Title: Genetic predisposition influences plasma lipids of subjects on habitual diet, but not the response to reductions in dietary intake of saturated fatty acids

Authors: Walker, CG\textsuperscript{1}, Loos RJF\textsuperscript{2}, Olson AD\textsuperscript{1}, Frost GS\textsuperscript{3}, Griffin, BA\textsuperscript{4}, Lovegrove JA\textsuperscript{5}, Sanders, TAB\textsuperscript{6}, Jebb SA\textsuperscript{1}

On behalf of the RISCK Study Group

1. MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK
2. MRC Epidemiology Unit, Institute of Medical Science, Cambridge, UK
3. Nutrition and Dietetic Research Group, Imperial College London, London, UK
4. Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK
5. Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading, UK
6. Nutritional Sciences Division, Kings College, University of London, London, UK

Corresponding Author:
Dr Celia Walker
MRC Human Nutrition Research
Elsie Widdowson Laboratory
Fulbourn Road
Cambridge CB1 9NL
UK

Email: celia.walker@mrc-hnr.cam.ac.uk
Phone + 44 (0) 1223 426 356
Fax: + 44 (0) 1223 437 515

Sources of funding: The RISCK dietary intervention trial was funded by the Food Standards Agency project number NO2031. Genetic analyses were funded by the participating centres.
Abstract

Objective: SNPs identified from genome-wide association studies associate with lipid risk markers of cardiovascular disease. This study investigated whether these SNPs altered the plasma lipid response to diet in the ‘RISCK’ study cohort.

Methods: Participants (n=490) from a dietary intervention to lower saturated fat by replacement with carbohydrate or monounsaturated fat, were genotyped for 39 lipid-associated SNPs. The association of each individual SNP, and of the SNPs combined (using genetic predisposition scores), with plasma lipid concentrations was assessed at baseline, and on change in response to 24 weeks on diets.

Results: The associations between SNPs and lipid concentrations were directionally consistent with previous findings. The genetic predisposition scores were significantly associated with higher baseline concentrations of plasma total (P=0.02) and LDL (P=0.002) cholesterol, triglycerides (0.001) and apolipoprotein B (P=0.004), and with lower baseline concentrations of HDL cholesterol (P<0.001) and apolipoprotein A-I (P<0.001). None of the SNPs showed significant association with the reduction of plasma lipids in response to the dietary interventions, with the exception of the HDL-C-predisposition score which was associated with a greater reduction in apolipoprotein A-I. There was no evidence of diet-gene interactions; however there were additive effects of low-fat diet and high genetic predisposition score to augment reductions in apo A-I.

Conclusion: Increased genetic predisposition was associated with an unfavourable plasma lipid profile at baseline, but did not influence the improvement in lipid profiles by the low-saturated-fat diets. The exception was the unfavourable decrease in apo A-I, a principal component of HDL.

Keywords: Dietary saturated fat, plasma lipids, SNP, genetic predisposition score, lipoprotein, gene-nutrient-interaction
**Introduction:**

Plasma lipids are risk factors for cardiovascular disease (CVD) and are known to be sensitive to dietary change (1). Plasma cholesterol can be altered by changes to the quality and quantity of fat in the diet; in particular total plasma cholesterol can be lowered by reductions in dietary total or saturated fat (2, 3). However, replacement of SFA with carbohydrate also reduces HDL cholesterol (HDL-C) with an associated increase in CVD risk (4). Replacement of SFA with monounsaturated fatty acids (MUFA) achieves a decrease in total (TC) and LDL cholesterol (LDL-C) without a decrease in HDL-C (4, 5). Genetic factors also exert a strong influence on the regulation of plasma lipids, with heritability estimates for fasting plasma lipids ranging from 35–60% (6-8). There is growing interest in the interplay between genetic and environmental factors, which may help to explain the variation between individuals in response to diet (6).

Genome-wide association (GWA) studies have identified a number of SNPs robustly associated with traits of dyslipidaemia in cross-sectional studies (9-16). However, it remains unknown whether these common lipid-associated SNPs also alter the responses to dietary interventions. We hypothesised that the mechanisms underlying genetic predisposition to dyslipidaemia would impair the improvement in plasma lipid status which can be produced by modifying the amount and type of dietary fat. This hypothesis was tested in a cohort of 490 participants in the RISCK trial; a highly-controlled intervention to reduce dietary saturated fat based on replacement with either carbohydrate (CHO) or monounsaturated fat (MUFA) (2). We examined the association of 39 lipid-associated SNPs, individually as well as combined, on plasma lipid measures at baseline and on the change in response to 24 weeks dietary intervention.

**Methods:**

Original RISCK trial study design
Full details of the RISCK trial have been published elsewhere (2). Briefly, men and women aged 30-70 years \((n = 720)\) were recruited from the general population. To participate in the trial, subjects had to be at increased risk of developing metabolic syndrome and CVD according to a study-specific scoring system (2). Self-reported ethnicity was recorded as White; South and South East Asian, Black African, or other.

The reference and intervention diets (described in detail in Moore et al. (17)) were designed to be iso-energetic, but varied in the amount and type of fat and carbohydrate. For the purposes of the current study the dietary intervention groups differing in carbohydrate quality were combined to focus the analyses on the manipulation of dietary fat, from which the impact in CVD risk was expected to be greater. The resulting three dietary groups were; ‘reference diet’ (REF) designed to reflect saturated fat intake in a ‘Western diet’ \((\sim 18\% \text{ of energy (E)}\) SFA, 12% MUFA, 38% total fat, 45% CHO); ‘MUFA diet’ in which SFA was reduced and replaced with MUFA \((\sim 10\% \text{ SFA, 20% MUFA, 38% total fat, 45% CHO}); ‘LF diet’, in which SFA was reduced through replacement of total fat with carbohydrate \((\sim 10\% \text{ SFA, 11% MUFA, 28% total fat, 55% CHO}).\)

All participants underwent a 4 week run-in period on the REF diet, after which anthropometry was measured and fasting blood samples were taken. Measurements taken after the run-in diet are referred to in this study as ‘baseline’ measurements. All participants followed their randomly prescribed diets for 24 weeks, after which a further blood sample was collected and anthropometry measured.

Ethical approval for the RISCK study (ISRCTN29111298) was granted from the National Research Ethics Service and written informed consent from participants was obtained including subsequent genetic analyses.

**Characteristics of study cohort**
Of the 720 participants, 549 completed the study and DNA was available for 512 participants. Based on self-reported ethnicity, we distinguished individuals of White (80%), South and South East (S, SE) Asian (9.5%); Black African (8%) and "other" (2.5%) ancestry. Analyses were stratified by self-reported ethnicity into three sub-groups with participants in the "other" subgroup excluded from this analysis (n=11). The characteristics of the participants in this study, stratified by the three main ethnic groups, are presented in Table 1.

The majority (91%) of the participants were overweight or obese and/or had elevated waist circumference (>94 cm for males and >84 cm for females) indicating the presence of central obesity.

We observed no significant differences for age, gender, BMI, body fat (%), waist circumference, triglycerides, HDL-C, apo B or apo A-I between the ethnic groups. Glucose concentrations were lower in S, SE Asians compared to Whites, insulin concentrations were higher in Black-Africans compared to Whites, and TC lower in Black-Africans compared to Whites (Table 1).

Plasma lipid analyses and response to dietary intervention

TC, HDL-C and triglyceride (TG) were analysed at King’s College London by enzymatic assay on a Bayer Advia Model Analyser using reagents supplied by the manufacturer (Bayer Diagnostics Europe, Newberry, Berks, UK). LDL-C was calculated using the Friedwald formula only if fasting TG concentrations were <4.49 mmol/L. Plasma apolipoproteins B (apo B) and A-I (apo A-I) were determined by immunoprecipitation assays (Randox Laboratories, Crumlin, UK) at the University of Surrey (see Jebb et al. (2) for further details).

As reported in full in Jebb et al. (2) plasma TC, LDL-C and apo B were significantly reduced in response to 24 weeks on the LF and MUFA diets. There was also a significant reduction in plasma HDL-C and apo A-I in response to the LF but not the MUFA diet. There was no change in plasma TG.
SNP selection and genotyping

Forty lipid-associated SNPs were identified from GWA studies published prior to April 2009. SNPs were only selected from GWA studies with at least 1000 individuals in the discovery stage, with replication in at least one independent population, and which reached the threshold of genome-wide significance of $P<5 \times 10^{-8}$ (9-16). Priority was given to SNPs that were plausible biological targets of lipid metabolic pathways, and that were identified by at least two independent GWA studies or meta-analyses. Where multiple SNPs resided in or near the same gene, SNPs in low linkage disequilibrium ($L^2 < 0.3$) were selected with a maximum of three SNPs per gene.

Genotyping was performed in the 501 participants of the three main ethnic groups who completed the study, and for which there was DNA available.

Genotyping was performed by KBiosciences (Hoddesdon, Herts, UK) using a fluorescence-based competitive allele-specific PCR (KASP) technology and all SNPs had a call rate $>95\%$. Individuals were excluded if genotyping was unsuccessful in $>10\%$ of SNPs (11 subjects). All genotype distributions were tested for deviation from the Hardy-Weinburg Equilibrium using the Log likelihood ratio chi-square test for association ($P<0.001$); and one SNP was excluded (rs174547) from analyses due to deviation ($P<0.0001$). This resulted in 14 HDL-C-associated SNPs in or near 11 genes; 12 LDL-C-associated SNPs in or near 11 genes; five TC-associated SNPs in or near five genes; and ten TG-associated SNPs in or near nine genes (Supplementary Table 1). Some SNPs were associated with more than one lipid trait.

Genetic Predisposition Score

A risk-allele was defined as the allele associated with raised TC, LDL-C, TG or low HDL-C in previous GWA studies (9-16). An additive model was assumed and individual SNPs were coded as 0, 1 and 2 on the basis of the number of the risk-
alleles for that particular SNP based on previous GWA studies (9-16). As there is currently no evidence for interaction between SNPs, a simple addition of the associated risk alleles for each trait has been commonly adopted (18-20). For each individual, genetic predisposition scores (GPS) were calculated for each of the traits separately (HDL-C, LDL-C, TC, TG) by adding all of the scores for each risk-allele associated with that trait (Supplementary Table 1). For participants missing individual genotyping data, the average count of risk alleles for the respective SNP was substituted for the missing genotype for the purposes of calculating the GPS. All GPS were normally distributed.

The SNPs selected for analysis are presented in Supplementary Table 1 showing the risk-allele frequency in each ethnicity for this cohort. Previous studies have shown that LDL-C-associated SNPs also associate with apo B and HDL-C-associated SNPs associate with apo A-I (16, 18). Therefore LDL-C-associated SNPs and LDL-C GPS were also used to examine the effect on apo B and HDL-C-associated SNPs and HDL-C GPS were used to assess the association with apo A-I.

**Statistical Analysis**

Distributions of traits were tested for normality; and baseline TG was log-transformed for analyses and presented in tables in this form. Linear regression analysis was used to test for associations between each SNP (coded as 0,1 and 2 according to the number of risk-alleles) and the relevant traits at baseline, assuming an additive effect of each additional risk allele, while adjusting for age, gender and BMI. Next, we tested for association between each SNP and change in lipid concentration following 24 weeks of intervention, adjusted for baseline values of respective trait, age, gender and BMI. There was no linearity assumption of relevant traits with diet, so each diet was added in the model individually.
Associations between the GPSs and respective traits (baseline and change) were tested with linear regression in the same way individual SNPs were tested, adjusting for the same covariates. All analyses were stratified by the three main ethnic groups. Summary statistics of the ethnicity-specific associations were pooled using inverse-variance fixed effects meta-analysis (metan function in Stata) and heterogeneity between the ethnicities was assessed by $I^2$ statistic. Statistical analysis was conducted using Stata 11 (StataCorp, Texas, USA).

**Results**

**Effect of genetic predisposition on lipids and apolipoproteins at baseline**

The trait-specific GPSs were all significantly associated with the respective traits; i.e. the higher the score the less favourable the lipid profile (Table 2). Each additional risk-allele in the TC-GPS was associated with 0.08 mM higher TC concentration; the LDL-C-GPS was associated with a 0.06 mM higher LDL-C concentration per additional risk-allele and the TG-GPS with a 0.04 mM higher lnTG concentration per additional risk-allele (Table 2).

The LDL-C-GPS was also associated with higher apo B concentration and the HDL-C-GPS with lower apo A-I levels per additional risk allele (Figure 1).

We observed directionally consistent associations for four out of five individual TC SNPs, of which one reached nominal significance; for nine out of 12 LDL-C SNPs, of which two reached nominal significance; for nine out of 13 HDL-C SNPs of which four reached nominal significance; and for five out of seven TG SNPs of which three reached nominal significance, when data of all three ethnic groups were combined (Supplementary Table 2).

Ten out of 12 LDL-C risk SNPs, were positively associated with apo B (Supplementary Figure 1a), of which two reached nominal significance.
Eleven out of 13 HDL-C risk SNPs were negatively associated with apo A-I (Supplementary Figure 1b), six of which reached nominal significance, four of which were also significantly negatively associated with HDL-C (rs1800775 and rs9989419 - CETP locus; rs10468017 - LIPC locus; rs4846914 - GALNT2 locus).

There was little evidence of heterogeneity between the ethnic groups except two TG SNPs and one HDL-C SNP that was directionally consistent in Whites and S,SE Asians but not in Black Africans (Supplementary Table 2).

Effect of genetic predisposition on the change in lipids and apolipoproteins in response to dietary intervention to lower SFA

Following the dietary intervention, the decrease in apo A-I was significantly greater the higher the HDL-C GPS, with an effect size of -0.01 g/L per HDL-C risk allele (P<0.05) (Table 3). The reducing effects of the LF diet and HDL-C-GPS were additive (Figure 2) but there was no significant diet x GPS interaction effect for apo A-I (P>0.09 for all ethnicities) or any other traits (data not shown). TC tended (P=0.1) to be further reduced by 0.03 mM per allele with higher TC-GPS (Table 3). There was also a trend (P=0.09) towards a greater reduction in HDL-C of 0.01 mM per allele with higher GPS. GPS was not associated with change in TG or with reduction of LDL-C or apo B (Table 3).

There was no evidence of heterogeneity across the three ethnic groups for the effect of GPS on the change in lipid or apolipoprotein traits.

Of the five TC SNPs, one in the APOB locus tended (P=0.06) to augment the diet-induced reductions in TC levels; of the 11 LDL-C SNPs, only one in the PCSK9 locus was significantly (P=0.04) associated with impeding the diet-induced reductions in LDL-C. No individual SNPs were associated with change in HDL-C, TG, apo B or apo A-I following the dietary intervention (Supplementary Table 3).
There was some indication ($P<0.1$) of heterogeneity between the ethnic groups for the effect of individual SNPs on change in traits with one TC SNP on the change in TC; 1 LDL-C SNP on the change in LDL-C and four on the change in apo B; one HDL-C SNP on change in HDL-C and two on change in apo A-I (Supplementary Table 3). Generally, associations were directionally consistent between the Whites and S,SE Asians but not Black-Africans.

While the intervention diets were designed to be iso-energetic, there was a significant ($P<0.001$) incidental weight loss on the low fat diet during the trial (2). As weight loss has been shown to improve lipid profiles (21), the analyses were repeated with and without weight change. There was no difference in the effect of SNP or GPS on change in plasma lipids (data not shown).

**Discussion**

Our results show the genetic predisposition scores were significantly associated with the respective lipid trait at baseline on a ‘habitual’ diet high in SFA. We also confirmed that many of the SNPs identified in GWA studies showed directionally consistent associations with lipid traits, and with effects of a similar magnitude in this cohort of subjects at increased cardiometabolic risk. We found no evidence that genetic predisposition to dyslipidaemic traits impaired the beneficial effects of a dietary intervention of reduced SFA intake to lower plasma TC, LDL-C and apo B. Furthermore, there was a tendency for greater improvements in plasma TC the higher the genetic predisposition to raised TC. However, unfavourable reductions in HDL-C and apo A-I were augmented by genetic predisposition to low HDL-C. This was an exploratory analysis in a relatively small cohort, but the results from this analysis suggests that further research is warranted to explore the role of these common SNPs as ‘effect modifiers’ in dietary intervention studies to improve plasma lipids and apoproteins.
Very few studies have examined the relationship between commonly occurring SNPs and plasma apolipoproteins. Previous GWA studies (16) and studies using the CardioChip (18) showed a close association between LDL-C-SNPs and apo B and HDL-C-SNPs and apo A-I. In the RISCK study cohort, we have shown that a cumulative GPS, composed of LDL-C-SNPs, was positively associated with apo B and that a cumulative GPS composed of HDL-C-SNPs was negatively associated with apo A-I at baseline. In response to 24 weeks dietary intervention both the LF diet and the HDL-C-GPS were significantly associated with a reduction in apo A-I. Although not significant, the association of GPS on change in HDL-C (P=0.09) was also negative. A disadvantage of a low fat diet can be a resultant decrease in HDL-C (4), as low HDL-C and apo A-I are in themselves markers of metabolic syndrome and CVD risk (22-24). This study shows that those genetically predisposed to low HDL-C, and who also have low apo A-I may have further adverse effects on lipid profile following a LF diet.

In this study, the opportunities to examine single SNPs were limited due to small sample size and the small effect size of the SNPs. Many of the selected SNPs were located in or near genes involved in lipid metabolic pathways (6). Many SNPs were also located in genes where rare monogenic mutations underlie causes of severe dyslipidaemia (25). Notably, SNPs of CETP (Cholesteryl Ester Transfer Protein) and LIPC (hepatic lipase) loci were significantly associated with baseline HDL-C and apo A-I as found previously both in GWA studies (9, 11, 13, 16) and in candidate gene studies (26, 27). However, these individual SNPs did not significantly alter the HDL-C and apo A-I response to dietary intervention. The rs4846914 SNP from the GALNT2 locus was also significantly associated with both baseline HDL-C and apo A-I in the current study. An association between this SNP and HDL-C was recently reported in a large meta-analysis of GWA studies of common variants and lipid traits, with follow-up functional studies in mice that...
implicated GALNT2 as a biological mediator of HDL-C concentration (28). In the current study, there was also no association of this SNP with change in apo A-I, but it was associated with a significant increase (0.03 ± 0.01 mM, P=0.03) in HDL-C in the White participants only, indicating a potential cardio-protective effect (Supplementary Table 2).

In the current study, there was little evidence of heterogeneity for individual SNPs or GPS on lipid traits between different ethnic groups, which is consistent with a recent large study that showed no evidence of heterogeneity between European, South and South-East Asian and African American populations in 95 lipid-related SNPs (28). There was a greater degree of heterogeneity between ethnic groups in the effects of SNPs on the change in lipid traits, however this finding can only be viewed as exploratory in view of the very small sample size of the Asian and Black subgroups. Nevertheless there was evidence that the effects of some SNPs in response to diets were greater in some ethnicities. For example SNPs rs693 (APOB locus) and rs3846662 (HMGCR locus) had a significantly greater reduction in plasma TC (effect size -0.26 and -0.31 mM per risk allele) in Black-Africans group but no significant effect in Whites (supplementary Table 3). As many of the SNPs reside outside coding regions of the genes, this may reflect that these SNPs were simply markers for other SNPs, and that the LD between the marker and functional SNP varies with ethnicity (29). This warrants further investigation in larger cohorts.

In conclusion, high plasma TC, TG, LDL-C and apo B and low apo A-I and HDL-C were influenced by genetic predisposition in this cohort of overweight men and women, who were identified at increased cardiometabolic risk. A dietary manipulation to lower SFA was successful in lowering plasma TC, LDL-C, and apo B, most notably in those genetically predisposed to dyslipidaemia. Conversely, genetic predisposition to a low plasma apo A-I, and possibly HDL-C, is
exacerbated in overweight subjects when dietary saturated and total fat is reduced in exchange for carbohydrate.

Acknowledgements

The RISCK Study Group comprised of: Carmel Moore, Mark Chatfield, Les Bluck, Christine Williams, Hannah Farrant, Claire Lawrence, Edel Magee, Kit Tsoi, Darren Cole, Steve Austin, Hanneke Mfuni, Kate Guberg, Anna Gent, Celia Greenberg, Caroline Stokes, Mario Siervo, Rosemary Hall, Louise Goff, Claire Howard, Namrata Dhopatkar, Bushra Siddiqui, Anne Dornhurst, Fiona Lewis, Samantha Bowen, L Chen, Robert Gray, Nuala Booth, Gary Moore, Roy Sherwood, Anthony Leeds, A Shah, G Saran, J Niehuser-Saran, JA Cockburn, Rachel Gitau, Katie Newens, Sean Lovegrove, Ana Rodriguez-Mateos, John Wright, Margaret Griffin, Nicola Harman. Special thanks to Aseel Al Saleh (Kings College London) for DNA extraction of samples.

The authors and their research groups have a number of links with the food industry. In a personal capacity GSF is a consultant to Coca-Cola, Premier Foods, and Unilever; and TABS has acted as a consultant to Seven Seas, is a member of the Scientific Advisory Committee for the Global Dairy Platform, the external scientific review committee of the Malaysian Palm Oil Board, and chairs Cadbury’s Global Nutrition Advisory Panel. TABS, BAG, JAL, SAJ, and GSF have received ad hoc honoraria for lectures or writing articles. CGW, RJLF and ADO reported no conflicts of interest.

In a non-personal capacity, BAG was formerly a member of an expert group known as the Fat Panel, which was supported by Dairy Crest, Kerry Gold, and Unilever; SAJ is a member of Scientific Advisory Boards for Coca-Cola, Heinz, PepsiCo, Nestlé and Kellogg’s; SAJ and JAL sit on government advisory boards that also include food industry members. All research groups received products from a range of food companies gratis for research purposes, including Archer Daniel Mills, Croda, Matthews Foods, Nestle, PepsiCo, Jordan, GSK, and Unilever.
Figure 1
(a) Variation of apo B at baseline by LDL-C genetic predisposition score (GPS) and
(b) Variation of apo A-I at baseline by HDL-C genetic predisposition score (GPS).
The squares represent the mean and standard error values of (a) apo B and (b)
apo A-I (right y-axes) for each GPS score category defined by the number of (a)
LDL-C and (b) HDL-C risk alleles per individual (x-axes). The histograms denote
the number of individuals in each GPS score category (left y-axes).

Figure 2
Apo A-I stratified by genetic predisposition score (GPS) before and after the
dietary intervention in the total cohort, and split into dietary intervention groups.
Data are presented as mean ± SE before and after 24 weeks on a dietary
intervention for a) the response in the total cohort and split into b) the REF diet
group; c) the high MUFA diet group; and d) the LF diet group. HDL-C-GPS was
stratified into 4 groups [≤11; 12-13; 14-15; ≥16 risk alleles]. The number of
subjects in each group was as follows: Total (GPS-1:129, GPS-2:138, GPS-3:149,
GPS-4:53); REF (GPS-1:17, GPS-2:28, GPS-3:22, GPS-4:9); MUFA (GPS-1:58,

Supplementary Figure 1
The effect of LDL-C-associated SNPs on apo B (a) and HDL-C-associated SNPs on
apo A-I (b) at baseline.
Data are presented as the effect size ± 95% CI from the meta-analysis of
summary statistics from linear regression analyses performed in the three
ethnicities between (a) individual LDL-C-SNPs and apo B and (b) individual HDL-C
SNPs and apo A-I at baseline. The models were adjusted for age, gender and
BMI.
Nominally significant (P<0.05) associations are in black symbols.
† rs1800588 (LIPC) was significantly heterogeneous (P<0.0001) for the 3 ethnicities, the meta-analysed data for the White and Asian subgroups only (Heterogeneity = 0.256) are presented for this SNP.
References


