toenail Se, which yielded the same top locus reported for GWAS of blood Se (6), further corroborated by significant polygenic overlap between the two traits, we performed a sample-weighted meta-analysis across all GWAS of toenail and blood Se. We also sought the association between our top loci and plasma Hcy levels in a previously reported GWAS in the Women's Genome Health Study (11).

Expression quantitative trait loci

We examined associations between our top Se SNPs and expression of nearby genes in whole blood from a recent meta-analysis of 5300 samples (GEO:GSE36382, GSE20142, GSE20332, GSE33828, GSE33321, GSE47729; ArrayExpress:E-TABM-1036, E-MTAB-945, E-MTAB-1708) (9).

Supplementary Material

Supplementary Material is available at HMG online.

Acknowledgements

We thank everyone who has contributed to the data collection, genotyping and analysis of the individual cohorts, as well as all the study participants.

Conflict of Interest statement. None declared.

Funding

The NHS and HPFS are supported by the National Cancer Institute (NCI, P01CA087969, R01CA137178); the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, R01DK058845); and the National Heart, Lung, and Blood Institute (NHLBI, R01HL35464), with additional support for the collection and management of genetic data. The NHS Breast Cancer GWAS (dbGaP:phs000147.v1.p1) was performed as part of the Cancer Genetic Markers of Susceptibility initiative of the NCI (R01CA40356, U01CA98233). The NHS and HPFS type 2 diabetes GWAS (dbGaP:phs000091.v2.p1) were funded as part of the Gene Environment-Association Studies (GENEVA) project under the NIH Genes, Environment, and Health Initiative (U01HG004399). Genotyping for the NHS and HPFS coronary heart disease GWAS was supported by Merck/Rosetta Research Laboratories, North Wales, PA. CARDIA is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with the University of Alabama at Birmingham (HHSN268201300025C and HHSN268201300026C), Northwestern University (HHSN268201300027C), University of Minnesota (HHSN268201300028C), Kaiser Foundation Research Institute (HHSN268201300029C) and Johns Hopkins University School of Medicine (HHSN268200900041C). CARDIA is also partially supported by the Intramural Research Program of the National Institute on Aging (NIA) and an intra-agency agreement between NIA and NHLBI (AG0005). Genotyping of the CARDIA participants and statistical data analysis was supported by grants U01-HG-004729 from the National Human Genome Research Institute and R01-HL-084099 from the NHLBI to M.F. The CARDIA Trace Element Study was also supported by the NHLBI (R01HL081572). This manuscript has been reviewed by CARDIA for scientific content. JoCo was supported in part by the Association of Schools of Public Health and the Centers for Disease Control and Prevention (S043, S1733 and S3486); and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases P60-AR30701 and P-60-AR49465; genotyping was supported by Algenomics, Inc. M.C.C. is supported, in part, by the American Diabetes Association Grant 7-13-JF-15.

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