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Genetic variation at the *SLC23A1* locus is associated with circulating levels of L-ascorbic acid (Vitamin C). Evidence from 5 independent studies with over 15000 participants

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Abstract

Background—L-ascorbic acid is an essential part of the human diet and has been associated with a wide-range of chronic complex diseases including cardiovascular outcomes. To date, there are no confirmed genetic correlates of circulating levels of L-ascorbic acid.

Objectives—We aimed to confirm the existence of association between common variation at the *SLC23A1* gene locus and circulating levels of L-ascorbic acid.

Design—We employed a two-stage design which used a discovery cohort (the British Women's Heart and Health Study) and a series of follow-up cohorts and meta-analysis (totalling 15087

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Disclosures
None

Contributions
NJT conceived, performed analysis for and wrote the paper. GDS, DAL, RMH and MJB provided analytical and writing support for the paper. All other authors provided data, cohort information and support throughout the drafting process.

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participants) to assess the relationship between variation at *SLC23A1* and circulating levels of L-ascorbic acid.

Results—In the discovery cohort, variation at rs33972313 was associated with a reduction in circulating levels of L-ascorbic acid (−4.15μmol/L (95%CI −0.49, −7.81), p=0.03 reduction per minor allele). Pooled analysis of the relationship between rs33972313 and circulating L-ascorbic acid across all studies confirmed this, showing that each additional rare allele was associated with a reduction in circulating levels of L-ascorbic acid of −5.98μmol/L (95%CI −8.23, −3.73), $p=2.0\times10^{-7}$ per minor allele.

Conclusion—Work here has identified a genetic variant (rs33972313) in the *SLC23A1* vitamin C active transporter locus that is reliably associated with circulating levels of L-ascorbic acid in the general population. This finding has implications more generally for the epidemiological investigation of relationships between circulating L-ascorbic acid and health outcomes.

Keywords

Vitamin C; genotype; L-ascorbic acid

Introduction

Humans are unable to synthesise L-ascorbic acid (vitamin C) owing to a series of loss-of-function mutations in the gulonolactone oxidase (*GULO*) locus(1, 2). Consequently, L-ascorbic acid has to be acquired from dietary sources(3). L-ascorbic acid has long been known to be an essential part of the human diet, with its deficiency ultimately resulting in scurvy(4). More recently variation in L-ascorbic acid levels have been associated with a wide-range of chronic complex diseases. These associations are thought to result from a contribution of L-ascorbic acid to antioxidant mechanisms and the synthesis of biological entities such as collagen and hormones(5-11).

Specifically, L-ascorbic acid is an electron donor(12) and through this mechanism contributes to the prevention of oxidative damage, which is thought to contribute to human diseases such as atherosclerosis (through the oxidation of low density lipoprotein cholesterol(13-15)), type 2 diabetes (through oxidative stress on the beta cell(9, 16)) and cancer (through oxidation-related DNA and DNA repair mechanism damage)(7). In addition L-ascorbic acid is essential for the biosynthesis of collagen and L-carnitine (important to membrane integrity during pregnancy(3, 17, 18)) and for the conversion of dopamine to norepinephrine(19).

L-ascorbic acid is obtained from the diet and is transported across the cell membrane, including intestinal cells, through both facilitated and active modes of transport. The active transport is achieved by sodium L-ascorbic acid (“vitamin C”) co-transporters (SVCTs) which transport ascorbate into the cell(20, 21). There are two isoforms of SVCT, hSVCT1 (slc23a1) and hSVCT2 (slc23a2) coded for by the genes *SLC23A2* and *SLC23A1* respectively(22, 23). The actual roles of SVCT1 and SVCT2 differ, with the two forms having different capacity for L-ascorbic acid transport. SVCT1 exhibits a higher maximum velocity and is recognized as a high capacity/low affinity carrier. The role of SVCT1 in sodium dependent L-ascorbic acid uptake has been recorded and confirmed in functional studies. Not only have these implicated SVCT1 in the absorption of dietary L-ascorbic acid across the intestinal barrier, but this work has highlighted the role of SVCT1 in kidney based reabsorption(24-26). As a result, SVCT1 is primarily involved in whole body homeostasis and transport of L-ascorbic acid and SVCT2 is primarily involved in the regulation of L-ascorbic acid in specific metabolically active tissues(27).

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Genetic variation in the sodium channel proteins *SLC23A2* and *SLC23A*1 shows differential patterns of linkage disequilibrium in each locus suggesting the possible action of different selection pressures through human population history(28). Variation in *SLC23A2* appears constrained in human populations (being consistent across both European and African populations), however variation is present in *SLC23A1*(28) and may be associated with changes in physiological function which have escaped immediate selection pressure. In this study, we have measured part of the genetic variation at *SLC23A1* in a series of European population based cohorts in order to identify single nucleotide polymorphisms (SNPs) robustly associated with circulating levels of L-ascorbic acid.

Subjects and Methods

The study design for this investigation had three parts. Initially, genetic associations were tested for in a discovery population. From this, further genotyping and replication analyses would be undertaken in first and second stage replication studies to validate initial findings. The studies involved are described below.

Discovery study

British Women's Heart and Health Study (BWHHS)

Between 1999 and 2001 4,286 women aged 60 to 79 years, were randomly selected from 23 British towns and were interviewed, examined and completed medical questionnaires. Methods used at baseline assessment have been previously described(29, 30). Within the BWHHS, 12 women were described by the examining nurse as non-white and were excluded from further analyses.

SNPs were genotyped using the KASPar chemistry which is a competitive allele-specific PCR SNP genotyping system using FRET quencher cassette oligos. All genotyping was performed by KBioscience (http://www.kbioscience.co.uk). Three stages of internal quality control were employed during genotyping. Known locations of non-DNA test controls were used to assure unique plate identity, a small sample of duplicate DNAs were genotyped for all SNPs and initial assay validations were performed on a sub-sample of 96 chromosomes before genotyping the whole sample set. A total of 3425 women (80% of the 4258 eligible white women who gave consent for genetic studies) had complete genotype and phenotype data and were included in analyses.

Blood samples were taken after a minimum 6-hour fast and samples were used for assessment of circulating L-ascorbic acid (which was assayed in duplicate, with the mean of the two values used in all analyses - see Supplementary Material)(31).

In this discovery study we also examined whether genotype and phenotype associations might be confounded. A priori we thought that due to population selection allele frequency might vary by birth geography and that since mobility in this cohort is generally low that this might also be related to variation in L-ascorbic acid levels and therefore this might confound gene-phenotype associations. In order to account for the geographic origin of participants, we matched the town/city of birth (reported by women on their first assessment) to geographical grid references giving the distance North and East of these locations from the British National Grid reference (located close to the Isle of Scilly, measured in metres). These measurements give an indication of latitude (distance North) and longitude (distance East) of place of birth. For other replication studies information on birth geography were not available and therefore we used the effect of adjusting for latitude and longitude of birth in BWHHS on the main association to consider how likely the unadjusted findings in other studies were to be influenced by this confounding. A number of

characteristics affect variation in vitamin C levels including socioeconomic position, physical activity, alcohol consumption and cigarette smoking, but we a priori assumed these would not be associated with genetic variants(32). However, we examined this assumption by assessing genotype associations with these characteristics as well as phenotype associations with these. Full details of how these characteristics were measured are presented in the Supplementary Material. These associations were not further explored in the replication studies.

Replication studies

Full details, including how DNA was extracted, genotype measured and L-ascorbic acid levels measured of all replication studies can be found in the Supplementary Material.

European Prospective Investigation of Cancer Norfolk Study (EPIC-Norfolk)

As first stage replication, we analysed data from a random sub-study of 5000 participants (EPIC5000) from the Norfolk arm of the European Prospective Investigation on Cancer (EPIC-Norfolk) study. Described in detail previously(33), EPIC-Norfolk is an ongoing prospective study of men and women aged between 40 and 79 years, resident in Norfolk, UK. For analyses here between 4500 and 4600 individuals were available with both genotypic and phenotypic data (the number varied depending on the SNP assessed).

MIDSPAN family study

The name MIDSPAN is given to 4 separate occupational and general population cohort studies based in Scotland. The 3 original studies took place between 1964 and 1976. Twenty years later in 1996 the next generation was studied when offspring of couples in the original Renfrew/Paisley Study were recruited into the Family Study(34). This latter group is the subject of the present analysis. For analyses here 1814 samples were available with genetic, phenotype and family data available.

10 Towns

The Ten Towns Study was a longitudinal study of the development of cardiovascular risk among children and adolescents in ten British towns, five with high and five with low adult cardiovascular mortality rates(35, 36). Analyses presented here are restricted to 1359 children of white European origin with genetic and phenotype data available.

British Regional Heart Study (BRHS)

In a study design very similar to that described in the BWHHS 7735 men aged 40-59 were recruited in 1978-80 from a single general practice in each of 24 towns across Great Britain, and have been followed ever since(37). In 1998-2000, when the participants were aged 60-79 years, 4252 were re-examined and most provided a whole blood sample. For analyses here 3740 samples were available with genetic and phenotype data available.

Genetic variation at *SLC23A1*

The locus *SLC23A1* was chosen as a locus of interest on the basis of previous available evidence suggesting a role for this locus in L-ascorbic acid transport(17, 22). Having identified this locus, SNPs were chosen on the basis of linkage disequilibrium and known genetic variation in this region in four populations in the USA (www.snp500cancer.nci.nih.gov)(28). The four SNPs chosen for analysis within the discovery cohort were distributed equally across the *SLC23A1* locus and tagged variation at this locus both in un-translated and genic regions. The location of SNPs across the *SLC23A1* locus is summarized in Figure 1.

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Statistical methods

Discovery study analyses

Hardy-Weinberg equilibrium was tested at each SNP locus using an exact test(38). Linkage disequilibrium (LD) estimates were quantified by D′ and r^2 values calculated using the Stata 11 (Stata Corp) package "pwld" (www-gene.cimr.cam.ac.uk/clayton).

We used linear regression to assess the association of circulating L-ascorbic acid levels with genetic variation at *SLC23A1* assuming an additive genetic model. L-ascorbic acid was not transformed for analysis as its variance (assessed from duplicate assays in BWHHS data), increased roughly linearly but only weakly with its mean, and a square-root transformation (which provided the best approximation to a normal distribution) would considerably hinder interpretability of the results. Although the distribution of L-ascorbic acid was somewhat positively skewed, the large sample sizes ensure robustness of the statistical methods to non-normality(39).

To examine the associations of L-ascorbic acid with potential confounding factors, continuously measured variables (socioeconomic position score, longitude and latitude) were split into tertiles. Mean differences in L-ascorbic acid by tertile of these variables and by category of categorical confounding variables were examined by linear regression. To examine genotype/confounder associations, mean differences of continuous variables and proportion of categorical variables are presented by genotype and were assessed again through the use of linear regression.

Replication study analyses

Associations between genetic variation and circulating L-ascorbic acid level in the discovery study (BWHHS) were first replicated in the EPIC replication study to determine whether primary results for rs10063949, rs6596473 and rs33972313 were robust. The same analyses were performed for the association between genotype and L-ascorbic acid adjusting for age and sex.

Following this, the sole replicating signal (rs33972313) was genotyped in the remaining three independent studies. In the MIDSPAN study linear regression of phenotype on genotype was carried out using a linear mixed model with the Stata 11 (Stata Corp) command "xtmixed" including rs3397213, age, sex, L-ascorbic acid and a variable representing family identity. This ensured that the standard errors were correct for non-independence of participants.

Summary statistics (regression estimates and standard errors) from the regressions of circulating L-ascorbic acid (with sex and age included in the model and assuming an additive genetic contribution) on rs33972313 were pooled using meta-analysis with appropriate metrics for consistency(40). As the BWHHS, BRHS and 10 Towns studies were all assayed for L ascorbic acid in the same way and EPIC and MIDSPAN by different protocols, we anticipated the presence of some heterogeneity in pooled estimates. We accounted for this by using a random effects model and also conducted separate sensitivity meta-analyses in groups determined by assay protocol for purposes of comparison. Meta-analysis was performed in Stata 11 (Stata Corp), using the command "metan".

Results

The median value for circulating L-ascorbic acid level in the BWHHS (mean age 68.9) was 39.78μmol/L(inter-quartile range 21.22, 60.14). Comparison of the duplicate measures of

vitamin C in the BWHHS showed reasonable repeatability in this measurement (Figure 2) (standard deviation of difference 3.2μmol/L).

Within the BWHHS minor (^{m}) allele frequencies at SNPs rs10063949(T/C^m), rs6596473(G/C^m), rs6596471(A/G^m) and rs33972313(C/T^m) were 0.32, 0.28, 0.25 and 0.03, respectively. rs10063949, rs6596473 and rs33972313 all adhered to HWE (p>0.3), though rs6596471 showed a nominal departure consistent with a slight over-representation of heterozygotes (p=0.01). Measurements of the degree of linkage disequilibrium (LD) between these variants are shown in Table 1. Variants rs6596473 and rs33972313 were correlated with each other (independent of allele frequency, r^2>0.8), but all other pairwise comparisons showed low LD.

In the BWHHS, three SNPs showed evidence for association with circulating L-ascorbic acid. For each additional minor allele of rs10063949 and rs6596473, there was an associated increase in circulating levels of L-ascorbic acid (1.91μmol/L (95%CI 0.47, 3.34), p=0.009 and 2.86μmol/L (95%CI 1.39, 4.33), p=0.0001 per minor allele respectively). In contrast, for rs33972313 the addition of each rare allele was associated with a reduction on circulating levels of L-ascorbic acid (−4.15μmol/L (95%CI −0.49, −7.81), p=0.03 reduction per minor allele). These findings are summarized in Table 2.

In the BWHHS, there was evidence for the association of circulating L-ascorbic acid with six of eight potential confounding factors assessed (Table 3). Analysis of the relationship between the confounding features measured in the BWHHS and genetic variation at the *SLC23A1* locus showed there to be no strong evidence for any associations (Table 4).

First stage replication of genotype-L-ascorbic acid association in the EPIC study showed null results for rs10063949 (Table 5). In contrast to this, rs33972313 and rs6596473 showed association results consistent with those found in the BWHHS (−8.31μmol/L (95%CI −10.51, −6.11), p=1.7×10^{-13} and 1.01μmol/L (95%CI 0.14, 1.87), p=0.02 per minor allele respectively). Given the nature of the LD between these loci (Table 1) and the nature of their effects, we decided to follow-up the single SNP rs33972313 within three further studies (details of which can be seen in Supplementary Table S1).

Pooled analysis of the relationship between rs33972313 and circulating L-ascorbic acid across all five studies showed that each additional rare allele was associated with a reduction in circulating levels of vitamin C of −5.98μmol/L (95%CI −8.23, −3.73), p=2.0×10^{-7} per minor allele (Figure 3). There was evidence of heterogeneity when all five studies were included in the pooled analysis (I^2 value of 55.2 (95%CI 0.0, 83.0)%, p^{het} value = 0.06). This appeared to be largely accounted for by the differing assay protocols used to measure L ascorbic acid and in sensitivity analyses (shown in Supplementary Table S2 & Figure 3) pooling the BWHHS, the BRHS and the Ten Towns studies (L-ascorbic acid measured for these in the same laboratory with the same protocol), the per allele effect was −4.07μmol/L (95%CI −6.26, −1.87) with the pooled result for MIDSPAN and EPIC being −8.09μmol/L (95%CI −9.97, −6.22).

Discussion

We have identified a genetic variant (rs33972313) in the *SLC23A1* vitamin C active transporter locus that is reliably associated with circulating levels of L-ascorbic acid in the general population. We have also shown that unlike direct measurements of L-ascorbic acid, genotypes associated with circulating levels are not confounded by a series of factors which often make the interpretation of observational data difficult. This variant provides a reliable, non-confounded proxy for variation in L-ascorbic acid at the population level and has the

potential for application to applied epidemiological investigations concerned with the impact of chronic variation in L-ascorbic acid levels in the general population (32, 41-43).

The variant implicated in this study is found to lie within the *SLC23A1* gene which encodes the active L-ascorbic acid transporter SVCT1 and confirms a predicted association between variation at this locus and circulating levels of L-ascorbic acid(44). Although functional assays of this variant have not been performed, rs33972313 is known to lie in exon 8 of *SLC23A1* and to yield a missense change delivering a methionine ($Meth/A_{TG}$) form in the presence of the rare A allele as opposed to the common G allele derived valine (Val/G_{TG}) form. It is this rare allelic form which is associated with lower levels of circulating L-ascorbic acid and which is assumed to be the by-product of a conformational change or protein failure which impairs active transport. Knockout experiments have shown that $Slc23a1^{-/-}$ mice exhibit lower plasma L-ascorbic acid, failure to accumulate L-ascorbic acid, very high levels of body store L-ascorbic acid excretion and a surprising level of compensatory L-ascorbic acid synthesis (an ability not found in humans)(44). Furthermore, *Slc23a1* null offspring had greater perinatal mortality associated with their lower plasma L-ascorbic acid levels, although this could be avoided by supplementation during pregnancy. Notably, this supplementation not only rescued perinatal mortality (through placental transfer), but also raised maternal L-ascorbic acid levels suggesting that other routes to intestinal absorption do exist.

As well as being involved in the regulation of circulating levels of L-ascorbic acid, SVCT1 has been shown to be active in a series of locations including the intestine(24, 45), the kidney(26) and the respiratory system(27). The involvement of the sister isoform of SVCT1 (SVCT2) in both similar functions (relating to active ascorbic acid transport in the brain, respiratory system, intestine, adrenal glands, bone and the eye (46-48)) and with health outcomes directly(49) goes further to substantiate the likely role for *SLC23A1* variation in vitamin C regulation, however the direct relationship between rs33972313 and the function of SVCT2 remain unknown.

There are several limitations to the work presented in this investigation. Firstly, genotypic data available across the *SLC23A1* locus does not represent all genetic variation in this region for all populations. Whilst we are able to capture and assess the reliability of association signals at specific SNPs, this is not a comprehensive screen of the *SLC23A1* locus. Not only are our results limited in inference to genetic variation specific to populations of European descent, we have only examined variation at or around the *SLC23A1* gene. Eck et al(28) examine both variation here and in the related *SLC23A2* gene and suggest that whilst variation at the former may be tolerated (and as such provide informative variation in genomic code for the purpose of association studies), it appears that such variation has been selectively removed from the population in the case of *SLC23A2*. Other than an interesting population genetic observation, this does suggest that variation at this “protected” locus may be more relevant to biological function and indeed may be valuable to the exploration of inherent differences in L-ascorbic acid transport (an issue highlighted by the specific tissue activity of SVCT2(27)). Lastly, we acknowledge that methods used to measure circulating L-ascorbic acid levels were not uniform across the five studies and this may have contributed to some of the heterogeneity that we found between studies. Despite this, the findings from the five studies on associations between circulating levels and the *SLC23A1* locus were directionally similar and broadly consistent lending validity to our conclusions.

Despite these limitations, this report brings attention to a confirmed genetic associate of genetic variation in *SLC23A1* and circulating measures of L-ascorbic acid with this association being robust across people from five independent studies. This finding is

important for understanding the mechanisms involved in variation in this essential vitamin between humans. It also has potential implications for the assessment of causal relationships between circulating L-ascorbic acid and health outcomes through the application of this genetic variant as a proxy measure for variation in circulating L-ascorbic acid. Specifically, this genetic variant could be used as an instrumental variable to determine the causal effect of lifetime variation in vitamin C levels on risk of cardiovascular disease, diabetes, cancer and other chronic disease outcomes that have been found to be associated with variation in vitamin C but for which causality remains debated(32, 41-43). This is because this genetic variant (like most genetic variation) is likely to be unrelated to many of the common characteristics that confound the association of circulating L-ascorbic acid with these chronic diseases and since genotype is allocated at conception its association with disease outcomes could not be affected by reverse causality(32, 43). The use of genetic variation at *SLC23A1* will, however, require a single study with very large sample size or pooling of several large studies(43).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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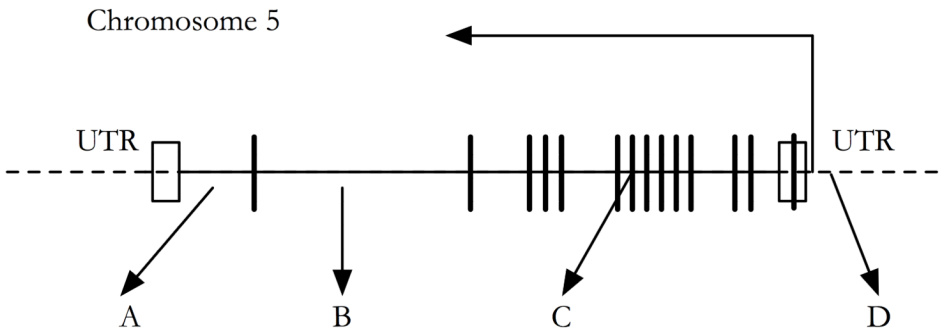

Figure 1.
Schematic of genetic variation assessed across the *SLC23A1* locus.
Variants assessed in discovery cohort, A - rs6596471 (138733487), B - rs6596473 (138738475), C - rs33972313 (138743401), D - rs10063949 (138747425). Open boxes represent 5′ and 3′ untranslated regions, strong bars represent exons. Base positions from Reference Assembly NC_000005.8

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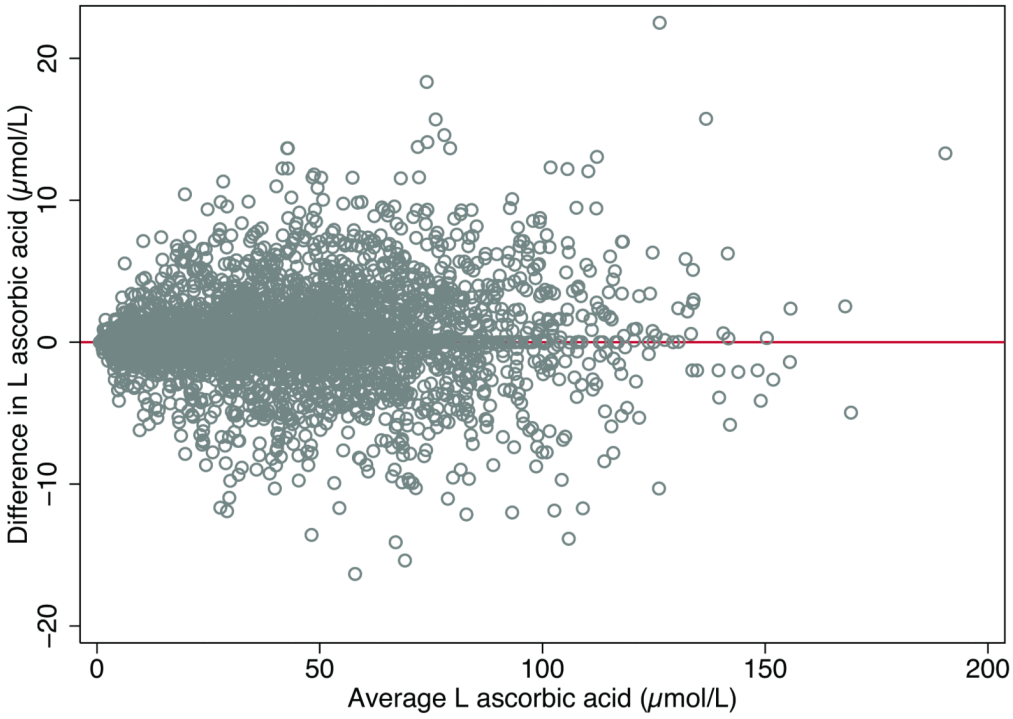

Figure 2.
Relationship between mean and difference in two repeat measurements of circulating L ascorbic acid (Bland Altman plot) in the British Women's Heart and Health Study (n = 3592).

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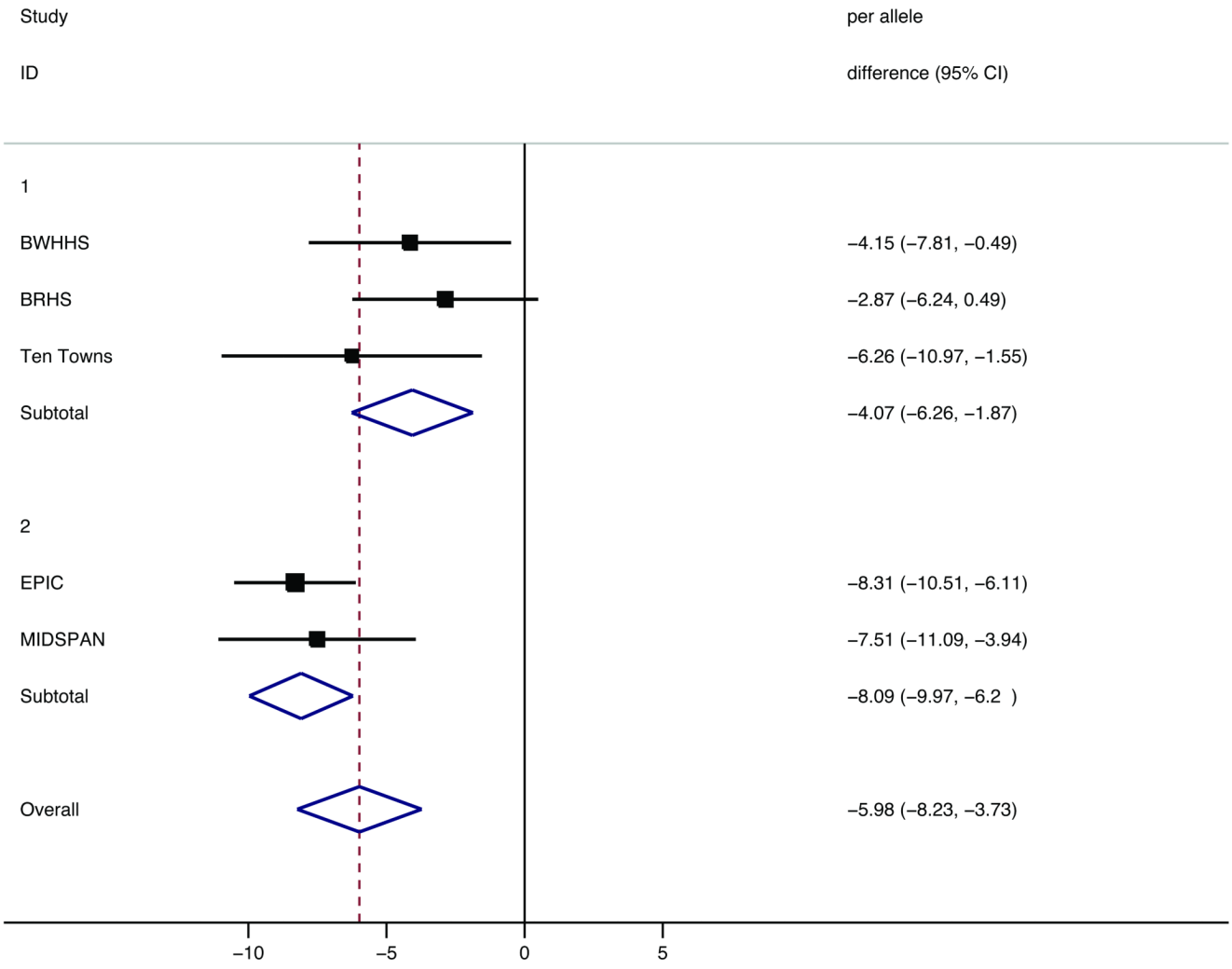

Figure 3.

Meta-analysis summary of rs33972313 association with circulating vitamin C from discovery and replication studies.

X axis represents associated difference in L-ascorbic acid per rare allele at rs33972313 (μmol/L). Sections 1 and 2 show sub-analyses by L-ascorbic assay type. BWHHS n=3425, BRHS n=3740, Ten Towns n=1359, EPIC n=4501, MIDSPAN n=1814. Overall - a meta-analysed pooled estimate of per allele association (random effects).

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Table 1

Linkage disequilibrium between SNPs across *SLC23A1* in the BWHHS.

Pos	SNP				
138733487bp	**rs6596471**	*0.25**			
138738475bp	**rs6596473**	0.48	*0.28**		
138743401bp	**rs33972313**	0.01	0.01	*0.03**	
138747425bp	**rs10063949**	0.37	0.82	0.06	*0.32**
		rs6596471	**rs6596473**	**rs33972313**	**rs10063949**

LD values (r^2)

HAPMAP build 36 chromosomal order (chr 5)

Values on diagonal (italics*) represent minor allele frequency of variants

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Table 2

Circulating L ascorbic acid by allelic variation at *SCL23A1* in the British Women's Heart and Health Study.

Genotype at rs33972313 (n=3252)					
L ascorbic acid µmol/L	**GG**	**GA**	**AA**	**Per allele effect**	**p**
	43.77 (42.80, 44.74)	38.63 (34.90, 42.37)	52.61 (30.16, 75.06)	−4.15 (−7.81, −0.49)	0.03
Genotype at rs10063949 (n=3365)					
L ascorbic acid µmol/L	**AA**	**AG**	**GG**	**Per allele effect**	**p**
	42.54 (41.15, 43.93)	43.85 (42.40, 45.30)	47.01 (44.07, 49.96)	1.91 (0.47, 3.34)	0.009
Genotype at rs6596473 (n=3215)					
L ascorbic acid µmol/L	**CC**	**CG**	**GG**	**Per allele effect**	**p**
	42.04 (40.72, 43.36)	44.35 (42.86, 45.83)	48.56 (45.31, 51.81)	2.86 (1.39, 4.33)	0.0001
Genotype at rs6596471 (n=3184)					
L ascorbic acid µmol/L	**TT**	**TC**	**CC**	**Per allele effect**	**p**
	43.01 (41.73, 44.29)	43.68 (42.17, 45.19)	45.56 (41.55, 49.58)	0.95 (−0.63, 2.53)	0.2

Means (95%CI) by genotype are adjusted for age.

Per allele effects and adjusted means are derived from linear regression.

Table 3

Circulating L ascorbic acid levels by levels of continuous and binary confounding factors in the British Women's Heart and Health Study

		Mean (95%CI) L-ascorbic acid			
Confounder	**n**	**Tertile 1**	**Tertile 2**	**Tertile 3**	**p**
SEP	2968	49.33 (47.71, 50.94)	42.72 (41.15, 44.29)	36.50 (34.26, 38.74)	2.8×10^{-20}
Latitude (m)	3404	46.43 (44.82, 48.05)	39.08 (37.43, 40.74)	43.16 (41.58, 44.74)	0.006
Longitude (m)	3404	40.89 (39.32, 42.45)	44.23 (42.66, 45.80)	43.95 (42.20, 45.70)	0.007
		Mean (95%CI) L-ascorbic acid			
Confounder	**n**	**No**	**Yes**		**p**
<1hr vigorous activity/wk	2972	43.56 (42.45, 44.67)	41.24 (38.93, .55)		0.08
< 2 drinks/day	3281	42.09 (41.04, 43.14)	49.49 (47.31, 51.67)		2.2×10^{-09}
Parental Cardiovascular disease	3320	43.54 (42.19, 44.89)	43.89 (42.54, 45.25)		0.7
Hormone replacement therapy	3397	43.06 (41.98, 44.14)	45.94 (43.85, 48.04)		0.02
Current smoker	3590	44.67 (43.71, 45.64)	32.47 (29.71, 35.22)		3.4e-16

Mean (95%CI) L ascorbic acid levels by continuous and binary confounding variables.

SEP denotes socioeconomic position score.

Tests of difference by confounder levels were derived from linear regression.

Table 4

Potential confounding factors by allelic variation at rs33972313 of *SCL23A1* in the British Women's Heart and Health Study

		Mean or proportion (95%CI) of each confounder			
		Genotype at rs33972313			
Confounder	**n**	**GG**	**GA**	**AA**	**p**
*SEP	3062	4.42 (4.33, 4.50)	4.54 (4.21, 4.88)	2.75 (0.50, 5.00)	0.8
*Latitude (m)	3512	395251.4 (389175.8, 401327)	397481.8 (374060, 420903.5)	300689.8 (158551.1, 442828.4)	0.8
*Longitude (m)	3512	405369.2 (401935.5, 408802.8)	408850.3 (395613.5, 422087)	378235.2 (297905.6, 458564.9)	0.8
<1hr vigorous activity/wk (y/n)	3125	18.39 (17.00, 19.86)	19.58 (14.81, 25.43)	20.76 (11.74, 34.04)	0.7
< 2 drinks/day (y/n)	3385	18.64 (17.32, 20.03)	19.26 (14.63, 24.93)	19.92 (11.32, 32.64)	0.8
Parental CVD (y/n)	3419	50.12 (48.39, 51.86)	52.12 (45.61, 58.56)	54.16 (41.13, 66.65)	0.5
HRT (y/n)	3507	18.56 (17.12, 20.08)	20.91 (16.23, 26.52)	23.68 (14.27, 36.66)	0.3
Current smoker (y/n)	3703	10.87 (9.87, 11.96)	8.53 (5.67, 12.65)	6.68 (2.87, 14.77)	0.3

Proportions/means (95%CI) of confounding features by allele derived from linear regression.

SEP denotes socioeconomic position score.

*Indicates continuous variable.

Table 5

Circulating L ascorbic acid by allelic variation at *SCL23A1* in the European Prospective Investigation of Cancer study.

	Mean (95%CI) L-ascorbic acid				
	Genotype at rs33972313 (n=4501)				
	GG	**GA**	**AA**	**Mean difference in L-ascorbic acid per minor allele**	**p**
L ascorbic acid μmol/L	56.66 (56.08, 57.24)	48.21 (45.99, 50.43)	43.38 (28.02, 58.73)	−8.31 (−10.51, −6.11)	1.7×10^{-13}
	Genotype at rs6596473 (n=4614)				
	CC	**CG**	**GG**	**Mean difference in L-ascorbic acid per minor allele**	**p**
L ascorbic acid μmol/L	55.54 (54.73, 56.35)	56.90 (56.05, 57.75)	57.09 (55.24, 58.94)	1.01 (0.14, 1.87)	0.02
	Genotype at rs10063949 (n=4539)				
	AA	**AG**	**GG**	**Mean difference in L-ascorbic acid per minor allele**	**p**
L ascorbic acid μmol/L	56.16 (55.31, 57.01)	56.19 (55.36, 57.03)	55.98 (54.29, 57.68)	−0.05 (−0.90, 0.80)	0.9

Means (95% CI) by genotype are adjusted for age and sex.

Per allele effects and adjusted means are derived from linear regression.