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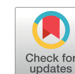
Gene interactions observed with the HDL-c blood lipid, intakes of protein, sugar and biotin in relation to circulating homocysteine concentrations in a group of black South Africans



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ABSTRACT

Background: Elevated homocysteine (Hcy) is associated with several pathologies. Gene–diet interactions related to Hcy might be used to customize dietary advice to reduce disease incidence. To explore this possibility, we investigated interactions between anthropometry, biochemical markers and diet and single-nucleotide polymorphisms (SNPs) in relation to Hcy concentrations. Five SNPs of Hcy-metabolizing enzymes were analyzed in 2010 black South Africans.

Results: Hcy was higher with each additional methylenetetrahydrofolate reductase (*MTHFR*) C677T minor allele copy, but was lower in methionine synthase (*MTR*) 2756AA homozygotes than heterozygotes. Individuals harboring cystathionine β synthase (*CBS*) 833 T/844ins68 had lower Hcy concentrations than others. No interactive effects were observed with any of the anthropometrical markers. *MTHFR* C677T and *CBS* T833C/844ins68 homozygote minor allele carriers presented with lower Hcy as high density lipoprotein cholesterol (HDL-c) increased. Hcy concentrations were negatively associated with dietary protein and animal protein intake in the TT and TC genotypes, but positively in the CC genotype of *CBS* T833C/844ins68. Hcy was markedly higher in TT homozygotes of *MTHFR* C677T as added sugar intake increased. In *CBS* T833C/844ins68 major allele carriers, biotin intake was negatively associated with Hcy; but positively in those harboring the homozygous minor allele.

Conclusions: The Hcy–SNP associations are modulated by diet and open up the possibility of invoking dietary interventions to treat hyperhomocysteinemia. Future intervention trials should further explore the observed gene–diet and gene–blood lipid interactions.

1. Background

Elevated circulating homocysteine (Hcy), which distinguishes hyperhomocysteinemia (HHcy), has been associated with several pathologies, including Alzheimer's disease [1], mental disorders such as schizophrenia [2], impaired bone health [3], type 2 diabetes [4], inflammatory bowel disease [5], adverse obstetrical outcomes [6], cancer [7] and cardiovascular diseases [8]. What complicates the etiology of pathologies contingent on Hcy is the fact that the molecule has its own set of environmental and genetic determinants. Of these determining

Abbreviations: A, adenine; Ala, alanine; Asp, aspartic acid; bp, base pairs; C, cytosine; *CBS*, cystathionine β synthase; CI, confidence intervals; CV, coefficient variation; d, Cohen's d-value; ES, effect size; G, guanine; GGT, gamma glutamyl transferase; GLM, generalized linear model; Gly, glycine; HbA1c, glycated hemoglobin; Hcy, homocysteine; HDL-c, high-density lipoprotein cholesterol; HHcy, hyperhomocysteinemia; HW, Hardy Weinberg; HWE, Hardy–Weinberg equilibrium; ID, identity; ISAK, International Society for the Advancement of Kinanthropometry; Ile, isoleucine; ins, insertion; LD, pairwise linkage-disequilibrium; LDL-c, low density lipoprotein cholesterol; MAF, minor allele frequency; MRC, Medical Research Council; MT, mutant type; *MTHFR*, methylenetetrahydrofolate reductase; *MTR*, methionine synthase; PA, physical activity; PCR, polymerase chain reaction; %TCHO, percentage total carbohydrate intake; %TE, percentage of total energy; PURE, Prospective Urban and Rural Epidemiology; QFFQ, quantitative food frequency questionnaire; RFLP, restriction fragment length polymorphism; T, thymine; SD, standard deviations; SE, standard error; SFA, saturated fatty acids; SNP, single-nucleotide polymorphism; T, thymine; Thr, threonine; THUSA, Transition and Health during Urbanization in South Africa; Val, valine; WT, wild type.

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factors, the exposure to environmental agents is modifiable, thus raising the prospect of intervention to reduce the likelihood of disease; however, non-modifiable factors such as age and genetics should also be taken into account.

By acknowledging the dietary aspect of Hcy metabolism, studies have demonstrated that Hcy and dietary methyl groups are interactively linked. Vitamin B_2 (riboflavin), vitamin B_6, vitamin B_{12} (cobalamin), folate, choline and betaine play key roles in the clearance of Hcy from the circulation [9]. Even though, interrelationships between biotin, folate and vitamin B_{12} have been reported [10–12], biotin has not been studied in relation to Hcy yet. Evidence also indicates the importance of macronutrients like protein, carbohydrates and dietary fats in influencing Hcy [13–18]. Hcy is synthesized in the liver as a response to the breakdown of the essential dietary amino acid, methionine. Acute methionine loading increases Hcy and is used to diagnose problems in Hcy metabolism [19–21]. Conversely, long-term increased protein (methionine) intake is inversely associated with Hcy [16–18]. Furthermore, there is a positive relationship between insulin resistance and Hcy [14,22,23]. However, studies investigating sugar intake in relation to Hcy are scarce [24]. Additional research exploring the relationship between dietary factors, especially macronutrients and Hcy, is needed.

Among the non-modifiable factors that are inherent in the functioning of Hcy, the methylenetetrahydrofolate reductase (*MTHFR*) C677T (rs1801133) polymorphism is a well-known genetic variant that results in an amino acid substitution (Alanine222Valine). This cytosine (C) to thymine (T) transition at nucleotide position 677 (c.C677T) causes a reduction of enzyme activity, which leads to inadequate methionine metabolism and the HHcy phenotype. Other variants—such as the insertion of 68 base pairs (bp) at position 844 (c.844ins68) in the cystathionine β synthase (*CBS*) gene that usually co-exists with the *CBS* T to C substitution at base 833 (c.T833C) (rs5742905), and the adenine (A) to guanine (G) substitution at position 2756 (c.A2756G) (rs1805087) within the methionine synthase (*MTR*) gene—also influence Hcy concentrations. CBS plays a role in Hcy clearance by regulating vitamin B_6-dependent *trans*-sulfuration. The A2756G polymorphism of the *MTR* gene decreases vitamin B_{12}-dependent remethylation of Hcy to methionine, which can possibly cause an increase in Hcy concentrations. Li et al. [25] reported that *MTHFR* 677TT, and *MTR* 2756AG + GG are independently correlated with high risk of folate deficiency and increased Hcy concentrations.

Although both dietary factors and genetic susceptibility have major effects on Hcy status, very few investigations have integrated genetic and dietary exposures. Such studies include that of Hustad et al. [26], who reported that vitamin B_2 modulated Hcy in homozygous *MTHFR* 677TT adults; and of Silaste et al. [27] and Kluijtmans et al. [28] that similarly showed this for folate; and of Nilsson et al. [29] for vitamin B_{12}. The minor G allele carriers of the *MTR* A2756G locus had a more prominent reduction in Hcy concentrations during high-folate intake than those with the homozygous major allele [27]. Lu et al. [30] showed that folate-deficient *CBS* variant carriers (p.D47E, c.T141A) had elevated Hcy compared with non-carriers, but that this difference disappeared after folate replacement. Studies that investigated possible interactions focused on folate and vitamin B_{12} only and neglected other dietary factors also involved in Hcy metabolism [9].

Our overall aim here is to report on whether there are interactions between anthropometry, nutritional biochemical markers and nutritional factors with specific genetic variations of genes, coding for enzymes involved in Hcy metabolism (*MTHFR* C677T, *CBS* T833C/844ins68, *CBS* G9276A and *MTR* A2756G), in relation to Hcy status in a black South African population. Proper understanding of gene–diet interactions may in future increase our ability to identify at-risk individuals who are particularly susceptible to HHcy and complex diseases contingent on Hcy as a result of dietary insult.

Here we record that the relationships of common genetic variations of genes in the Hcy metabolism with Hcy concentrations were modulated by dietary intake of sugar, protein and biotin (vitamin B_7/H), as well as one of the blood lipids, high-density lipoprotein cholesterol (HDL-c). Making this the first study of its kind, to our knowledge, to explore dietary factors other than coffee, alcohol, folate, vitamin B_{12} and vitamin B_2 intake, paving the way for future experiments exploring the newly identified gene–diet interactions.

2. Methods

This investigation was conducted on the baseline data of the South African arm of the Prospective Urban and Rural Epidemiology (PURE) study, which examined the prevalence of non-communicable disease risk factors in several countries experiencing urbanization [31]. For our study, only the data from the South African arm were used; sampling procedures and study design are described in detail elsewhere [32]. In short, selection of ostensibly healthy black participants, stratified according to urbanization level from a census including 6000 households, resulted in the recruitment of 4000 eligible individuals. Of those meeting the inclusion criteria, 2792 (1348 = urban, 1444 = rural) gave written informed consent to take part in the study and 2010 (1004 = urban, 1006 = rural) attended the measurement day.

Anthropometrical measurements were taken in accordance with the guidelines of the International Society for the Advancement of Kinanthropometry (ISAK) by ISAK-trained researchers. Measurements included height, weight and skinfolds as well as hip, waist and mid-upper arm circumferences.

Participants completed questionnaires verbally by means of interviews in the language of their choice. A standardized questionnaire was used to collect detailed demographic, health and lifestyle information. The dietary intake was assessed by means of a validated, interview-based quantitative food frequency questionnaire (QFFQ), which was developed in South Africa for the Transition and Health during Urbanization in South Africa (THUSA) study [33]. The QFFQ used for this study was validated against 7-day weighed-food records, 24-h urinary nitrogen excretion, as well as the estimated basal metabolic rate [34]. Food portion photograph books were specifically designed and standardized for this population. All the participants were asked to recall their usual food intake, including drinks, by reporting the frequency, amounts (models and food labels were used to demonstrate portion sizes) and preparation methods for the foods consumed during the previous month. The data obtained from the QFFQ were computerized, using the FoodFinder3® program [Medical Research Council (MRC)], Tygerberg, 2007) and sent to the South African MRC for nutrient analyses.

Blood samples were drawn after a 12-h overnight fast. Plasma for the quantification of total Hcy was separated with minimal delay and stored at −80 °C until analysis. Hcy concentrations were quantified by a pathology firm using the Abbott automated immunoassay analyzer (AxSYM), which is based on fluorescence polarization immunoassay technology [coefficient of variation (CV) = 4.52%].

Serum lipids and gamma glutamyl transferase (GGT) were measured using a sequential multiple analyzer computer, using the KonelabTM auto analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland), a clinical chemistry instrument for colorimetric, immunoturbidometric and ion-selective electrode methods [35]. Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald–Levy–Fredrickson formula [36].

Fasting glycated hemoglobin ($HbA1_C$) was determined using whole ethylenediamine tetra-acetic acid blood for measuring $HbA1_C$ values, with a D-10 hemoglobin testing system (Bio-Rad Laboratories, Hercules, CA, USA). Fasting plasma glucose was quantified by using a hexokinase method of the SynchronR System (Beckman Coulter Co., Fullerton, CA, USA).

Genomic DNA was isolated from buffy coat using an established

Table 1
Frequencies of SNPs in the *MTHFR*, *MTR* and *CBS* genes and their relationship with Hcy.

Gene	SNP (SNP ID; SNP location)	Genotype (genotype frequency)	Genotype frequency %	95% CI of genotype frequency (%)	MAF	Spearman correlation with Hcy		Hcy (μmol/L)	ES (*d*)
						r	*p*		
MTHFR	C677T; Ala222Val	CC (1579)	84	82.2–85.6	0.08	0.10	< 0.0001	10.1 (9.9; 10.3)$^{\partial\gamma}$	0.22
	(rs1801133;	CT (286)	15.2	13.7–17.0				11.1 (10.6; 11.6)$^{\partial\gamma}$	
	1:11796321)	TT (15)	0.8	0.37–1.17				18.5 (16.2; 20.7)$^{\gamma\gamma}$	
								GLM $p < .00001$	
MTR	A2756G; Asp919Gly	AA (1194)	63.7	61.1–65.4	0.21	−0.06	0.01	AA: 10.6 (10.3; 10.8)$^{\partial}$	0.16
	(rs1805087;	AG (590)	31.5	29.2–33.4				AG/GG: 9.9 (9.5; 10.2)$^{\partial}$	
	1:236885200)	GG (89)	4.8	3.76–5.68				GLM $p = .004$	
CBS	T833C; Ile278Thr	TT (997)	53	50.6–55.1	0.27	−0.02	0.41	10.5 (10.2; 10.7)	0.07
	(rs5742905;	TC (746)	39.7	37.3–41.7				10.2 (9.9; 10.5)	
	21:43063074)	CC (138)	7.3	6.14–8.49				GLM $p = .16$	
	844ins68 indel (no rs#)	Homozygous non-insert (WT) (998)	53	50.7–55.2	0.27	−0.02	0.41	10.5 (10.2; 10.7)	0.07
		Heterozygous (748)	39.8	37.4–41.8				10.2 (9.9; 10.5)	
		Homozygous insert (MT) (136)	7.2	6.04–8.38				GLM $p = .16$	
	G9276A	GG (977)	51.9	49.5–54.0	0.28	−0.02	0.44	10.5 (10.2; 10.7)	0.07
	(novel SNP no rs#)	GA (757)	40.3	37.9–42.3				10.2 (9.9; 10.5)	
	21:43071860	AA (146)	7.8	6.53–8.95				GLM $p = .17$	

Hcy concentrations are means adjusted for age and GGT (95% CI).
Significant ($p < .01^{\partial}$; $p < .001^{\gamma}$) differences between the subdivisions as indicated by post hoc test, i.e. t-tests, corrected for multiple comparisons.

method. All polymorphic variants were analyzed using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP), details of which are described elsewhere [32].

Haploview software version 4.2 (developed in Mark Daly's laboratory at the Broad Institute; http://www.broad.mit.edu/mpg/haploview) was used to calculate the level of pairwise linkage-disequilibrium (LD) between the *CBS* SNPs, using both D' and r^2 values [37]. One limitation of this software is that it does not allow for the entering of indels; therefore, LD between the *CBS* c.T833C and *CBS* c.844ins68 was determined using Statistica® (Statsoft Inc., Tulsa, OK, USA). Additionally, Haploview was used to compare the expected genotype frequencies according to the assumptions adhering to Hardy–Weinberg (HW) equilibrium (HWE) with those observed in our study.

Statistical analyses were performed using the computer software programs Statistica® and SAS System for Windows (SAS Institute Inc., Cary, NC, USA). Quantitative variables were subjected to normality testing by using the Shapiro–Wilks Normality test, as well as visual inspection using Q-Q plots. Skewed variables were log transformed and, in the cases where log transformation increased normality, parametric statistics were performed.

Descriptive statistics were calculated and continuous variables are presented as means ± standard deviations (SD) or medians (interquartile ranges) for normal and skewed data, respectively. Spearman correlations were computed to determine the relationships between two variables.

Differences in Hcy concentrations and other biochemical and nutritional variables among the genotypic subgroups were analyzed using general linear models (GLMs) adjusting for possible confounders, followed by post hoc tests. Differences in genotype frequencies among different Hcy strata and deviations from HWE were assessed by X^2 analysis. GLMs were used to determine visually how the genotypes should be coded. For the *MTHFR* c.C677T, a stepwise (upward) association was observed and an additive genetic model of action was constructed by dummy coding (0/1/2) to indicate the number of copies of the variant allele. For the *MTR* c.A2756G genotype, a dominant genetic mode of action for those containing the variant allele (minor allele) was observed and, therefore, we combined the heterozygotes with those homozygous for the variant alleles (0/1/1). No distinct patterns were present for the remaining genotypes, and as a result, coding was done by adding the heterozygotes to the homozygotes for the minor alleles similar to the dominant genetic mode of action. Subsequent correlations to determine the association of the genotypes with the Hcy phenotype were calculated using the above genetic coding.

To investigate whether factors—including anthropometry, biochemical variables and dietary components—modulated Hcy polymorphisms and influenced Hcy concentrations, GLMs (factorial) with adjustment for possible confounders, which allowed the assessment of any interaction effect over and above the main effects in the model being tested, were performed. Parametric statistical tests are robust and only slightly influenced by violations of the assumptions. Interactions that remained after excluding possible statistical outliers were reported as being significant.

Effect sizes (ES), standardized difference between two groups, were calculated as estimations of meaningfulness for *t*-tests as well as for the GLMs, using Cohen's formulae $d = |x1 - x2| / S_{max}$ and $d = |x1 - x2| / \sqrt{MSE}$, respectively, where d = the ES; MSE = the mean square error; x1 = the mean of one of the groups; x2 = the mean of the other group; and S_{max} = the maximum standard deviation of the two means [38]. D values of 0.2 or less are regarded as small, 0.5 as moderate and 0.8 or more as large ES [38].

3. Results

For details of the descriptive characteristics of our population, see Supplemental Table 1. Mean Hcy values of the population and for men and women were 9.2, 11.3 and 9.8 μmol/L, respectively, with the difference between the genders reaching statistical significance ($p < .0001$). According to the definition of HHcy determined by Castañon et al. [39] (i.e. fasting plasma Hcy concentrations > 12 μmol/L), 25.1% of our participants were hyperhomocysteinemic, which may bode adversely for them in the future as HHcy is associated with a range of pathologies. Correlations between different variables and Hcy can also be seen in Supplemental Table 1. Hcy correlated weakly with several dietary components and biomarkers; however, the positive correlations with age and GGT are noteworthy. Therefore, in subsequent statistical analyses they were adjusted for.

3.1. Genotyping and the individual influence of the SNPs on Hcy concentrations

The *CBS* T833C and *CBS* 844ins68 SNPs were found to be in linkage [32] and will be reported as *CBS* T833C/844ins68. Genotype information about the participants is presented in Table 1. All genotype distributions were in accordance with HW predictions. Most of the minor allele frequencies (MAFs) are comparable with those reported for African populations, whereas the MAF for the *CBS* T833C/844ins68 deviated from what has previously been reported [40]. For a full discussion of the genotypes' distributions, please refer to Nienaber-Rousseau et al. [32]. Additionally, Table 1 provides details on the frequencies of the investigated SNPs and their relationships with Hcy. When assuming the stepwise genetic model, *MTHFR* C677T correlated positively with Hcy concentrations. The only other significant, albeit weak, correlation with Hcy was a negative association with *MTR* A2756G when assuming a dominant genetic mode.

GLMs indicated that Hcy concentrations increased with each addition of the T allele at the *MTHFR* 677 locus (see Table 1). The *MTR* 2756AA homozygotes presented with significantly higher Hcy than their 2756AG peers, but heterozygotes did not differ from those homozygous for the minor allele. When combining the heterozygotes with the homozygote variants for this polymorphism, a definite lowering of Hcy concentrations was observed. Although statistically insignificant, *CBS* 833 T/844ins68 carriers had slightly lower Hcy concentrations than non-carriers. The difference in Hcy concentrations between the *CBS* 9276GG, 9276GA and 9276AA genotypes did not reach statistical significance although a trend towards higher Hcy in 9276G carriers was observed when compared with the other genotypes. Even though the *CBS* T833C/844ins68 and *CBS* 9276 polymorphisms did not associate with Hcy, they were taken into consideration when determining possible interactions with diet.

3.2. Interaction effects

As mentioned before, several dietary components were associated with Hcy; however, the Spearman's rho values were weak and exceeded 0.1 only for alcohol intake, added sugar expressed as a percentage of carbohydrate intake, total fat and saturated fatty acid (SFA) intake ($p < .01$). The interactions between nutrition-related dietary components and the different genotypes in relation to Hcy are indicated in Table 2. To explore the identified interaction further, correlations between the exposure in the interaction with Hcy were calculated stratified by genotype. These associations per subgroup can be compared to those provided in Supplementary Table 1 for the whole population, irrespective of genetic make-up. However, our focus is on the interactions and these are described below.

As can be seen in Table 2, we observed HDL-c blood lipid interactions with the *MTHFR* C677T and *CBS* T833C/844ins68 SNPs. At both loci, minor allele carriers presented with lower Hcy than the heterozygote and homozygote major allele carriers (interaction $p = .02$; $p = .001$, respectively) as HDL-c increased. Significant interactions occurred between the *CBS* T833C/844ins68 polymorphisms and total dietary protein intake as well as dietary animal protein intake (interaction $p < .001$; $p = .02$, respectively). In terms of the interaction, the homozygote minor allele carriers displayed an increase in Hcy as total dietary protein intake and animal protein intake increased, whereas Hcy decreased in the major homozygote TT and heterozygote TC genotypes when consumption was high. The interaction with animal protein remained significant after adjusting for B-vitamins and saturated fat intake.

Added sugar as percentage of energy and the *MTHFR* C677T polymorphism interacted so that the TT genotype carriers, when compared with the other allele carriers (interaction $p = .004$), presented with a prominent increase in Hcy as sugar intake increased.

Significant interactions were observed for the *CBS* T833C/844ins68 polymorphisms and biotin (interaction $p = .04$) in modulating Hcy concentrations. In carriers of the *CBS* T833C major allele, elevated biotin intake was associated with lowered Hcy, whereas Hcy was elevated in those harboring the homozygous minor allele. The interaction remained significant even after adjusting for other B-vitamins and lipids ($p = .04$).

4. Discussion

We hypothesized that certain individuals might be more susceptible to HHcy when their genetic make-up was combined with adverse dietary factors. Here we analyzed five genetic polymorphisms of Hcy-metabolizing enzymes in 2010 individuals, and quantified their interactions with anthropometric characteristics and biochemical markers influenced by diet and dietary components in modulating Hcy concentrations. Whereas previous research focused on vitamin B_2, folate and vitamin B_{12}, we investigated biotin and other neglected dietary factors as possible modulators of Hcy concentrations, thereby extending

Table 2
Effect of *MTHFR,* and *CBS* gene polymorphism interactions with markers of nutritional status on Hcy concentrations.

Interaction	Interaction *p* value	Genotype	*r*	*p* value	Slope[a] (± SE)	Slope *p* value
MTHFR C677T-HDL-c (mmol/L)	0.02	CC	0.18	< 0.000001	1.05 (0.18)	< 0.000001
		CT	0.25	< 0.0001	2.17 (0.41)	< 0.000001
		TT	−0.29	0.32	−2.94 (5.54)	0.61
CBS T833C/844ins68-HDL-c (mmol/L)	0.001	TT	0.23	< 0.000001	1.73 (0.24)	< 0.000001
		CT	−0.18	< 0.00001	0.91 (0.26)	0.001
		CC	−0.02	0.79	−0.17 (0.57)	0.77
CBS T833C/844ins68-protein (%TE)	< 0.001	TT	−0.08	0.02	−0.17 (0.08)	0.02
		CT	−0.04	0.30	−0.11 (0.08)	0.18
		CC	0.21	0.02	0.72 (0.20)	< 0.001
CBS T833C/844ins68-animal protein intake (g/day)	0.02	TT	−0.10	< 0.01	−0.03 (0.01)	0.01
		CT	−0.10	0.01	−0.03 (0.01)	< 0.01
		CC	0.16	0.07	0.03 (0.02)	0.09
MTHFR C677T-added sugar intake (%TCHO)	0.004	CC	−0.13	< 0.000001	−0.10 (0.02)	< 0.00001
		CT	−0.17	< 0.01	−0.12 (0.05)	0.01
		TT	0.48	0.08	0.60 (0.45)	0.21
CBS T833C/844ins68-biotin intake (μg/day)	0.04	TT	−0.10	< 0.01	−0.02 (0.01)	0.05
		CT	−0.09	0.02	−0.01 (0.01)	0.05
		CC	0.14	0.10	0.03 (0.02)	0.05

[a] In the regression line y = mx + c, 'slope' refers to m. All interactions presented in the table are adjusted for age and GGT, but the interaction with protein is additionally adjusted for SFA and the other B-vitamins while the interaction with biotin is additionally adjusted for the other B-vitamins (these results are discussed in text and not shown in the table).

existing knowledge. While examining the frequencies of selected SNPs of interest in the *MTHFR*, *MTR* and *CBS* genes and their relationship with Hcy, we determined that individuals harboring the *MTHFR* 677TT and *MTR* 2756AA genotypes presented with significantly higher Hcy concentrations than the other alternative genotypes. We also observed that, when the *MTR* 2756 heterozygotes and homozygote minor alleles were combined, a definite lower Hcy concentration was observed than homozygote major allele carriers. Several gene–diet and gene–blood lipid interactions were observed, i.e. *CBS* T833C/844ins68-HDL-c, *CBS* T833C/844ins68-protein intake (expressed as percentage of total energy), *CBS* T833C/844ins68-animal protein intake, *MTHFR* C677T-added sugar intake (expressed as percentage total carbohydrate intake) and *CBS* T833C/844ins68-biotin intake. In our study, alcohol intake and the liver function marker GGT correlated positively with Hcy concentrations. For a detailed discussion on this matter, please refer to Nienaber-Rousseau et al. [41].

The Hordaland Hcy study [42,43] reported that high intakes of SFA were associated with high plasma Hcy concentrations; we, however, found that SFA intake ($p < .01$) had a negative correlation with Hcy, as well as added sugar expressed as a percentage of carbohydrate intake and total fat intake. To explain this unexpected phenomenon, we reviewed previous studies, which indicated that a higher sugar and SFA intake in this particular population studied here, indicated a higher socio-economic status which, in turn, led to improved micronutrient status and better overall diet quality [31,44]. Improved diet quality and micronutrient intake assist in lowering Hcy concentrations, which explains the negative correlation observed in this population.

The *CBS* T833C/844ins68 polymorphisms showed the highest number of interactions, i.e. with blood lipids, protein and vitamin intake in relation to Hcy when compared with the other SNPs. The minor allele carriers of *CBS* T833C/844ins68 presented with lower Hcy concentrations as HDL-c blood lipids increased in those participants. In the same minor allele carriers, an increase in Hcy concentrations was observed as total dietary protein and animal protein intake increased ($p < .001$; $p = .02$), respectively; however, Hcy decreased in the major homozygote TT and heterozygote CT groups when consumption was high. Remarkably, biotin was the only vitamin in our investigation modulated Hcy concentrations ($p = .04$), where homozygotes of the major T allele indicated that increasing biotin intake was associated with lowered Hcy, whereas Hcy was elevated in those harboring the homozygous CC minor allele genotype. Given that the other B-vitamins besides biotin, on which Hcy metabolism depends, did not modulate the relationships of the SNPs in relation to Hcy, we postulate that variations in B vitamin intake across the genotypes was not large enough to produce any effects. It has been known for several decades that biotin, folate and vitamin B_{12} are interrelated and that they are involved in methylation [10–12]. Administration of vitamin B_{12} to rats on a biotin-free diet delayed biotin deficiency symptoms, while supplementation of biotin to biotin- and folate-deficient rats resulted in the restoration of liver folate concentrations [11,12]. Furthermore, Hcy's conversion to methionine is decreased in the livers of rats deficient in folate and biotin, partly because biotin influences the availability of folate coenzymes [10].

Previous studies support our findings that plasma Hcy is significantly and inversely correlated with HDL-c [45–49]. However, to our knowledge, none exists which took into account the *CBS* polymorphism when investigating the correlation between Hcy and HDL-c. Some examinations focused on CBS enzyme deficiency and determined that HHcy was more pronounced when serum levels of HDL-c were low [46,47,50]. Mechanisms have been identified which inhibit HDL-c biosynthesis in HHcy and reverse cholesterol transport, which leads to the negative correlation with Hcy (full review by Liao et al. [47]). As with the *CBS* T833C/844ins68, the well-known *MTHFR* C677T polymorphism minor allele carriers presented with lower Hcy concentrations as HDL-c increased. A previous study also found a correlation between plasma Hcy concentrations and plasma HDL-c, where subjects with the TT genotype had higher plasma Hcy values in association with lower HDL-c levels [51].

The literature indicates that serum Hcy concentrations are inversely correlated with daily total protein intake [52], which corresponds with what we observed in the major homozygote TT and heterozygote CT genotype carriers of *CBS* T833C/844ins68. We postulated that, for those harboring the T allele, prudent daily intake of protein had a protective effect on plasma Hcy concentrations as a result of protein-originated vitamin action. To test our hypothesis, we additionally adjusted for the B-vitamins and found that the interaction remained significant and, therefore, it is the protein intake per se that seems to be important.

One of the other interactions observed for *MTHFR* C677T and Hcy was with added sugar as a percentage of energy intake from carbohydrates, where TT genotype carriers presented with a noticeable increase in Hcy concentrations as sugar intake increased. We speculate that sugar poses an additional threat to increasing Hcy concentrations in those with the 677 TT genotype, because both the T allele [53] and higher glucose concentrations per se reduce MTHFR activity [15]. This underscores the potential benefits that may be gained by improving the dietary control of the glycemic milieu and a decreased intake of added sugar. Limited evidence on interactions between sugar intake and Hcy concentrations is available, especially for gene–sugar intake, which creates possible future research opportunities. Studies exploring nutrigenetic effects with regard to *MTHFR* C677T have, to date, focused on vitamin B_2, folate and vitamin B_{12} intake and their effects on Hcy concentrations. We, however, explored other dietary factors, which are less known or have not been previously investigated.

Here we contribute to the body of evidence underpinning Hcy nutrigenetics in a black South African population by using a large dataset. However, our population is not representative of all black South Africans and future studies should include other black South African ethnicities as well and preferably increase the sample size to account for SNPs with low frequencies. We recognize the inherent limitations of the QFFQ for dietary assessment including recall bias. Our method underestimated biotin intake and does not enable the determination of betaine or choline intake as a result of shortcomings to the South African food composition tables in terms of completeness. Because of the former, we predict that the interactive influence of biotin could be even more pronounced. To complement our work, researchers should use biochemical measures of vitamin intake including betaine/choline in order to account for differences in bioavailability, absorption and metabolism, which is not possible when using intake from questionnaire data alone.

5. Conclusions

The associations between Hcy and the genetic variants in the *MTHFR* and *CBS* genes are modulated by diet in this South African population, which implies that genotype-guided dietary intake might be warranted. For the general population, biotin consumption may reduce Hcy concentrations, whereas for those homozygous for the rare minor allele of *MTHFR* and *CBS* polymorphisms, consumption of this vitamin does not seem to have this benefit. The minority of the population harboring the minor allele of the *MTHFR* and *CBS* SNPs may, however, benefit in terms of Hcy from increasing levels of HDL-c, which is inversely associated with Hcy concentrations and interacts with these SNPs to lower Hcy. However, since high Hcy and low HDL-c might share the same lifestyle determinants they might not be causally related and could both be a marker of the same underlying conditions. Moderate to high protein intake as well as a healthy nutritional status presented with a seemingly protective action against increasing Hcy concentrations. Whether dietary manipulations of Hcy in the presence of certain genetic characteristics will actually result in Hcy lowering and subsequent disease risk reduction still needs to be determined in future studies. Our study supports the use of genetic information to

guide an individual's diet therapy; such information may have implications for health-care workers, especially doctors and dieticians, when they treat HHcy. As described here, new opportunities for improved risk-stratification tailored to treat HHcy in population subtypes according to their genotype are likely to emerge in the future.

Ethics approval and consent to participate

Ethical approval, in accordance with the Declaration of Helsinki as revised in 2004, was obtained for the larger study from the Health Research Ethics Committee of the Faculty of Health Sciences, North-West University (NWU–HREC, ethics number: 04 M10). Ethical approval was also granted for this affiliated study (ethics number: NWU-00332-16-S1). Goodwill permission was granted to the PURE study by mayors, household heads, community leaders of the communities included, and tribal chiefs before the research started. Written informed consent was obtained from all participants prior to participation.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Authors' contribution statement

JPdP performed the statistical analysis with CN-R, JPdP analyzed the data with CN-R and wrote the manuscript with CN-R; AM-B acquired funding for the project and co-authored the paper; LZ read and corrected the manuscript; CN-R isolated the DNA, performed the genotyping of the *MTHFR C677T*, *MTR A2756G*, *CBS T833C/844ins68* and *CBS G9276A*, conceptualized the paper, performed the statistical analysis with JPdP, and was involved in the writing of the manuscript with JPdP. All authors approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2019.100556.

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