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Title: Gene-nutrient interactions and gender may modulate the association between *ApoA1* and *ApoB* gene polymorphisms and metabolic syndrome risk

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## Abstract

**Objective:** Dyslipidemia is a key feature of the metabolic syndrome (MetS), which is determined by both genetic and dietary factors.

**Methods:** We determined relationships between *ApoA1* and *ApoB* polymorphisms and MetS risk, and whether dietary fat modulate this in the LIPGENE-SU.VI.MAX study of MetS cases and matched controls (n = 1754).

**Results:** *ApoB* rs512535 and *ApoA1* rs670 major G allele homozygotes had increased MetS risk (OR 1.65 [CI 1.24, 2.20] $P = 0.0006$, OR 1.42 [CI 1.08, 1.87] $P = 0.013$), which may be, partly, explained by their increased abdominal obesity and impaired insulin sensitivity ($P < 0.05$) but not dyslipidemia. Interestingly these associations derived primarily from the male GG homozygotes (*ApoB* rs512535 OR 1.92 [CI 1.31, 2.81] $P = 0.0008$, *ApoA1* rs670 OR 1.50 [CI 1.05, 2.12] $P = 0.024$). MetS risk was exacerbated among the habitual high-fat consumers (> 35% energy) (*ApoB* rs512535 OR 2.00 [CI 1.14, 3.51] $P = 0.015$, OR 1.58 [CI 1.11, 2.25] $P = 0.012$ for *ApoA1* rs670). In addition a high monounsaturated fat (MUFA) intake (> 14% energy) increased MetS risk (OR 1.89 [CI 1.08, 3.30] $P = 0.026$ and OR 1.57 [CI 1.10, 2.40] $P = 0.014$ for *ApoB* rs512535 and *ApoA1* rs670, respectively). MetS risk was abolished among the habitual low-fat consumers (< 35% energy). Saturated and polyunsaturated fat intake did not modulate MetS risk.

**Conclusion:** *ApoB* rs512535 and *ApoA1* rs670 may influence MetS risk. Apparent modulation of these associations by gender and dietary fat composition suggest novel gene-gender-diet interactions.


Supplementary key words: apolipoproteins, genetic polymorphisms, metabolic syndrome, insulin sensitivity, monounsaturated fatty acids

**Gene-nutrient interactions and gender may modulate the association between *ApoA1* and *ApoB* gene polymorphisms and metabolic syndrome risk**

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Abbreviations:

| | |
|---|---|
| Apolipoprotein | Apo |
| BMI | Body mass index |
| C3 | Complement component 3 |
| CRP | C reactive protein |
| CVD | Cardiovascular disease |
| HOMA | Homeostasis model assessment |
| MetS | Metabolic syndrome |
| MUFA | Monounsaturated fatty acid |
| PUFA | Polyunsaturated fatty acid |
| QUICKI | Quantitative insulin-sensitivity check index |
| SFA | Saturated fatty acid |
| Si | Insulin sensitivity index |
| SNP | Single nucleotide polymorphism |
| T2DM | Type 2 diabetes mellitus |
| TAG | Triacylglycerol |

1

## 1. Introduction

The metabolic syndrome (MetS) is a common, multi-component, condition characterised by dyslipidemia, abdominal obesity, insulin resistance and hypertension that promotes atherosclerosis and increases risk of cardiovascular disease (CVD) and type 2 diabetes (T2DM) [1]. Chronic low-grade inflammation is also thought to play a role in the pathogenesis of these conditions [2], with elevated concentrations of the inflammatory biomarkers complement component 3 (C3) and C reactive protein (CRP) associated with insulin resistance, diabetes, CVD and the MetS [3-5]. Elevated triglyceride and decreased high density lipoprotein (HDL) cholesterol concentrations are hallmarks of the dyslipidemic profile associated with the development of these conditions [6]. Apolipoprotein B (ApoB) and A1 (ApoA1) are the major structural and functional protein constituents of the triglyceride-rich lipoproteins and of HDL, respectively. Perhaps not surprisingly then the ApoB/A1 ratio has been associated with insulin resistance, the MetS and CVD [7, 8]. The genes encoding *ApoB* and *ApoA1* are highly polymorphic and common single nucleotide polymorphisms (SNPs) of these genes have been studied extensively in relation to plasma lipid and lipoprotein concentrations [9].

However the current global epidemic in the incidence of the MetS and T2DM highlight the contribution of both environmental and genetic factors to diet-related polygenic disorders. Dietary fat is an important environmental factor, wherein excessive exposure (high-fat, obeseogenic, insulin de-sensitising diets) and interaction with genetic factors plays a key role in the development of the MetS and CVD [10-14]. It is well known that dietary fat modifications alter plasma lipid metabolism differentially between individuals. This inter-individual variability in response to dietary modification is most likely due to genetic factors. Apolipoprotein E (ApoE)

genotype is the most widely studied genotype in this context [15]. Data from the FINGEN study, which examined the effect of long chain n-3 polyunsaturated fatty acid (LC n-3 PUFA) supplementation and ApoE genotype on plasma lipid related measurements, reported greater triacylglycerol (TAG) lowering effects following dietary intervention in ApoE4 males than in females [16]. Genetic variation at the *ApoB* locus has also been shown to influence TAG levels and TAG response to dietary fat modification, in particular to a monounsaturated fatty acid (MUFA) rich diet [17]. Data from the Framingham Heart Study showed that dietary PUFA intake modulates the effect of the *ApoA1* -75G/A polymorphism (rs670) on plasma HDL cholesterol concentrations in women but not in men [18]. Such data demonstrate how both dietary fat background and gender can influence genotype-phenotype associations. While ApoB and ApoA1 play important roles relevant to lipid metabolism and responsiveness to dietary fat, no studies to date have examined whether *ApoB* and *ApoA1* genotype and interaction with dietary fat intake predict the development of the MetS. Therefore this case-control study investigated the potential relationship between common genetic polymorphisms of *ApoB* and *ApoA1* and the MetS and its phenotypes, and whether they are modulated by gender and gene-nutrient interactions with dietary fatty acid intake.

## 2. Methods

### *2.1. Subjects, MetS classification and study design*

This study is part of a prospective case control candidate gene study of LIPGENE, an EU Sixth Framework Programme Integrated Project entitled "Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis". Participants were selected from an existing French SU.VI.MAX cohort

including 13,000 adults studied over 7.5 y beginning in 1994 to 2002 [19]. The LIPGENE-SU.VI.MAX study is a nested case control study of MetS consisting of women aged 35-60 y and men aged 45-60 y recruited from SU.VI.MAX. Additional ethical approval from the ethical committee (CCPPRB of Paris-Cochin Hospital) included an additional clause (n° Am 2840-12-706) to perform the biochemical analysis and genetic analysis required for the LIPGENE study. LIPGENE participants were informed of the study objectives and signed a consent form. Participants were invited to provide a 24 h dietary record every two months, for a total of six records per year. Baseline daily dietary intake data was estimated by using food composition tables validated for the French population [20].

Baseline and 7.5 y follow up data including full clinical examination records were made available to LIPGENE. This data was used to identify cases, individuals who developed elements of MetS, over the 7.5 y follow up period and controls. MetS cases were selected according to the NCEP-ATP III criteria for MetS [21]. Participants were required to fulfill at least three of the following five criteria: increased waist circumference [>94cm (men) or >80cm (women)], elevated fasting blood glucose [≥5.5 mmol/L or treatment for diabetes], elevated triacylglycerol (TAG) [≥ 1.5 mmol/L or treatment for dyslipidemia], low HDL cholesterol [<1.04 mmol/L (men) or < 1.29 mmol/L (women)] and elevated systolic/diastolic blood pressure [≥ 130/85 mmHg or antihypertensive treatment]. MetS cases were defined as both men and women with ≥ 3 abnormalities, and controls were defined as men and women with no abnormalities or men with ≤1 abnormality. Cases and controls (n=1754) were matched according to age (± 5 y), gender and number of dietary records available.

*2.2. Biochemical analysis*

Fasting glucose, TAG, HDL and total cholesterol were measured as previously described [19]. Insulin was determined by electrochemiluminescence immunoassays (Roche Diagnostics, France). NEFA and LDL cholesterol were measured by enzymatic colorimetric methods (Randox Laboratories, UK and Roche Diagnostics, France). Homeostasis model assessment (HOMA), a measure of insulin resistance, was calculated as: [(fasting plasma glucose x fasting serum insulin)/ 22.5] [22]. Quantitative insulin-sensitivity check index (QUICKI), a measure of insulin sensitivity, was calculated as = [1/(log fasting insulin + log fasting glucose + log fasting NEFA)] [23]. Total plasma C3 and CRP were measured as previously described [12].

*2.3. DNA extraction and genotyping*

DNA extraction from buffy coats and whole genome amplification of low yielding samples (<10 ng) was performed as previously described [12]. *ApoB* and *apoA1* genotype data from HapMap v1.1 (www.hapmap.org) was uploaded into HITAGENE, a web-based combined database and genetic analysis software suite developed by Hitachi Dublin Laboratory. Haplotype frequencies were estimated by implementation of the expectation maximization algorithm. Using a 5% cut-off for individual haplotype frequency and >70% for the sum of all haplotype frequencies, haplotype tagged SNPs were identified using SNP Tagger (www.broad.mit.edu/mpg/tagger/server.html). Together with SNPs identified in the literature, the following polymorphisms (*ApoB* rs1042031, rs1367117, rs512535, rs676210, rs679899, rs693, *ApoA1* rs5069, rs5070, rs5081 and rs670) were genotyped as part of the entire genotyping component of the LIPGENE study by Illumina Inc., (San Diego, CA, USA) using the Golden Gate Assay on a BeadStation 500G

genotyping system. We achieved an average genotyping success rate of 99% and call rate of 99%. Linkage disequilibrium between SNPs and departure of genotype distributions from Hardy-Weinberg equilibrium (HWE) were assessed in HITAGENE.

*2.4. Statistical analysis*

Statistical analysis was performed using SAS for Windows™, version 9.0 (SAS Institute, USA). Data is expressed as means ± SEM. After checking for skewness and kurtosis, glucose, insulin, TAG, QUICKI and HOMA were normalised by logarithmic transformation. Genotype frequencies were compared between cases and controls in HITAGENE using Fishers exact test. Conditional logistic regression determined associations between genotypes and the MetS. Three genotype groups were first considered to check different inherent models (additive, dominant and recessive). Where a dominant or recessive effect existed analysis was repeated comparing carriers versus non-carriers of that particular allele. Where gender differences were observed formal tests of heterogeneity (Breslow-Day) between the odds ratios of the gender-specific estimates were performed. To determine modulation by dietary fatty acids, logistic analyses were repeated using the median concentration of control subjects to dichotomise fatty acids and associations were examined below and above the fatty acid median. The generalised estimating equation (GEE) linear regression [24] investigated associations between genotypes and continuous MetS phenotypes. Analyses were performed on the whole study population and then stratified by gender to ascertain the homogeneity of genetic effects. Potential confounding factors used in the adjusted multivariate analysis included age, gender, BMI, smoking status, physical

activity, energy and alcohol intake and use of medications. A *P*-value of $< 0.05$ was considered as significant.

## 3. Results

### *3.1 ApoA1 and ApoB polymorphisms influence MetS risk*

**Tables 1** and 2 detail the *ApoB* and *ApoA1* polymorphisms studied. All SNPs were in HWE ($P > 0.05$). Examination of allele distributions revealed differences between MetS cases and controls for *ApoB* rs512535 (OR 1.22 [CI 1.07, 1.40] $P = 0.004$) and *ApoA1* rs670 (OR 1.32 [CI 1.09, 1.59] $P = 0.004$), whereby the major G alleles of both SNPs were more frequent in the MetS cases. Genotype frequencies were also different between MetS cases and controls for *ApoB* rs512535 ($P = 0.0003$) and *ApoA1* rs670 ($P = 0.015$). The association between both SNPs and MetS risk remained significant in the multivariate logistic regression analysis. MetS risk conferred by *ApoB* rs512535 GG homozygosity was over 65% higher relative to the A allele carriers (OR 1.65 [CI 1.24, 2.20] $P = 0.0006$) and was just over 40% higher in the *ApoA1* rs670 GG homozygotes compared to the A allele carriers (OR 1.42 [CI 1.08, 1.87] $P = 0.013$). Therefore we focused our analyses on these two polymorphisms.

### *3.2 Genetic influence on MetS risk and gender*

Homogeneity of the genotype effects of the *ApoB* rs512535 and *ApoA1* rs670 polymorphisms on MetS was assessed by stratifying according to gender. This analysis revealed that the association between *ApoB* rs512535 and MetS primarily derived from the male subjects (OR 1.92 [CI 1.31, 2.81] $P = 0.0008$, GG homozygotes relative to the A allele carriers). Although the effect was in the same direction in the female subjects it was not significant (OR 1.35 [CI 0.87, 2.12] $P = 0.18$). Similarly for *ApoA1* rs670 the association with MetS was more evident in the male subjects (OR 1.50 [CI 1.05, 2.12] $P = 0.024$) but failed to reach statistical

significance in the female subjects (OR 1.20 [CI 0.75, 1.92] $P$ = 0.44), GG homozygotes relative to the A allele carriers). Formal tests of heterogeneity (Breslow-Day) between the odds ratios of the gender-specific estimates confirmed the existence of gender-specific associations with MetS ($P < 0.05$).

*3.3 Clinical characteristics according to genotype*

The clinical characteristics and dietary fat intakes of the subjects according to *ApoB* rs512535 and *ApoA1* rs670 genotype are presented in **Table 3**. In terms of their phenotype, *ApoB* rs512535 GG homozygotes had elevated insulin concentrations ($P$ = 0.009), impaired insulin sensitivity ($P$ = 0.0007) and increased insulin resistance ($P$ = 0.017) compared to the A allele carriers. In addition GG homozygotes had greater BMI ($P$ = 0.019) and waist circumference ($P$ = 0.029) compared to the A allele carriers. Interestingly GG homozygotes also displayed higher C3 ($P$ = 0.0012) and CRP concentrations ($P$ = 0.043). Examination of the metabolic parameters for *ApoA1* rs670 revealed that GG homozygotes had higher insulin concentrations ($P$ = 0.044) and reduced insulin sensitivity ($P$ = 0.018), lower HDL cholesterol ($P$ = 0.004) and ApoA1 ($P$ = 0.002) concentrations, with a more inflammatory phenotype as reflected by their CRP status ($P$ = 0.044), relative to the A allele carriers. GG homozygotes also had greater BMI ($P$ = 0.001) and waist circumference ($P$ = 0.009) compared to the A allele carriers. Age, gender distribution, medication use, total energy, alcohol and dietary fat intake were not different between genotypes for either SNP. We also examined the clinical characteristics across genotypes according to gender and found that for both SNPs the results for the male, but not the female, subjects reflected the findings for the entire cohort (Supplementary Table 1).

*3.4 Gene-nutrient and gene-nutrient-gender interactions may modulate MetS risk*

We examined the influence of dietary fat intake on MetS risk by stratifying according to the control median fat intake. Interestingly MetS risk appeared to be modulated by dietary fat status and composition, whereby the risk conferred by GG homozygosity was accentuated among individuals who consumed a high-fat diet (> 35% energy), *P* for interaction (0.021) *ApoB* rs512535 and (0.017) *ApoA1* rs670, resulting in the following ORs: OR 2.00 [CI 1.14, 3.51] *P* = 0.015 and OR 1.58 [CI 1.11, 2.25] *P* = 0.012 for *ApoB* rs512535 and *ApoA1* rs670, respectively. Of note MetS risk was abolished among individuals who consumed a low-fat diet (< 35% energy), (OR 1.39 [CI 0.74, 2.62] and OR 1.08 [CI 0.75, 2.41] for *ApoB* rs512535 and *ApoA1* rs670, respectively *P* = ns). Each of the individual fat classes (PUFA, MUFA and saturated fat (SFA)) were higher in the high-fat consumers compared to the low-fat consumers (*P* <0.0001). Examination of the individual fatty acid classes identified a gene-nutrient interaction with MUFA (*P* for interaction (0.039) *ApoB* rs512535 and (0.024) *ApoA1* rs670), whereby GG homozygotes with high MUFA intake (> 14% energy) had increased MetS risk (OR 1.89 [CI 1.08, 3.30] *P* = 0.026 and OR 1.57 [CI 1.10, 2.40] *P* = 0.014 for *ApoB* rs512535 and *ApoA1* rs670, respectively). Dietary PUFA and SFA intake did not modulate MetS risk (data not shown). Due to the gender effect on genetic susceptibility to the MetS identified in this study we examined the impact of gender on the gene-nutrient interaction by analysing male and female subjects separately. The modulation of MetS risk by dietary fat intake observed in the entire cohort was reflected by the male high-fat consumers only (*P* for interaction (0.002) *ApoB* rs512535 and (0.01) *ApoA1* rs670), (OR 1.97 [CI 1.37, 2.86] *P* = 0.0003 and OR 1.57 [CI 1.10, 2.25] *P* = 0.012 for *ApoB* rs512535 and *ApoA1* rs670, respectively), in particular the male high-MUFA consumers (OR 2.04 [CI 1.38,

2.92] $P$ = 0.0003 and OR 1.57 [CI 1.09, 2.24] $P$ = 0.014 for *ApoB* rs512535 and *ApoA1* rs670, respectively).

*3.5 Gene-nutrient interactions may influence MetS phenotypes*

As the anthropometric and metabolic measurements were significantly different between genotypes for both *ApoB* rs512535 and *ApoA1* rs670, we additionally investigated the combined impact of dietary fat intake and genotype on these phenotypes (**Figure 1**). Interestingly, among the high-fat consumers further impairments to insulin concentrations (**Figure 1A**), insulin resistance (**Figure 1B**) and BMI (**Figure 1C**) were evident in the GG homozygotes of both SNPs compared to the A allele carriers and particularly to the GG homozygotes with low fat intake ($P$ < 0.05). It was also interesting to note that *ApoB* rs512535 and *ApoA1* rs670 genotype did not affect any of these phenotypes among individuals who habitually consumed a low-fat diet. Interaction analyses confirmed these gene-nutrient interactions ($P$ < 0.05).

Additionally we examined the impact of dietary fat intake and genotype on inflammatory and lipid related risk factors associated with atherosclerosis including concentrations of CRP and C3 and the ratios of LDL/HDL and ApoA1/B, indices of atherogenic risk. Of note gene-nutrient interactions for these parameters (CRP, C3 and LDL/HDL) were observed only with *ApoA1* rs670. In particular among the high-fat consumers, greater CRP (2.48±0.23 vs. 1.85±0.22 mg/L, $P$ < 0.01) and C3 concentrations (1.55±0.03 vs. 1.42±0.03 g/L, $P$ < 0.05) and also a higher LDL/HDL ratio (2.60±0.06 vs. 2.45±0.06, $P$ < 0.05) were noted in the GG homozygotes relative to the A allele carriers. No differences were observed between genotypes among the low-fat consumers.

## 3. Discussion

Associations between *ApoB* and *ApoA1* polymorphisms and plasma lipids, important risk factors for CVD and the MetS, have been extensively studied [9]. Nutrigenetic research has demonstrated that dietary fat background and gender can influence such genotype-phenotype associations [10, 16-18, 25, 26]. To our knowledge this is the first study to investigate whether *ApoB* and *ApoA1* genotype and interaction with dietary fat intake and gender can influence the development of the MetS. In this study we identified associations between common genetic variants of *ApoB* and *ApoA1* with increased MetS risk. This may be, in part, explained by higher fasting concentrations of insulin and the pro-inflammatory markers C3 and CRP, reduced insulin sensitivity and greater BMI/abdominal obesity in the GG homozygotes relative to the minor A allele carriers of *ApoB* rs512535 and *ApoA1* rs670. Additionally lower HDL cholesterol and ApoA1 concentrations were noted in the *ApoA1* rs670 GG homozygotes. Interestingly the genetic associations with MetS risk appeared to be modulated by gender and dietary fat intake and composition.

The *ApoA1* rs670 A allele has been associated with phenotypes related to reduced risk of CVD and diabetes such as higher ApoA1 and HDL cholesterol concentrations [27]. On the other hand *ApoA1* rs670 AA homozygotes have been associated with increased risk of impaired glucose tolerance and T2DM [28]. Such inconsistencies between studies may reflect differences in population size, study design, gender, genetic heterogeneity or indeed the dietary environment of the populations studied. In the current study, A allele carriers had reduced MetS risk, most likely due to their higher ApoA1 and HDL cholesterol concentrations and improvements in their metabolic and anthropometric phenotypes relative to the GG homozygotes, which should in turn also reduce their CVD and diabetes risk. Lifestyle

intervention programmes including dietary fat modification [18, 25, 26] have been shown to modulate the effect of the *ApoA1* rs670 on plasma HDL and LDL cholesterol concentrations as well as HDL sub-fraction distribution. Interestingly *ApoA1* rs670 A allele carriers were more responsive to dietary fat changes than the GG homozygotes [25, 26]. Genetic variation at the *ApoB* locus has also been shown to influence TAG concentrations and TAG response to dietary fat intervention, in particular to a MUFA-rich diet [17]. Gender modulated these and other gene-nutrient interactions [16, 18, 26]. We also noted gender differences for the associations between *ApoB* rs512535 and *ApoA1* rs670 and MetS risk. While the effect was in the same direction in the female subjects it did not reach statistical significance, which may reflect limited statistical power due to the smaller number of female subjects (60/40 male/female).

An individuals' phenotype represents a complex interaction between the genetic background and environmental factors over the course of an individuals' lifetime. Dietary fat composition is recognised as an important environmental factor which may alter MetS risk [10-14]. In the current study MetS risk appeared to be modified by dietary fat intake, whereby the deleterious effects conferred by GG homozygosity for *ApoB* rs512535 and *ApoA1* rs670 were exacerbated among individuals consuming a high-fat diet, particularly one high in MUFA. Consistent with the hypothesis that the genetic association with MetS may be modulated by dietary fat, we found that among the top 50$^{th}$ percentile, GG homozygotes also had further impairments to insulin resistance and greater BMI compared to the A allele carriers and especially to the GG homozygotes with the lowest fat intake. Importantly among individuals who habitually consumed a low-fat diet genotype did not seem to affect these phenotypes. One interpretation could be that individuals who are

genetically predisposed to the MetS are most sensitive to dietary fat, such that high total fat intake accentuates genetic susceptibility of developing the MetS.

It is generally accepted that high-fat diets, in particular high SFA diets, have detrimental effects on obesity and insulin sensitivity, promoting the development of MetS, T2DM and CVD [14, 29, 30]. While replacing SFA with MUFA may improve insulin sensitivity [31], intervention trials to confirm potential functional effects are mixed, perhaps reflecting genetic heterogeneity and interaction with dietary fat exposure. In contrast it has been suggested that dietary oleic acid (the major MUFA) may be more readily oxidized than SFA which may in turn have a negative effect on insulin sensitivity [32]. As dietary fatty acids were calculated as percentage intake of total energy the amount of each fatty acid is relative to the remaining fatty acids. This approach makes direct inference regards individual fatty acid effects difficult. However SFA and PUFA did not modulate MetS risk in this study, suggesting a MUFA specific effect. Additionally as oleic acid is mostly derived from animal products and not olive oil, at least outside of the Mediterranean region, it is difficult to fully differentiate the effects of SFA from MUFA. This may account for some of the inconsistencies observed in the above studies and it is also possible that what appears as a MUFA specific effect in the current study may be, in part, related to SFA. The mechanism whereby fatty acids could potentially modulate the genetic risk conferred by these polymorphisms is unknown and functional studies are required to ascertain the biological significance of such gene-nutrient interactions. We attempted to replicate our findings in a separate independent LIPGENE MetS case only cohort ($n$ = 464) [33]. Interestingly we replicated our original finding that risk phenotypes were modulated by a potential gene-nutrient interaction in a MUFA dependant fashion. Among the low MUFA consumers, the "risk" GG homozygotes for *ApoB* rs522535

had lower insulin concentrations (8.86±0.38 vs 10.79±0.96 mmol/L $P$ = 0.026) and displayed increased insulin sensitivity (Si 3.16±0.17 vs 2.61±0.19 $P$ = 0.046) relative to the A allele carriers. No differences were observed among the high MUFA consumers.

Several features of the current study (prospective nature, comprehensive phenotypic characterisation, large number of male and female cases and matched controls from all socio-economical categories and areas in the country) make this study particularly robust. Nevertheless, some limitations can be identified. As dietary consumption was self-reported by food-frequency questionnaire, some misclassification of exposure, due to deficiencies in nutrient databases, accuracy of memories or willingness to divulge these details, was inevitable. The number of dietary records used was minimal (3 in a small number of subjects) but was necessitated in order to maximise the number of matched cases and controls. The focus of the current analysis was on dietary fat composition but other food components such as carbohydrate or fibre can play a role in the development of the MetS.

The apoB/A1 ratio has been associated with insulin resistance, the MetS and CVD [7, 8], in keeping with these data we also observed significant differences comparing MetS cases to controls (0.87±0.01 vs 0.67±0.01 $P$ < 0.0001), despite the lack of a genotype effect on the apoB/A1 ratio. The promoter location of *ApoB* rs512535 and *ApoA1* rs670 has the potential to affect the level, location or timing of expression of their respective genes. Indeed altered transcription efficiency has been demonstrated with *ApoA1* rs670 [34, 35] and we report higher ApoA1 concentrations in the A allele carriers. Furthermore FASTSNP, a functional analysis tool [36], identified *ApoA1* rs670 as lying in a sequence homologous to a binding site for the nuclear factor SP-1.

Interestingly *ApoA1* is thought to be stimulated by insulin through SP-1 binding elements [37]. There is no functional data on *ApoB* rs512535 thus we can only speculate about mechanisms underlying our findings. Examination of the HAPMAP data indicates allele frequency differences between ethnic groups for *ApoB* rs512535. Whereas the allele frequency in the current study is not far from that in the European HAPMAP population, the opposite is true in Sub-Saharan Africans where the G allele is the minor allele. It is also possible that these SNPs may be surrogate markers for other functional SNPs of *ApoB* or *ApoA1,* or other genes in these regions. Therefore our results require further investigation and functional studies are needed to ascertain their biological significance.

In conclusion, this study provides new data on *ApoB* and *ApoA1* genotype and MetS risk. The novel gene-nutrient interactions between SNPs of these genes and dietary fat suggest that genetic predisposition to MetS may be more evident in individuals with a high habitual dietary fat intake, in particular MUFA. Functional characterisation and replication of these potentially important gene-nutrient interactions in an independent case-control cohort should be valuable regards their validation. Unravelling the molecular mechanisms underlying such gene-nutrient interactions may be useful in terms of developing personalised dietary recommendations wherein genetic profile may determine choice of dietary therapy to aid responsiveness to dietary fatty acid interventions and reduce risk of MetS and CVD.

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1 **FIGURE LEGENDS**

2

3 **FIGURE 1**

4 Influence of dietary fat intake and *ApoB* rs512535 and *ApoA1* rs670 genotype on anthropometric and metabolic measurements

5

Gene-nutrient interactions between *ApoB* rs512535 and *ApoA1* rs670 genotype and dietary fat intake on plasma insulin concentrations (**Figure 1A**), insulin resistance (**Figure 1B**) and BMI (**Figure 1C**). Values are means ± SEM. The black bars represent the A allele carriers ($n$ = 1289 for *ApoB* rs512535 and $n$ = 524 for *ApoA1* rs670) and the white bars represent the GG homozygotes ($n$ = 465 for *ApoB* rs512535 and $n$ = 1230 for *ApoA1* rs670). $P$ values were calculated by GEE linear regression adjusting for potential confounding factors including age, gender, BMI, smoking status, energy intake, physical activity and medication use. * $P < 0.05$, ** $P < 0.005$ and *** $P < 0.001$ different from GG homozygotes with high fat intake. **TABLE 1. Polymorphisms investigated and comparison of their allele frequencies between MetS cases and controls**

| | | | | MetS cases | | Controls | | | |
|---|---|---|---|---|---|---|---|---|---|
| Locus | Locus alias | Location | Allele | n | % frequency | n | % frequency | Fishers *P* value | Odds Ratio (95% CI) |
| *ApoB* rs512535 | G-837A | Promoter | A | 798 | 0.455 | 894 | 0.509 | 0.004 | 1.22 (1.07 - 1.40) |
| | | | G | 956 | 0.545 | 860 | 0.491 | | |
| *ApoB* rs1042031 | Lys 4181Glu | Exon 29 | A | 342 | 0.195 | 351 | 0.200 | 0.572 | 1.05 (0.89 - 1.25) |
| | | | G | 1412 | 0.805 | 1403 | 0.800 | | |
| *ApoB* rs676210 | Leu2739Pro | Exon 26 | A | 377 | 0.215 | 350 | 0.200 | 0.289 | 1.10 (0.93 - 1.29) |
| | | | G | 1377 | 0.785 | 1404 | 0.800 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *ApoB* rs693 | Thr2515Thr | Exon 26 | T | 869 | 0.495 | 867 | 0.494 | 0.972 | 1.00 (0.87 - 1.15) |
| | | | C | 885 | 0.505 | 887 | 0.506 | | |
| *ApoB* rs679899 | Val618Ala | Exon 14 | A | 851 | 0.485 | 798 | 0.455 | 0.091 | 1.13 (0.98 - 1.29) |
| | | | G | 903 | 0.515 | 956 | 0.545 | | |
| *ApoB* rs1367117 | Ile98Thr | Exon 4 | A | 544 | 0.310 | 587 | 0.335 | 0.149 | 1.12 (0.96 - 1.29) |
| | | | G | 1210 | 0.689 | 1167 | 0.665 | | |
| *ApoA1* rs5070 | C317T | Intron 2 | A | 528 | 0.301 | 570 | 0.325 | 0.144 | 1.12 (0.96 - 1.29) |
| | | | G | 1226 | 0.698 | 1184 | 0.675 | | |
| *ApoA1* rs5081 | | 3' region | T | 1722 | 0.981 | 1732 | 0.987 | 0.208 | 1.47 (0.84 - 2.58) |
| | | | A | 32 | 0.019 | 22 | 0.013 | | |
| *ApoA1* rs670 | G-75A | Promoter | A | 254 | 0.145 | 320 | 0.182 | 0.004 | 1.32 (1.09 - 1.59) |
| | | | G | 1500 | 0.855 | 1434 | 0.818 | | |

Allele frequencies were compared between cases and controls in HITAGENE using Fishers exact test.

**TABLE 2. Comparison of genotype frequencies between MetS cases and controls**

| | | MetS cases | | Controls | | | | |
|---|---|---|---|---|---|---|---|---|
| Locus | Genotype | n | % frequency | n | % frequency | Fishers *P* value | Dominant model | Recessive model |
| *ApoB* rs512535 | A/A | 193 | 0.22 | 210 | 0.24 | 0.0003 | 0.523 | 0.00007 |
| | A/G | 412 | 0.47 | 474 | 0.54 | | | |
| | G/G | 272 | 0.31 | 193 | 0.22 | | | |
| *ApoB* rs1042031 | A/A | 35 | 0.04 | 35 | 0.04 | 0.795 | 0.613 | 0.683 |
| | A/G | 272 | 0.31 | 281 | 0.32 | | | |
| | G/G | 570 | 0.65 | 561 | 0.64 | | | |
| *ApoB* rs676210 | A/A | 35 | 0.04 | 26 | 0.03 | 0.547 | 0.510 | 0.339 |
| | A/G | 307 | 0.35 | 298 | 0.34 | | | |
| | G/G | 535 | 0.61 | 553 | 0.63 | | | |
| *ApoB* rs693 | T/T | 202 | 0.23 | 210 | 0.24 | 0.707 | 0.606 | 0.651 |
| | C/T | 465 | 0.53 | 447 | 0.51 | | | |
| | C/C | 210 | 0.24 | 219 | 0.25 | | | |
| *ApoB* rs679899 | A/A | 202 | 0.23 | 193 | 0.22 | 0.099 | 0.560 | 0.034 |
| | A/G | 447 | 0.51 | 412 | 0.47 | | | |
| | G/G | 228 | 0.26 | 272 | 0.31 | | | |
| *ApoB* rs1367117 | A/A | 88 | 0.10 | 96 | 0.11 | 0.321 | 0.473 | 0.155 |
| | A/G | 368 | 0.42 | 395 | 0.45 | | | |
| | G/G | 421 | 0.48 | 386 | 0.44 | | | |
| *ApoA1* rs5070 | A/A | 83 | 0.09 | 100 | 0.11 | 0.330 | 0.228 | 0.258 |
| | A/G | 362 | 0.41 | 370 | 0.42 | | | |
| | G/G | 432 | 0.49 | 407 | 0.46 | | | |
| *ApoA1* rs5081 | T/T | 845 | 0.96 | 855 | 0.98 | 0.204 | 0.205 | 1.000 |
| | A/T | 32 | 0.04 | 22 | 0.03 | | | |
| | A/A | 0 | 0.00 | 0 | 0.00 | | | |
| *ApoA1* rs670 | A/A | 20 | 0.02 | 30 | 0.03 | 0.015 | 0.186 | 0.005 |
| | A/G | 214 | 0.24 | 260 | 0.30 | | | |
| | G/G | 643 | 0.73 | 587 | 0.67 | | | |

Genotype frequencies were compared between cases and controls in HITAGENE using Fishers exact test.

For the dominant model, minor allele homozygotes were compared to the combined major allele carriers.
For the recessive model, major allele homozygotes were compared to the combined minor allele carriers.

**TABLE 3. Clinical characteristics and dietary fat intakes of all subjects according to *ApoB* rs512535 and *ApoA1* rs670 genotypes**

| | ***ApoB* rs512535** | | ***ApoA1* rs670** | |
|---|---|---|---|---|
| | AG + AA | GG | AG + AA | GG |
| n | 1289 | 465 | 524 | 1230 |
| Male/Female, % | 60/40 | 60/40 | 54/46 | 55/45 |
| Age, y | 58±0.17 | 58±0.20 | 58±0.24 | 58±0.15 |
| Insulin, mmol/L | 7.27±0.19 | 7.55±0.21 * | 6.98±0.27 | 7.62±0.17 * |
| Glucose, mmol/L | 5.24±0.03 | 5.28±0.03 | 5.26±0.05 | 5.26±0.03 |
| QUICKI | 0.34±0.00 | 0.32±0.00 * | 0.32±0.00 | 0.30±0.00 * |
| HOMA | 1.76±0.05 | 2.00±0.08 * | 1.72±0.08 | 1.88±0.05 |
| BMI, kg/m$^2$ | 25.3±0.14 | 26.8±0.16 * | 25.5±0.19 | 26.2±0.13 * |
| Waist, cm | 88±0.42 | 90±0.46 * | 87±0.56 | 89±0.38 * |
| CRP, mg/L | 2.09±0.08 | 2.39±0.16 * | 1.99±0.12 | 2.37±0.14 * |
| C3, g/L | 1.48±0.02 | 1.58±0.03 * | 1.50±0.02 | 1.53±0.02 |
| Total cholesterol, mmol/L | 5.71±0.03 | 5.72±0.03 | 5.75±0.04 | 5.70±0.03 |
| HDL cholesterol, mmol/L | 1.46±0.01 | 1.49±0.02 | 1.52±0.02 | 1.46±0.01 * |
| LDL cholesterol, mmol/L | 3.56±0.04 | 3.50±0.04 | 3.57±0.05 | 3.52±0.03 |
| TAG, mmol/L | 1.27±0.02 | 1.26±0.03 | 1.27±0.03 | 1.26±0.02 |
| LDL/HDL | 2.58±0.03 | 2.61±0.05 | 2.57±0.04 | 2.60±0.04 |
| ApoA1, g/L | 1.57±0.00 | 1.59±0.01 | 1.60±0.00 | 1.56±0.01* |
| ApoB, g/L | 1.16±0.00 | 1.18±0.01 | 1.16±0.01 | 1.16±0.01 |
| ApoA1/ApoB | 0.76±0.01 | 0.77±0.01 | 0.75±0.01 | 0.76±0.01 |
| SBP, mm Hg | 131±0.50 | 132±0.59 | 129±0.69 | 132±0.46 |
| DBP, mm Hg | 82±0.30 | 82±0.35 | 82±0.41 | 82±0.2 |
| Lipid lowering medication, % | 19 | 20 | 18 | 19 |
| Anti-diabetic medication, % | 4 | 3 | 3 | 3 |
| Hypertensive medication, % | 21 | 22 | 19 | 21 |
| Total dietary fat intake, % energy | 35.27±0.23 | 35.19±0.26 | 35.42±0.31 | 35.18±0.21 |

| | | | | |
|---|---|---|---|---|
| MUFA intake, % energy | 14.19±0.11 | 14.24±0.13 | 14.32±0.15 | 14.17±0.10 |
| PUFA intake, % energy | 5.63±0.07 | 5.67±0.08 | 5.71±0.10 | 5.61±0.06 |
| n-6 PUFA intake, % energy | 5.05±0.07 | 5.12±0.08 | 5.14±0.09 | 5.05±0.06 |
| n-3 PUFA intake, % energy | 0.57±0.01 | 0.55±0.01 | 0.57±0.01 | 0.56±0.01 |
| SFA intake, % energy | 15.46±0.13 | 15.29±0.14 | 15.39±0.16 | 15.39±0.11 |
| Alcohol intake, % energy | 6.58±0.25 | 6.80±0.40 | 6.65±0.32 | 6.73±0.19 |

Values are means ± SEM. * indicates $P < 0.05$ for linear regression adjusted for age, gender, BMI, smoking status, physical activity, energy and alcohol intake and use of medications.

1

**Figure 1**

**Fig 1A** ***ApoB*** **rs512535** ***ApoA1*** **rs670**

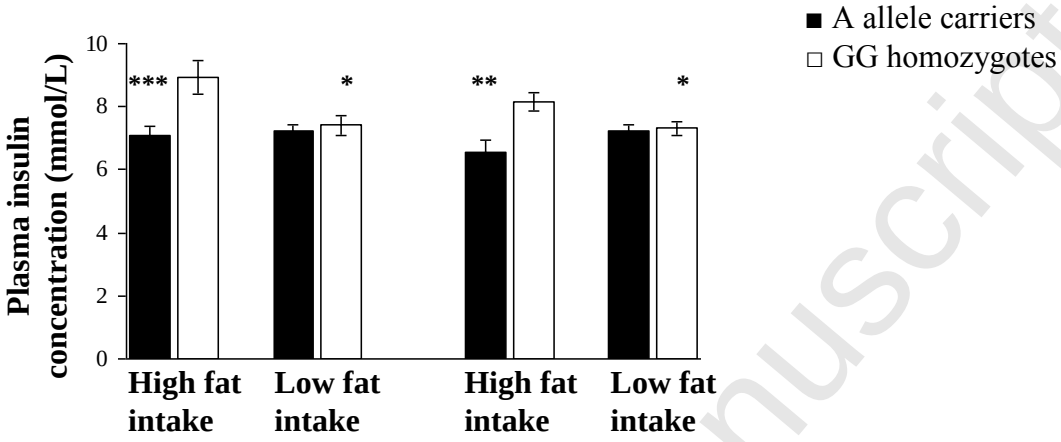


**Fig 1B** ***ApoB*** **rs512535** ***ApoA1*** **rs670**

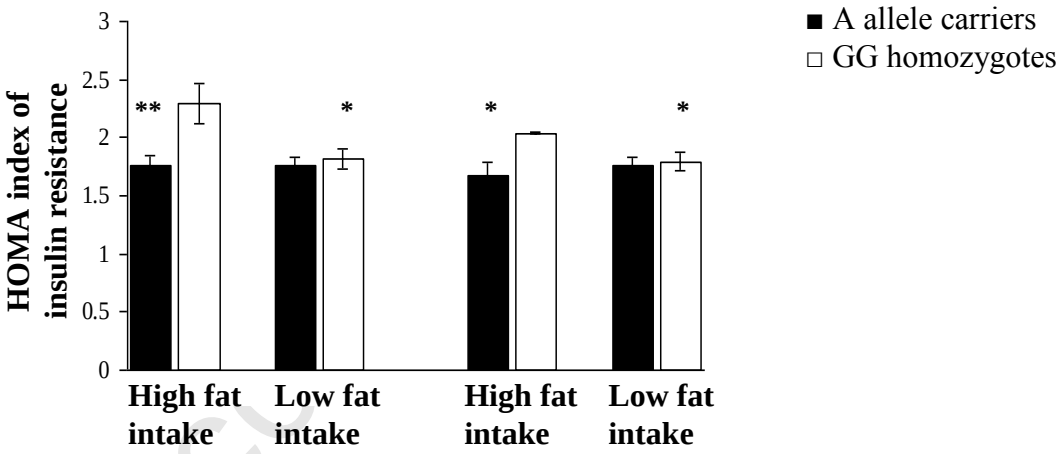


**Fig 1C** ***ApoB*** **rs512535** ***ApoA1*** **rs670**