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A nutrigenetic approach to examine the relationship between vitamin B12 status and cardio-metabolic traits in multiple ethnic groups: Findings from the GeNuIne Collaboration

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Abstract

Low vitamin B12 concentration has been shown to be a risk factor for cardio-metabolic traits in numerous observational studies; however, the relationship has remained inconsistent. It is possible that certain genotypes jointly contribute to cardio-metabolic diseases and vitamin B12 deficiency, and these may be modulated by dietary factors. The main objective of this article is to summarise the findings from the *GeNuIne* (Gene-Nutrient Interactions) Collaboration on the effect of gene–nutrient interactions on vitamin B12 concentrations and cardio-metabolic disease risk factors in population-based studies from different ethnic groups. Interactions between vitamin B12-related single-nucleotide polymorphisms (SNPs) and protein energy intake (%) on waist circumference ($P_{interation}$ = 0.002) and body fat percentage ($P_{interaction}$ = 0.034) were observed in Sri Lankan and Indonesian populations, respectively. In the study in Brazilian adolescents, the metabolic and vitamin B12-related SNPs showed a significant interaction with dietary carbohydrate and protein intakes on oxidised low-density lipoprotein cholesterol and homocysteine concentrations, respectively. In the Asian Indian population, an association between obesity-related SNPs and vitamin B12 concentrations (P = 0.018) was observed. In summary, these studies in multiple ethnic groups show that the association between genetically low vitamin B12 concentrations and cardio-metabolic traits may be modified by dietary intake. Further studies utilising larger sample sizes are needed to confirm or refute our findings.

Keywords: GRS, metabolic traits, nutrigenetics, obesity, SNP, vitamin B12 pathway

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Introduction

Vitamin B12 is an essential water soluble micronutrient, which participates as a cofactor for the synthesis of DNA, fatty acids and myelin (Smith *et al.* 2018). It has long been known that vegans, lacto-ovo vegetarians and older individuals are at risk of vitamin B12

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deficiency (Green *et al*. 2017). Vitamin B12 deficiency can also be caused by having inadequate amounts of intrinsic factor (IF), gastric atrophy, intestinal disease, gastric surgery, bacterial overgrowth in the small intestine, alcohol consumption, a tapeworm infection, drug–nutrient interactions and some genetic defects (Institute of Medicine 2000; Allen 2008; O'Leary & Samman 2010). Additionally, alarmingly high prevalence rates of low plasma vitamin B12 status have been recognised to exist in certain regions of the world including the Indian subcontinent, Mexico, Central and South America and selected areas in Africa (Stabler & Allen 2004).

Vitamin B12 functions as a coenzyme of several key enzymes in one-carbon metabolism, which in turn are essential for nucleotide synthesis and methylation reactions (Selhub 2002). The two active forms of vitamin B12, methylcobalamin and 5-deoxyadenosylcobalamin, are essential coenzymes for the homeostasis of methionine and methylmalonic acid (MMA), respectively (Koury & Ponka 2004; Sobczyńska-Malefora *et al*. 2014). Observational studies have shown that low vitamin B12 concentrations are accompanied by a wide range of chronic diseases and conditions, including obesity, insulin dysregulation and adverse cardiometabolic outcomes (Baltaci *et al*. 2013; Mahalle *et al*. 2013; Adaikalakoteswari *et al*. 2014; Narang *et al*. 2016; Chakraborty *et al*. 2018; Guarnizo-Poma *et al*. 2018; Jayashri *et al*. 2018). The aetiology between low vitamin B12 concentrations and metabolic phenotypes is still under debate and could be the result of several mechanisms. Vitamin B12 deficiency could possibly lead to adipocyte dysfunction by modulating lipid metabolism and enhancing cellular inflammation (Kumar *et al*. 2013). Several studies have suggested that vitamin B12 deficiency is more prevalent in individuals who are obese as result of either low dietary vitamin B12 consumption (Thomas-Valdés *et al*. 2017; Sun *et al*. 2019) or increased vitamin B12 demands secondary to an increased surface area of the body (Wall 1998; Pinhas-Hamiel *et al*. 2006).

Genetic studies have implicated several gene loci associated with circulating vitamin B12 (Surendran *et al*. 2018). To date, only three Mendelian randomisation studies have explored the relationship between a genetically determined decrease in serum vitamin B12 concentrations on cardio-metabolic traits such as body mass index (BMI) (Allin *et al*. 2017), blood pressure (Husemoen *et al*. 2016) and cardio-metabolic risk (Moen *et al*. 2018). However, these studies were unable to support the causal role of decreased serum vitamin B12 concentrations with these cardio-metabolic traits. Further to this, dietary factors play an important role in the development of vitamin B12 deficiency, but this relationship may differ across countries, due to the variation in food consumed worldwide (Vimaleswaran 2017). Studies have shown that the intrauterine imbalance of vitamin B12 and folate can affect DNA methylation and 'programme' the offspring to develop metabolic disorders later in life (Yajnik & Deshmukh 2012) providing evidence for interactions between genes and nutrients in the development of cardio-metabolic disease, including type 2 diabetes, hypertension, obesity and cardiovascular disease (CVD). The aim of this article is to provide an overview of the findings from the four studies in the gene–nutrient interactions (*GeNuIne*) Collaboration that used a genetic approach to explore the relationship between cardio-metabolic disease risk factors and vitamin B12 status and to investigate whether these relationships were modified by macronutrient intake (carbohydrate, fat and protein intake) (Vimaleswaran 2017).

Importance of studying gene–diet interactions in different genetic groups

It has been established that previous genetic studies looking at vitamin B12 status in healthy adults, especially large-scale ones, have been unable to capture the level of diversity which exists worldwide, as they are mainly based on individuals of European ancestry (Surendran *et al*. 2018). The under-representation of diverse ethnic groups hampers our full understanding of the genetic architecture of vitamin B12 concentrations (Sirugo *et al*. 2019). Furthermore, the limited genetic data on non-Caucasian populations in relation to genetic susceptibility to vitamin B12 deficiency can also impede our ability to translate genetic research into clinical care and will exacerbate health inequalities (Sirugo *et al*. 2019). Given that vitamin B12 status can also be determined by environmental factors, it is also important to explore gene–diet interactions in different ethnic groups. It is important to note that different ethnic groups respond differently to specific dietary interventions (Vimaleswaran 2017). Therefore, using estimates of genetic risk for vitamin B12 deficiency from European-based studies in non-Europeans may result in an inaccurate assessment of risk of vitamin B12 deficiency and could result in an inappropriate environmental intervention (dietary or physical activity) in under-studied populations. To address all these issues, the *GeNuIne* Collaboration (Vimaleswaran 2017) was initiated to investigate the effect of

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gene–lifestyle interactions on cardio-metabolic disease risk factors using population-based studies from various ethnic groups in lower-middle income countries (LMICs): Brazil (Surendran & Vimaleswaran 2019), Sri Lanka (Surendran *et al*. 2019b), India (Surendran *et al*. 2019c) and Indonesia (Surendran *et al*. 2019a). Another objective of the *GeNuIne* Collaboration was to look at gene–lifestyle interactions on vitamin B12 concentrations (Matusheski *et al*. 2021). A summary of the general characteristics of the populations and the SNPs investigated are provided in Tables 1 and 2.

Impact of genes and diet on homocysteine, vitamin B12, folate and lipids in a Brazilian adolescent population

Epidemiological studies have suggested that increased homocysteine concentrations may contribute to up to 25% of coronary events (Stanger *et al*. 2004) and have been shown to inversely correlate with B-complex vitamins, such as folate and vitamin B12 (Surendran & Vimaleswaran 2019). While B-complex vitamins have a role in the reduction of homocysteine

Table 1 Single-nucleotide polymorphisms (SNPs) under investigation: A comparison of studies in the Brazilian, Sri Lankan, Indian and Indonesian populations

	Brazil (n = 113)[a]	Sri Lanka (n = 109)	India study (n = 548)[c]	Indonesia study (n = 117)[b]
B12-related SNPs analysed	• Fucosyltransferase [*FUT2*]-rs602662 • Transcobalamin 2 [*TCN2*]-rs1801198 • 5-methyltetrahydrofolate-homocysteine methyltransferase or methionine synthase [*MTR*]-rs1805087 • 5-methyltetrahydrofolate-homocysteine methyltransferase reductase or methionine synthase reductase [*MTRR*]-rs1801394 • Betaine-homocysteine S-methyltransferase [*BHMT*]-rs3797546 and rs492842 • methylenetetrahydrofolate reductase [*MTHFR*]-rs1801131 and rs1801133	• *MTHFR*-rs1801133 • Carbamoyl-phosphate synthase 1 [*CPS1*]-rs1047891 • Cubulin [*CUBN*]-rs1801222 • CD320 molecule [*CD320*]-rs2336573 • *TCN2*-rs1131603 • Citrate lyase beta like [*CLYBL*]-rs41281112 • *FUT2*-rs602662 • Transcobalamin 1 [*TCN1*]-rs34324219 • Fucosyltransferase 6 [*FUT6*]-rs778805 • Methylmalonyl-CoA mutase [*MUT*]-rs1141321)	Not applicable	• *MTHFR*-rs1801133 • *CPS1*-rs1047891 • *CUBN*-rs1801222 • *CD320*-rs2336573 • *TCN2*-rs1131603 • *FUT2*-rs602662 • *TCN1*-rs34324219 • *FUT6*-rs778805 • *MUT*-rs1141321
Metabolic disease SNPs analysed	Catechol-o-methyl transferase [*COMT*]-rs4680 and rs4633	• Fat mass and obesity-associated [*FTO*]-rs9939609 and rs8050136 • Melanocortin 4 Receptor [*MC4R*]-rs17782313 and rs2229616 • Transcription factor 7-like 2 [*TCF7L2*]-rs12255372 and rs7903146 • Potassium voltage-gated channel subfamily J member 11 [*KCNJ11*]-rs5219 • Calpain 10 [*CAPN10*]-rs3792267, rs2975760 and rs5030952	*FTO*- rs8050136 and rs2388405	• *FTO*-rs9939609 and rs8050136 • *MC4R*-rs17782313 and rs2229616 • *TCF7L2*-rs12255372 and rs7903146 • *KCNJ11*-rs5219 • *CAPN10*-rs3792267 and rs5030952

[a]Brazilian adolescents who were overweight/obese and/or were previously diagnosed with dyslipidaemia, but not with cardiovascular disease, were included
[b]Only women were included for the Indonesian study
[c]Case-control study for type 2 diabetes in men and women

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Table 2 General characteristics, dietary intake and biochemical levels: A comparison of studies in the Brazilian, Sri Lankan, Indian and Indonesian populations

	Brazil (n = 113)[a]	Sri Lanka study (n = 109)	India study (n = 548)[c]	Indonesia study (n = 117)[b]
Study design	Cross-sectional	Cross-sectional	Case-control	Cross-sectional
General characteristics				
Age (years)	14 ± 2	38 ± 7	49.39 ± 11.45	40 ± 10
BMI (kg/m^2)	24.0 ± 4.9	24.6 ± 4.1	26.75 ± 5.04	25.1 ± 4.2
Waist-to-hip ratio	N/A	0.92 ± 0.11	0.90 ± 0.09	N/A
Fat (%)	N/A	27.25 ± 7.37	N/A	35.70 ± 7.00
Physical activity levels (%)				
Low	31	72.5	82.0	39.3
Medium	69	19.3	16.2	49.6
High		8.3	1.8	11.1
Dietary intake				
Total energy (Kcal/day)	2522 ± 586	2098 ± 456	2597 ± 773	1774 ± 609
Dietary carbohydrate (%)	47.7 ± 20.6	69.6 ± 8.8	64.3 ± 6.3	54.1 ± 9.4
Dietary protein (%)	17.0 ± 8.4	11.3 ± 2.3	11.3 ± 1.1	16.9 ± 3.3
Dietary fat (%)	25.4 ± 13.2	21.9 ± 5.3	23.8 ± 4.7	28.9 ± 8.0
Total fibre (g)	N/A	16.8 ± 8.2	32.2 ± 11.3	8.8 ± 4.5
Clinical parameter				
24 hours ambulatory systolic BP (mm Hg)	N/A	120 ± 15	129 ± 20	113 ± 9
24 hours ambulatory diastolic BP (mm Hg)	N/A	75 ± 16	80 ± 12	77 ± 6
Triacylglycerol (mg/dl)	94.05 ± 54.16	144.19 ± 86.81	146.54 ± 116.88	97.67 ± 42.80
HDL (mg/dl)	46.29 ± 11.79	42.56 ± 8.24	40.89 ± 8.85	58.99 ± 10.20
LDL (mg/dl)	90.28 ± 21.00	134.03 ± 28.60	114.37 ± 35.56	127.77 ± 39.17
VLDL (mg/dl)	18.85 ± 10.82	28.84 ± 17.36	N/A	N/A
Oxidised-LDL (U/l)	6.42 ± 13.69	N/A	N/A	N/A
Fasting plasma glucose (mg/dl)	N/A	86 ± 13	116 ± 49	92 ± 20
Fasting serum insulin (μlU/ml)	N/A	9.9 ± 7.2	9.23 ± 6.25	32 959 ± 26 327
Glycated haemoglobin (%)	N/A	5.4 ± 0.5	6.5 ± 1.7	N/A
B-vitamin biomarker status				
Vitamin B12 (pg/ml)	520 ± 232	516 ± 180	417 ± 255	591 ± 579
Homocysteine (μmol/l)	7.04 ± 2.99	N/A	13.67 ± 8.09	N/A
Folic acid (ng/ml)	11.02 ± 3.27	N/A	8.59 ± 5.81	N/A

BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

[a]Brazilian adolescents who were overweight/obese and/or were previously diagnosed with dyslipidaemia, but not with cardiovascular disease, were included

[b]Only women were included for the Indonesian study

[c]Case-control study for type 2 diabetes in men and women

concentrations, the effect of these vitamins on cardiovascular health remains ambiguous (Ntaios *et al*. 2009). Some studies have suggested that high folate and vitamin B12 status are associated with a lower risk of coronary heart disease (Voutilainen *et al*. 2001; Ishihara *et al*. 2008). Common variants in genes of the one-carbon metabolism pathway have been reported to influence concentrations of homocysteine, folate, vitamin B12 and lipids (Hazra *et al*. 2009). The aim of this study was to examine the association of ten SNPs involved in the one-carbon metabolism pathway with vitamin B12, folic acid, homocysteine and blood lipids, and to investigate whether environmental factors (dietary factors and physical activity levels) modified the association of the SNPs in 113 Brazilian adolescents (aged 10–19 years) with cardiovascular risk (overweight or obese and/or previously diagnosed dyslipidaemia, but not with CVD).

Since several SNPs were analysed in all the studies, correction for multiple testing was applied. No gene–lifestyle interactions were observed on vitamin B12 concentrations. This study provided evidence for interactions between the metabolic *COMT* SNP rs4680 and carbohydrate intake on ox-LDL concentrations and the B12-associated *FUT2* SNP rs602662 and protein intake on homocysteine concentrations. This is the first study to provide novel gene–diet interactions at the *COMT* and *FUT2* gene loci, on ox-LDL and

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homocysteine concentrations; hence, we have no other studies to compare our findings with. Given that ox-LDL and homocysteine are well-known independent risk factors for cardiovascular disease (Shenoy *et al*. 2014; Papageorgiou & Tousoulis 2016), these findings may have significant public health implications.

In addition to the genetic component of metabolic traits and markers of one-carbon metabolites, physical inactivity could be an important contributor that could interact with an individual's genetic predisposition. Our results showed that the *MTHFR* SNP rs1801131 showed a significant interaction with physical activity on folic acid concentrations. Folate plays a critical role in the methylation pathway and is involved in the methylation of DNA, creatine and acetylcholine, all of which are important for physical activity. It is important to note that physical activity levels may interact with folate metabolism by increasing intestinal folate absorption or stimulating the methionine synthase enzyme due to an increased metabolic demand and the associated possible increase in turnover of methylated molecules required for exercise (Kim *et al*. 2016). It is important that in the future, replications of this finding are made, preferably in a larger independent cohort with adequate statistical power utilising more direct measures of physical activity.

Impact of genes and diet on vitamin B12 concentrations and cardio-metabolic diseases in an Asian Sri Lankan population (*GOOD* study)

South Asians exhibit a unique phenotype collectively known as the 'South Asian Phenotype' which consists of higher levels of total and visceral fat, higher waist circumference and an increased susceptibility to type 2 diabetes (Mohan & Deepa 2006; Surendran *et al*. 2019b). Currently, over 34.4% of the Sri Lankan adult population are diagnosed as being overweight or obese (Katulanda *et al*. 2010). Although there is a strong genetic component to developing the 'South Asian Phenotype', consuming an unhealthy diet and leading a sedentary lifestyle can further contribute to this phenotype (Anjana *et al*. 2014; Vimaleswaran *et al*. 2016). Several studies have suggested that obesity is related to many micronutrient deficiencies including vitamin B12 (Astrup & Bügel 2010; Damms-Machado *et al*. 2012; Chakraborty *et al*. 2018). This is the first study to investigate the relationship between metabolic traits and vitamin B12 status using a gene-based approach in 109 Sinhalese adults [*Genetics Of Obesity and Diabetes (GOOD)* study; 61 men and 48 women, aged 25–50 years], and to investigate whether these relationships were modified by lifestyle factors (dietary factors/physical activity levels).

The unweighted genetic risk score (GRS) was calculated for each participant based on the sum of risk allele counts across each SNP which predicted a lower vitamin B12 status (B12-GRS). A second, unweighted GRS was created using allele markers previously reported to be associated with metabolic disease traits (metabolic-GRS). A numerical value of 0, 1 or 2 was assigned to each SNP, which indicates the number of risk alleles on that SNP. The scores were then calculated by adding the number of risk alleles across each SNP. The risk allele score was then divided by the median score into those carrying ≤9 risk alleles versus those with ≥10 risk alleles for the B12 GRS and ≤8 risk alleles versus those with ≥9 risk alleles for the metabolic-GRS.

The findings from this study demonstrated that a genetically lowered vitamin B12 concentration may be associated with central obesity (waist circumference) in the presence of a dietary influence (protein energy intake %). Additionally, the metabolic-GRS interacted with carbohydrate energy intake (%) to influence waist-to-hip ratio levels, where individuals carrying more than 9 risk alleles had a higher waist-to-hip ratio among those in the highest tertile of carbohydrate energy percentage compared to those in the lowest tertile (mean ± SD = 78.00 ± 7.90%; Fig. 1).

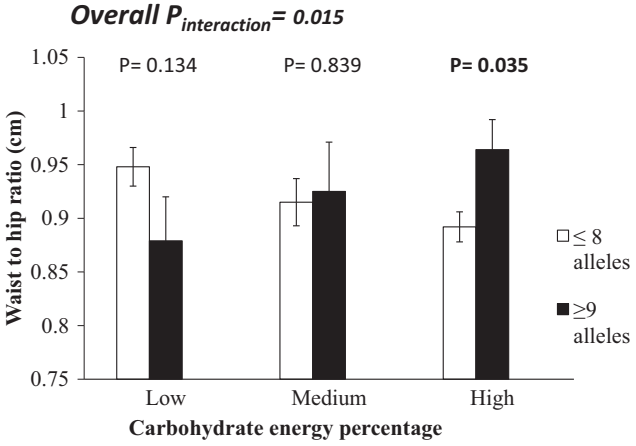

Figure 1 Interaction between the metabolic-genetic risk score and carbohydrate energy intake (%) on waist-to-hip ratio ($P_{interaction}$ = 0.015). Among those who consumed a high carbohydrate diet, individuals who carried nine or more risk alleles had significantly higher waist-to-hip ratios compared to individuals carrying eight or less risk alleles (P = 0.035)

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Further studies are warranted to investigate the mechanisms underlying the effect of these GRSs on metabolic traits. Given that the daily consumption of protein is low and carbohydrate intake is high in Sri Lankan adults (Jayawardena *et al*. 2014), these findings, if confirmed in randomised control trials, may have significant public health implications in terms of revising dietary guidelines for this population, which could prevent central obesity and its related CVD-related outcomes.

Impact of genes and diet on vitamin B12 concentrations and metabolic diseases in an Asian Indian population (*CURES* study)

Although vitamin B12 deficiency has been linked to obesity (Pinhas-Hamiel *et al*. 2006; MacFarlane *et al*. 2011; Baltaci *et al*. 2013) and diabetes (Yajnik *et al*. 2008; Krishnaveni *et al*. 2009; Knight *et al*. 2015), no study to date has tested the genetic link between metabolic traits and vitamin B12 status in an Asian Indian population (Surendran *et al*. 2019c). Hence, this study examined whether dietary intake and physical activity levels modified associations between a GRS using two previously studied *FTO* SNPs (rs8050136 and rs2388405) and vitamin B12 concentrations and metabolic disease-related outcomes in 548 Asian Indians (220 normal glucose-tolerant individuals, 152 individuals with prediabetes and 176 individuals with type 2 diabetes). In this study, participants were randomly recruited from the *Chennai Urban Rural Epidemiology Study (CURES)*, a cross-sectional case–control epidemiological study conducted on a representative population of Chennai in Southern India (Deepa *et al*. 2003).

To date, the *FTO* (fat mass and obesity associated) gene has been the strongest obesity risk loci in several populations (Vimaleswaran & Loos 2010). Thus, the *FTO* gene was considered as a suitable candidate to establish the genetic link between obesity-related traits and vitamin B12 concentrations and to determine whether this relationship was modified by lifestyle factors. We found no significant interactions between the *FTO*-GRS and lifestyle factors (diet and physical activity levels) in this study. However, a novel finding of this study indicated the potential association between the *FTO*-GRS and vitamin B12 concentrations, after adjustment for potential confounders. Carriers of more than one risk allele for the *FTO*-GRS had lower vitamin B12 concentrations (mean ± SD: 355 ± 189 pg/ml), compared to individuals carrying zero risk alleles (mean ± SD: 410 ± 202 pg/ml). The mechanism explaining the differences in vitamin B12 concentrations in the *FTO*-GRS could potentially be due to the *FTO* genotypes modulating gut microbiota and inducing metabolic inflammation, consequently impairing B12 absorption (Caesar *et al*. 2015; Chakraborty *et al*. 2018).

Given that low vitamin B12 concentrations in Asian Indians are common (Yajnik *et al*. 2006; Sivaprasad *et al*. 2016) and that 28–44% of Asians carry at least one copy of the *FTO* risk allele (Li *et al*. 2012), this study highlights the importance of considering obesity as a risk factor for vitamin B12 deficiency. This has implications for the possible targeting of relevant obesity prevention strategies, by focusing on increasing vitamin B12 intakes in obese or overweight individuals. Following such advice could substantially reduce vitamin B12 deficiency among Asian Indians.

Impact of genes and diet on vitamin B12 concentrations and metabolic diseases in an Indonesian women population (Minangkabau community: *MINANG* study)

Optimal vitamin B12 status is essential for women to maintain adequate maternal health and to avoid fetal developmental complications (Krishnaveni *et al*. 2009; Dror & Allen 2012; Surendran *et al*. 2019a). Additionally, low vitamin B12 concentrations have shown negative correlations with BMI in healthy women (Baltaci *et al*. 2017). Currently, the prevalence of low vitamin B12 status in healthy Indonesian women is unknown and studies on the relationship between low vitamin B12 status and obesity-related traits have yielded conflicting results (Wiebe *et al*. 2018). The Minangkabau population from Indonesia is of particular interest, as it is the largest matrilineal society in the world. Women in this society have relatively greater authority within the family compared to men and have a major role in dictating food choices and maintaining the welfare of the family (Stark 2013). In this population, GRSs based on nine B12-related SNPs and nine metabolic disease-related SNPs were constructed.

In the Indonesian study, a novel interaction between the B12-GRS and dietary fibre intake (g) on glycated haemoglobin was observed ($P_{\text{interaction}} = 0.042$; Fig. 2). Individuals who consumed a low-fibre diet (4.90 ± 1.00 g/day) and carried ≥9 risk alleles for vitamin B12 deficiency had significantly higher HbA1C concentrations compared to those carrying ≤8 risk alleles.

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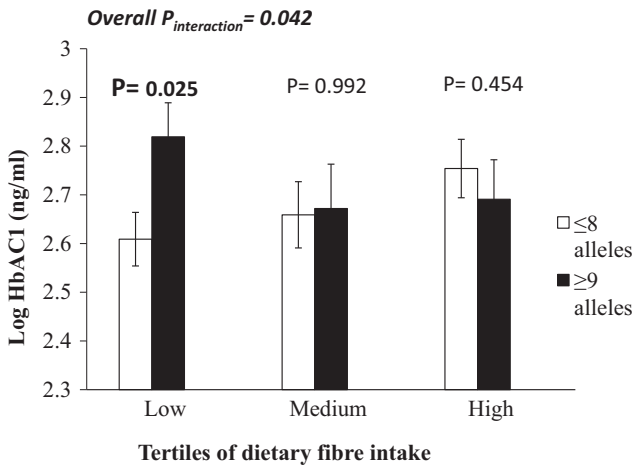

Figure 2 Interaction between the B12-genetic risk score and dietary fibre intake (g) on log HbAC1 (ng/ml; $P_{interaction}$ = 0.042). Among those who consumed a low-fibre diet, individuals who carried 9 or more risk alleles had significantly higher concentrations of log HbAC1 compared to individuals carrying eight or less risk alleles (P = 0.025)

Furthermore, interactions were also seen between the B12-GRS and protein (energy %) on log transformed body fat percentage.

Previous studies have shown that dietary fibre consumption is low in the Indonesian population, and thus, this finding, if confirmed in randomised controlled trials, could be the basis of a consumer education campaign centred around encouraging fibre intake to reduce HbA1C concentrations with those with genetic risk, which could improve glycaemic control in this population.

Ethnic-specific differences

The observations described in these studies confirm previous findings on population-based differences in vitamin B12 concentrations (McLean *et al*. 2008). The two South Asian populations demonstrated lower vitamin B12 concentrations than the Indonesian women population [Indian (417 ± 255 pg/ml) and Sri Lankan populations (516 ± 180 pg/ml) vs. Indonesian women (591 ± 579 pg/ml)]. Despite the Brazilian adolescent population exhibiting signs of cardiovascular risk, intermediate vitamin B12 concentrations were observed in this population (520 ± 232 pg/ml). It is difficult to generalise these findings meaningfully, as some of the populations included cohorts at risk of disease and furthermore the sample size of the population was limited (Table 2).

Several genetic loci have supported the presence of ethnic differences for metabolic traits and vitamin B12 status within the populations studied in this review. The *FTO* rs8050136 genotype is an example of genetic heterogeneity according to race. The minor allele 'A' of the SNP rs8050136 was present in 13% of Indian participants versus 23% of Indonesian participants and although India and Sri Lanka are geographically close, it was even more frequent in the Sinhalese population (34%).

The *FUT2* SNP rs602662 is one of the most commonly studied variants related to vitamin B12 status. The rs602662 SNP is an example of a B12 SNP that has demonstrated ethnic specificity, for example within the Indonesian population the minor allele frequency was extremely low (0.03%) in comparison to the Sri Lankan (31%) and Brazilian populations (41%). Although no genotyping errors were identified, the SNP rs602662 did not reach Hardy–Weinberg equilibrium (HWE), when using the chi-square test, within the Indonesian and Sri Lankan populations. It is possible that HWE was not reached in these populations due to the small sample size and the possibility of interbreeding (especially as consanguineous marriages are common in these populations).

How these findings could be translated into effective personalised and public health strategies

Obesity and perhaps vitamin B12 deficiency are important modifiable risk factors for a variety of chronic diseases. Obesity and vitamin B12 status are known to be associated, but the direction of the association and whether it is a causal relationship has been uncertain. Many genetic variants have been identified for vitamin B12 deficiency and metabolic diseases through candidate gene and genome-wide approaches (Bradfield *et al*. 2012; Willer *et al*. 2013; Surendran *et al*. 2018). However, it is possible that these genetic variants may not cause diseases without exposure to certain dietary factors (Rhee *et al*. 2012). Although this study has shown that low vitamin B12 concentrations are associated with metabolic diseases through a dietary interaction, the molecular and pathophysiological mechanisms remain unknown. It is important that mechanistic studies are carried out to determine how genetically low vitamin B12 concentrations interact with dietary factors to influence adipose tissue metabolism or how epigenetic mechanisms contribute to the development of metabolic diseases. Current literature also suggests that the genetic profile of an individual may shape the microbiome of the host, and an altered gut flora has been associated with vitamin B12 deficiency (Hall *et al*.

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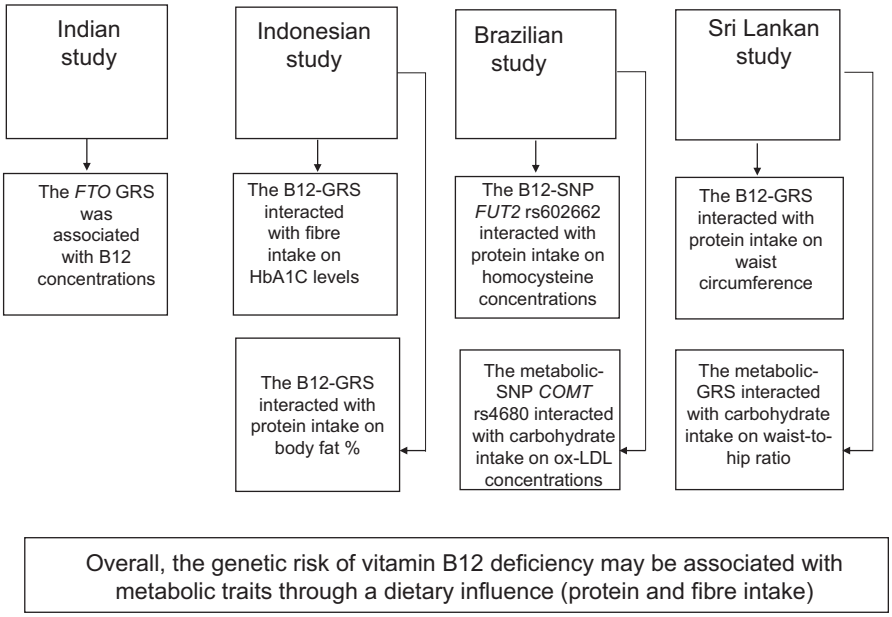

Figure 3 The main study findings of the *GeNuIne* Collaboration

2017; Surendran *et al*. 2018). This possibility requires investigation, given the known link between an altered distribution in gut microbiota and obesity. Hence, the combined application of nutrigenetics, nutrigenomics [dietary effects on gene expression (Ordovas *et al*. 2018)] and metabolomics (dietary effects on metabolite changes) is needed to clarify the gene–diet interactions identified in this project.

However, before the findings from these studies can be translated into personalised advice and/or public health strategies, they require confirmation from larger observational studies which include individuals from different ethnic groups and randomised controlled trials. It is difficult to truly isolate the macronutrient accountable for any nutrigenetic effects in observational studies, especially for fat and carbohydrates, as one macronutrient usually compensates the other (Sarzynski & Bouchard 2013). Therefore, dietary intervention studies are preferable as they can manipulate dietary intake more specifically. Dietary intervention studies also have the potential to avoid the measurement bias found within food frequency questionnaires and can demonstrate causal relationships between SNPs, diet and metabolic traits. Future studies should also measure intakes of specific dietary micronutrients to enable more detailed investigations of gene–diet interactions to be carried out. More studies that robustly measure both body composition (*e.g.* dual-energy X-ray absorptiometry, magnetic resonance imaging and/or computed tomography scans) and body size, and which measure vitamin B12 status using markers such as homocysteine, MMA and holotranscobalamin to investigate the effects of adiposity on vitamin B12 status across all body sizes are also needed (Wiebe *et al*. 2018).

Conclusion

In conclusion, the *GeNuIne* Collaboration identified novel interactions between vitamin B12-related genes and dietary factors (in particular protein intake and fibre intake) on cardio-metabolic traits within Brazilian, Sri Lankan and Indonesian populations. In addition, within an Indian population, individuals who were genetically predisposed to obesity had lower vitamin B12 concentrations, without influence from dietary factors (Fig. 3). Further understanding of the role of these gene–diet interactions at the molecular level is needed before diets can be tailored according to each ethnic subgroup.

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Conflict of interest

The authors have no conflict of interest.

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