

The association of common genetic variants in the *APOA5*, *LPL* and *GCK* genes with longitudinal changes in metabolic and cardiovascular traits

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Abstract

Aims/hypothesis Common genetic variants influence plasma triacylglycerol, HDL-cholesterol (HDL-C) and glucose levels in cross-sectional studies. However, the longitudinal effects of these established variants have not been studied. Our aim was to examine the longitudinal associations of four such variants in the apolipoprotein A-V (*APOA5*), lipoprotein

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lipase (*LPL*), and glucokinase (*GCK*) genes with fasting glucose or lipid levels.

Methods The individuals analysed were participants in the Busselton Health Survey ($n=4,554$). Cross-sectional analyses of family data used the total association test. Longitudinal association analyses of unrelated participant data ($n=2,864$) used linear mixed-effects models.

Results The findings of cross-sectional association analyses replicated those of previous studies. We observed associations of the G and C alleles at the *APOA5* single nucleotide polymorphisms (SNPs) rs662799 and rs3135506 with raised triacylglycerol levels ($p=0.0003$ and $p<0.0001$, respectively), the 447X allele at the *LPL* SNP rs328 with reduced triacylglycerol levels ($p=0.0004$) and raised HDL-C levels ($p=0.0004$), and the A allele of the *GCK* SNP rs1799884 with raised fasting glucose level ($p=0.015$). Longitudinal association analyses showed that most of these associations did not change in the same individuals over an average follow-up time of 17.4 years, though there was some evidence that the association of the 447X allele of rs328 with raised HDL-C level significantly increased with age ($p=0.01$), and that the association of the C allele of rs3135506 with raised triacylglycerol level significantly increased over time ($p=0.0007$).

Conclusions/interpretation The current study suggests that the effects of established gene variants on lipid and glucose traits do not tend to alter with age during adulthood or over time.

Keywords *APOA5* · Association · *GCK* · Longitudinal · *LPL* · SNP · Type 2 diabetes

Abbreviations

BHS Busselton Health Survey
HDL-C HDL-cholesterol

HOMA	homeostatic model assessment
LDL-C	LDL-cholesterol
SNP	single nucleotide polymorphism

Introduction

There are an increasing number of common genetic variants that are robustly associated with common diseases and quantitative traits. These include variants associated with type 2 diabetes, height, body mass index and inflammatory diseases [1–4]. Variants associated with lipid levels also continue to be identified through ongoing genome-wide association studies [5]. Most variants have been identified through recent genome-wide association studies of cases and controls or using cross-sectional data in population-based studies.

The effects of common variants on key metabolic and cardiovascular phenotypes within individuals as they age have not been well studied. There have been many cross-sectional studies of such genotype–phenotype associations in individuals of different ages [6–10]; however, these studies do not test the possible longitudinal effects of variants in the same individuals. Like the associated risks of many diseases, measures of quantitative traits of clinical relevance often alter with age. Metabolic traits are no exception, with triacylglycerol and glucose levels, body mass index and blood pressure all tending to increase with age [11–13], and HDL-cholesterol (HDL-C) level tending to decrease with age [14]. Very few studies have tested whether variants shown to be associated with these traits in cross-sectional studies have similar associations in the same individuals over time. One study, using 3,877 individuals from the Data from an Epidemiological Study on the Insulin Resistance syndrome (DESIR) cohort, reported possible differential effects of the *GCK*-30 variant on fasting glucose levels at baseline and after a 9 year follow-up, but there have been few other studies [15].

In this study, we use data from the Busselton Health Survey (BHS), where individuals have been followed up for an average time of 21.2 years (95% CI 13.6–28.8 years) over the last 28 years, with an average of 3.9 measures on each individual, to assess the longitudinal associations of four common variants with metabolic and cardiovascular traits. The variants selected were the apolipoprotein A-V (*APOA5*) single nucleotide polymorphisms (SNPs) rs662799 and rs3135506; the lipoprotein lipase (*LPL*) SNP rs328; and the glucokinase (*GCK*) SNP rs1799884, which have been shown in multiple cross-sectional studies to be associated with triacylglycerol (*APOA5* and *LPL*) [16–21], HDL-C (*LPL*) [20, 21] and fasting glucose (*GCK*) [9, 22]

levels, traits important in the risk of diabetes and cardiovascular disease.

Methods

Participants The BHS includes a series of seven cross-sectional population health surveys of adult residents of the Shire of Busselton in the south-west of Western Australia, undertaken between 1966 and 1990, and focused on respiratory and cardiovascular phenotypes [23–25]. In 1994/1995 a cross-sectional community follow-up study, which included the collection of blood for DNA extraction, was undertaken of all survivors of previous surveys. A total of 4,554 individuals participated in this follow-up, including 696 families with a median family size of three. The population is predominantly European–Australian. Population descriptives for individuals surveyed in 1994/1995 in this follow-up are presented in Table 1. A subset of this cohort, selected for longitudinal analysis, comprised 2,864 unrelated individuals who had participated in at least one of the eight cross-sectional surveys while aged between 18 and 80 years. Descriptives for individuals in this subset are presented in Table 2. These individuals had been followed up for a mean time of 21.2 years (95% CI 13.6–28.8 years) (Fig. 1) with an average of 3.9 (SEM 0.04) survey attendances. Fasting glucose, triacylglycerol and HDL-C levels were measured at 7, 6 and 4 time points respectively, with average follow-up times of 19.1, 17.2 and 15.9 years, respectively. All participants gave written informed consent and all study protocols were approved by the Human Research Ethics Committee of the University of Western Australia.

Clinical measurements The clinical measurements of individuals in the BHS have been described previously [23, 24]. A self-administered questionnaire was used to record participants' history of smoking, physician-diagnosed diabetes and use of lipid-lowering medication and insulin injections. Anthropomorphic measurements were taken according to standard clinical procedures. The presence of CHD was determined using the Rose questionnaire, electrocardiogram and self-reported history of physician-diagnosed angina or myocardial infarction. The presence of metabolic syndrome was determined according to the National Cholesterol Education Program Third Adult Panel (NCEP ATP III) criteria.

Laboratory measurements Lipid levels (HDL-C, LDL-cholesterol (LDL-C) and triacylglycerol concentrations), and fasting plasma glucose and insulin levels were determined from fasting venous blood samples as described previously [26]. Insulin resistance was determined using model-derived estimates of the homeostatic model assessment (HOMA)

Table 1 Description of the BHS whole study population at the 1994/1995 survey ($n=4,554$)

Variable (units)	Mean (SD)
Age (years)	50.6 (17.2)
BMI (kg/m^2)	26.0 (4.2)
Fasting glucose (mmol/l)	5.0 (1.4)
Triacylglycerol (mmol/l)	1.3 (0.9)
LDL-C (mmol/l)	3.6 (1.0)
HDL-C (mmol/l)	1.4 (0.4)
Total cholesterol (mmol/l)	5.6 (1.1)
Systolic BP (mmHg)	124.1 (17.9)
Diastolic BP (mmHg)	74.5 (10.2)
Male, % (n)	44.2 (2,014)
Smoking (ever smoked), % (n)	17.7 (805)
Diabetes (ever had diabetes), % (n)	6.0 (271)
CHD, % (n)	16.3 (744)
Metabolic syndrome (IDF definition), % (n)	19.1 (871)
Metabolic syndrome (NCEP definition), % (n)	15.9 (722)
Lipid-lowering medication, % (n)	2.5 (116)
Insulin injections, % (n)	0.7 (31)

IDF, International Diabetes Federation; NCEP, National Cholesterol Education Program

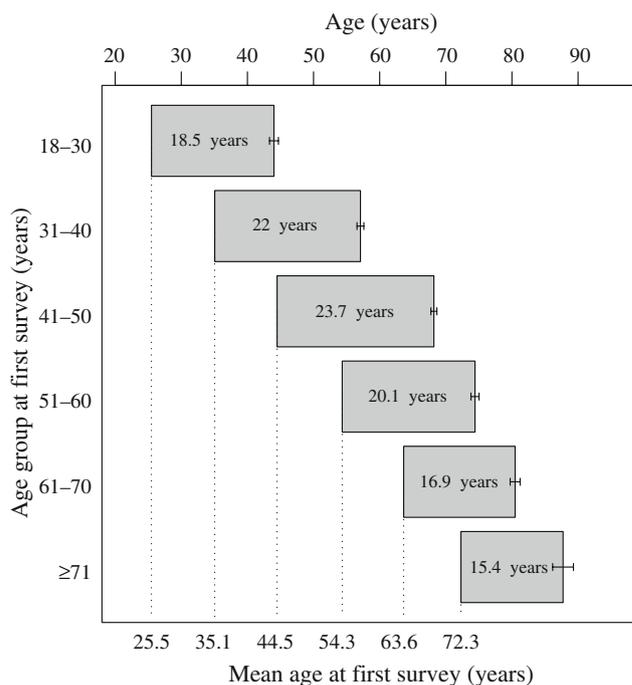
scores HOMA2-%B (steady-state beta cell function), HOMA2-%S (insulin sensitivity) and HOMA2-IR (insulin resistance) [27, 28], computed by the HOMA Calculator [29].

Genes and genotyping We selected four variants in three gene regions for analysis. These variants were the *APOA5* SNPs rs662799 and rs3135506; the *LPL* SNP rs328; and the *GCK* SNP rs1799884. Genomic DNA from all 4,554 study participants was available for genotyping. The SNPs were genotyped using TaqMan probes designed and supplied by Applied Biosystems (Scoresby, VIC, Australia). All primer sequences and experimental conditions are available

Table 2 Description of BHS populations of participants aged 18–80 years, for variables with longitudinal data, at the time of first assessment

Variable (units)	Unrelated population ($n=2,864$)	Unrelated male population ($n=1,234$)	Unrelated female population ($n=1,630$)
Age (years)	37.8 (11.6)	38.1 (11.6)	37.6 (11.6)
BMI (kg/m^2)	24.3 (3.6)	24.9 (3.2)	23.8 (3.8)
Fasting glucose (mmol/l)	5.0 (1.1)	5.2 (1.4)	4.8 (0.8)
Triacylglycerol (mmol/l)	1.2 (0.8)	1.4 (1.0)	1.1 (0.6)
HDL-C (mmol/l)	1.4 (0.4)	1.3 (0.3)	1.6 (0.4)
Total cholesterol (mmol/l)	5.6 (1.2)	5.6 (1.2)	5.6 (1.2)

Values are means (SD)

**Fig. 1** Mean follow-up times (95% CI) by age group at first survey

from the authors on request. Genotyping was performed by the PathWest Molecular Genetics Service (Nedlands, WA, Australia). Percentage call rates were at least 99.6% for all SNPs. Duplicate genotypes were run for ~10% of participants for all SNPs, with duplicate error rates of less than 0.5% in each case.

Statistical analyses Genotype frequencies at each SNP locus were tested for deviations from Hardy–Weinberg equilibrium using an exact test implemented in the statistical analysis package, R [30].

Cross-sectional total association analyses were performed on the whole cohort of 4,554 participants using 1994/1995 survey data. The outcome variables HDL-C, LDL-C, triacylglycerol, fasting glucose and fasting insulin levels, HOMA2-%B, HOMA2-%S, HOMA2-IR and BMI were analysed as continuous traits. All but LDL-C were natural log-transformed prior to analysis to achieve a normal distribution. Generalised linear models were used to model the effects of multiple covariates on quantitative outcomes in the unrelated sample, using the SimHap v1.0.0 program (<http://www.genepi.org.au/simhap.html>). Covariates associated with each outcome were identified from an initial set of relevant phenotypes through stepwise variable selection, so as to ensure that each covariate model included only covariates with independent, significant associations with the outcome, in the population under study. In addition to the full models derived using this procedure, analyses were performed using a simple cova-

riate model including only sex and age as covariates, for comparison.

The total association test (i.e. a general variance components model to partition observed phenotypic variance into genetic and non-genetic components), as implemented in the QTDT v2.6.0 program [31], was used to model the effects of covariates and *APOA5*, *LPL* and *GCK* genotypes on quantitative outcomes within families. Variances between individuals were modelled with environmental, polygenic and additive major-gene-effect variance components. The evidence for population stratification was also evaluated by comparing the between- and within-family components of association in the quantitative transmission disequilibrium test orthogonal model, using the QTDT program. The absence of a significant difference between the two components was taken to indicate that the results for the SNPs analysed were not influenced by any population structure.

Genotype-specific geometric means and 95% confidence intervals were calculated for triacylglycerol and HDL-C levels in unrelated individuals, and fasting glucose levels in unrelated, non-diabetic individuals, in three consecutive age groups (18–40, 41–60 and 61–80 years) using 1994/95 survey data only, to examine whether these traits differed with genotype in different age groups. The geometric mean and geometric standard deviation of a data set can be calculated by taking the antilogarithm of the arithmetic mean and arithmetic standard deviation, respectively, of the log-transformed data values. Geometric confidence intervals are calculated from these values in the same way as arithmetic confidence intervals. Geometric statistics better describe data that, like the triacylglycerol, HDL-C and fasting glucose data, follow a log-normal distribution (i.e. a distribution for which log-transformed values are normally distributed). Therefore, the use of geometric, rather than arithmetic, mean and confidence interval values, was most appropriate for the present analysis. Differences between the geometric means of different genotype groups within each age group were tested for significance using two-tailed *t* tests.

Full longitudinal association analyses were performed on the sub-cohort of 2,864 unrelated participants aged 18–80 years and, for analyses of the fasting glucose outcome variable only, on the 2,665 non-diabetic participants from this sub-cohort. The longitudinal outcome variables HDL-C, triacylglycerol and fasting plasma glucose levels were natural log-transformed prior to analysis to achieve a normal distribution, and age, age² and age³ covariates were mean-centred. Longitudinal association analyses were conducted using linear mixed-effects models [32] to incorporate correlation inherent within observations from the same individual, using the SimHap program. This involved the inclusion of time × SNP and/or age × SNP interaction terms in the models. Covariate selection was performed using step-wise elimination. In view of the low minor allele frequencies

of the variants, genotypes were coded into two classes (major allele homozygote=0, heterozygote or minor allele homozygote=1) and analysed under a dominant genetic model.

Statistical power All power calculations were performed using the Quanto software, version 1.2.3 [33]. It was determined that for all SNPs, analyses of quantitative phenotypes had at least 90% power at an alpha level of $p=0.05$ to detect a difference of 0.07 standard deviations in the case of longitudinal analyses and 0.06 standard deviations in the case of cross-sectional analyses, under a dominant genetic model.

Results

Population characteristics Genotype sample numbers and allele frequencies are given in Table 3. These frequencies were not different from those expected under Hardy–Weinberg equilibrium for all four SNPs in both study groups. Using the QTDT program, we found no significant evidence that population structure influenced the results for the SNPs analysed.

Cross-sectional association of SNPs with metabolic traits The results of cross-sectional total association analyses are given in Table 4. In analyses using full covariate models, we observed that the presence of the G and C alleles at the *APOA5* SNPs rs662799 and rs3135506, respectively, was associated with raised triacylglycerol levels ($p=0.0003$ and $p<0.0001$, respectively), that the 447X allele at the *LPL* SNP rs328 was associated with reduced triacylglycerol levels ($p=0.0004$) and raised HDL-C levels ($p=0.0004$), and that the A allele of the *GCK* SNP rs1799884 was associated with raised fasting glucose level ($p=0.015$). Similar results were obtained using the simple covariate model, with two exceptions. While analyses using the full covariate model showed no significant association between either rs662799 or rs3135506 and HDL-C levels, analyses using the simple covariate model showed that the presence of the G and C alleles at rs662799 and rs3135506, respectively, was associated with reduced HDL-C levels ($p=0.005$ and $p=0.01$, respectively). Subsequent analysis indicated that this is likely the result of a correlation between HDL-C and triacylglycerol levels. In analyses of fasting glucose and lipid levels in the unrelated sample at the 1994/95 survey, we observed that rs1799884 explained 0.3% of the variation in fasting glucose level, rs662799, rs3135506 and rs328 explained 0.1%, 0.4% and 0.3% of variation in triacylglycerol level, respectively, and rs328 explained 0.2% of variation in HDL-C level. When rs662799, rs3135506 and rs328 were included together in

Table 3 Genotypic and allelic frequencies for *APOA5*, *LPL* and *GCK* SNPs in BHS populations

Gene	SNP	Homozygous wild-type, <i>n</i> (%; genotype)	Heterozygote, <i>n</i> (%; genotype)	Homozygous mutant, <i>n</i> (%; genotype)	Minor allele frequency	Hardy–Weinberg equilibrium <i>p</i> value
Whole study population (<i>n</i> =4,554)						
<i>GCK</i>	rs1799884	3,086 (68.2%; GG)	1,311 (29.0%; GA)	130 (2.9%; AA)	0.174	0.534
<i>APOA5</i>	rs662799	4,011 (88.5%; AA)	504 (11.1%; AG)	18 (0.4%; GG)	0.060	0.595
<i>APOA5</i>	rs3135506	3,985 (87.7%; GG)	542 (11.9%; GC)	15 (0.3%; CC)	0.063	0.530
<i>LPL</i>	rs328	3,621 (79.8%; CC)	863 (19.0%; CG)	54 (1.2%; GG)	0.107	0.756
Unrelated, 18–80 year old population (<i>n</i> =2,864)						
<i>GCK</i>	rs1799884	1,929 (67.4%; GG)	839 (29.3%; GA)	78 (2.7%; AA)	0.175	0.269
<i>APOA5</i>	rs662799	2,525 (88.2%; AA)	318 (11.1%; AG)	10 (0.3%; GG)	0.059	1
<i>APOA5</i>	rs3135506	2,485 (86.8%; GG)	359 (12.5%; GC)	12 (0.4%; CC)	0.067	1
<i>LPL</i>	rs328	2,295 (80.1%; CC)	522 (18.2%; CG)	38 (1.3%; GG)	0.105	0.193

a multivariate model for the triacylglycerol level outcome, all three remained significantly associated with triacylglycerol level ($p=0.004$, $p=0.0002$ and $p=0.0008$, respectively) and, combined, accounted for 0.7% of the variation in this trait. No significant two-way interactions were observed between combinations of these three SNPs for the triacylglycerol level outcome. Across survey years, using simple models, the percentages of variation in glucose and lipid levels explained by each of the four individual SNPs showed no significant trends and varied by between 0.1% and 1.8%. The geometric means of glucose and lipid levels in different genotype and age groups in unrelated populations are presented in Table 5.

The results of cross-sectional total association analyses performed with additional metabolic outcomes are given in Electronic supplementary material (ESM) Table 1. The presence of the A allele at rs1799884 was found to be associated with reduced fasting insulin level, HOMA2-%B and HOMA2-IR, and raised HOMA2-%S.

Longitudinal association of SNPs with lipid and fasting glucose levels The results of the longitudinal association analyses are summarised in Table 6. For each phenotype tested, values fitted according to the full covariate, dominant genetic model used for analysis are plotted by genotype group against age in Figs 2 and 3. There was little evidence that the effect sizes of established associations altered with either age during adult life or time over the period from 1975 to 1994/1995. An exception was an association between the age \times rs328 interaction term and HDL-C level ($p=0.01$), indicating that the presence of the 447X allele at rs328 increases HDL-C levels with increasing age (Fig. 3f). There was no evidence for an association between the time \times rs328 interaction term and HDL-C level, indicating that the association between rs328 and HDL-C has not varied over time. A second exception was an association between the time \times rs3135506, but not age \times rs3135506, interaction term and triacylglycerol level ($p=0.0007$). This indicated that the presence of the C allele at rs3135506 has

Table 4 Results of 1994/1995 cross-sectional total association analyses of the whole study population (*n*=4,554)

Phenotype (log _e [mmol/l] scale)	Gene	SNP	Full covariate model ^a		Simple covariate model ^b
			Per allele effect size	<i>p</i> value	<i>p</i> value
Fasting glucose	<i>GCK</i>	rs1799884	0.01	0.01	0.003
Triacylglycerol	<i>APOA5</i>	rs662799	0.07	0.0003	<0.0001
Triacylglycerol	<i>APOA5</i>	rs3135506	0.08	<0.0001	<0.0001
Triacylglycerol	<i>LPL</i>	rs328	−0.05	0.0004	<0.0001
HDL-C	<i>APOA5</i>	rs662799	–	0.22	0.005
HDL-C	<i>APOA5</i>	rs3135506	–	0.67	0.01
HDL-C	<i>LPL</i>	rs328	0.03	0.0004	<0.0001

^a Phenotype adjustments, full covariate model:

Fasting glucose: sex, age, age², BMI, fasting insulin, systolic BP, history of diabetes

Triacylglycerol: sex, age, age², age³, WHR, fasting insulin, HDL-C, LDL-C, systolic BP, history of smoking

HDL-C: sex, age, age², age³, BMI, BMI², WHR, fasting insulin, triacylglycerol, history of diabetes

^b Phenotype adjustments for all outcomes, simple covariate model: sex, age

Table 5 Genotype-specific geometric means of lipid levels in unrelated participants and fasting glucose level in unrelated, non-diabetic participants, in three age groups, 18–40 years ($n=514$), 41–60 years ($n=1,105$), 61–80 years ($n=1,104$)

Age group	Gene	SNP	Geometric mean (95% CI)		<i>p</i> value
			Common hom.	Minor allele hom. + het.	
Fasting glucose (mmol/l)					
18–40 years	<i>GCK</i>	rs1799884	4.64 (4.59–4.69)	4.72 (4.64–4.80)	0.09
41–60 years	<i>GCK</i>	rs1799884	4.82 (4.78–4.86)	4.90 (4.83–4.97)	0.05
61–80 years	<i>GCK</i>	rs1799884	4.95 (4.90–5.00)	5.00 (4.92–5.08)	0.26
Triacylglycerol (mmol/l)					
18–40 years	<i>APOA5</i>	rs662799	0.88 (0.84–0.92)	1.08 (0.93–1.24)	0.01*
41–60 years	<i>APOA5</i>	rs662799	1.14 (1.10–1.18)	1.23 (1.11–1.36)	0.18
61–80 years	<i>APOA5</i>	rs662799	1.33 (1.29–1.37)	1.55 (1.40–1.71)	0.004*
18–40 years	<i>APOA5</i>	rs3135506	0.89 (0.84–0.93)	1.01 (0.90–1.14)	0.05
41–60 years	<i>APOA5</i>	rs3135506	1.13 (1.09–1.16)	1.35 (1.22–1.49)	0.001*
61–80 years	<i>APOA5</i>	rs3135506	1.33 (1.29–1.37)	1.47 (1.35–1.61)	0.03*
18–40 years	<i>LPL</i>	rs328	0.92 (0.87–0.96)	0.86 (0.78–0.94)	0.25
41–60 years	<i>LPL</i>	rs328	1.18 (1.14–1.22)	1.03 (0.96–1.11)	0.001*
61–80 years	<i>LPL</i>	rs328	1.38 (1.34–1.43)	1.20 (1.13–1.28)	<0.0001*
HDL-C (mmol/l)					
18–40 years	<i>APOA5</i>	rs662799	1.34 (1.31–1.37)	1.28 (1.21–1.36)	0.16
41–60 years	<i>APOA5</i>	rs662799	1.36 (1.33–1.38)	1.30 (1.23–1.37)	0.12
61–80 years	<i>APOA5</i>	rs662799	1.35 (1.32–1.37)	1.32 (1.26–1.40)	0.55
18–40 years	<i>APOA5</i>	rs3135506	1.35 (1.32–1.38)	1.25 (1.19–1.32)	<0.01*
41–60 years	<i>APOA5</i>	rs3135506	1.35 (1.33–1.38)	1.33 (1.26–1.40)	0.57
61–80 years	<i>APOA5</i>	rs3135506	1.35 (1.33–1.38)	1.31 (1.25–1.37)	0.20
18–40 years	<i>LPL</i>	rs328	1.33 (1.30–1.36)	1.33 (1.27–1.40)	0.98
41–60 years	<i>LPL</i>	rs328	1.34 (1.31–1.36)	1.42 (1.36–1.47)	0.008*
61–80 years	<i>LPL</i>	rs328	1.33 (1.30–1.35)	1.43 (1.37–1.48)	0.001*

* $p < 0.05$

het., heterozygote; hom., homozygote

increased triacylglycerol levels over time, without varying this effect with increasing age. Triacylglycerol level was also found to be significantly associated with the year at which it was surveyed, with levels tending to be lower at later

survey times. The observed association between the time \times rs3135506 interaction term and triacylglycerol level counteracts this trend over time. Similar results were obtained with both the full and simple covariate models. The results of

Table 6 Results of longitudinal association analyses of lipid levels in unrelated participants aged 18–80 years ($n=2,864$), and of fasting glucose level in the subset of non-diabetic participants ($n=2,665$)

Phenotype ^a (log _e [mmol/l] scale)	Gene	SNP	Age \times SNP interaction		Time \times SNP interaction	
			Beta (95% CI)	<i>p</i> value	Beta (95% CI)	<i>p</i> value
Fasting glucose	<i>GCK</i>	rs1799884	-1.76×10^{-4} (-7.66×10^{-4} , 4.13×10^{-4})	0.56	1.60×10^{-4} (-1.04×10^{-3} , 7.18×10^{-4})	0.72
Triacylglycerol	<i>APOA5</i>	rs662799	4.62×10^{-4} (-4.38×10^{-3} , 3.46×10^{-3})	0.79	-3.59×10^{-6} (-3.92×10^{-3} , 3.92×10^{-3})	1
Triacylglycerol	<i>APOA5</i>	rs3135506	-2.17×10^{-4} (-4.14×10^{-3} , 3.70×10^{-3})	0.89	7.35×10^{-3} (3.43×10^{-3} , 0.01)	0.0007*
Triacylglycerol	<i>LPL</i>	rs328	-1.09×10^{-3} (-3.05×10^{-3} , 8.68×10^{-4})	0.43	-5.38×10^{-4} (-4.46×10^{-3} , 3.38×10^{-3})	0.77
HDL-C	<i>APOA5</i>	rs662799	-8.91×10^{-5} (-2.05×10^{-3} , 1.87×10^{-3})	0.92	1.06×10^{-3} (-3.02×10^{-3} , 8.99×10^{-4})	0.38
HDL-C	<i>APOA5</i>	rs3135506	1.18×10^{-4} (-1.84×10^{-3} , 2.08×10^{-3})	0.89	-8.20×10^{-4} (-1.14×10^{-3} , 2.78×10^{-3})	0.46
HDL-C	<i>LPL</i>	rs328	1.81×10^{-3} (-1.51×10^{-4} , 3.77×10^{-3})	0.01*	1.16×10^{-4} (-1.84×10^{-3} , 2.08×10^{-3})	0.90

Results are for age \times SNP and time \times SNP interaction terms with full covariate, dominant genetic models* $p < 0.05$ ^aPhenotype adjustments, full covariate model:Fasting glucose: sex, age, age², age at first survey, time of surveyTriacylglycerol: sex, age, age², age³, sex \times age interaction, age at first survey, time of surveyHDL-C: sex, age, age², sex \times age interaction, age at first survey, time of survey

sex-stratified longitudinal association analyses are given in ESM Table 2.

Conclusion

In this study, we investigated the association of variants in the *GCK*, *APOA5* and *LPL* genes with fasting glucose and lipid levels on a total of 4,554 individuals using both population- and family-based study designs and incorporating both cross-sectional and longitudinal measures. The associations of these variants with fasting glucose and lipid measures in cross-sectional studies are well established but their longitudinal effects in the same individuals followed-up for a long period of time are not known. We used data from a cohort of predominantly European–Australian individuals from the BHS to test the associations of some common variants with longitudinal measures of metabolic and cardiovascular phenotypes. The BHS is a very comprehensively phenotyped resource, with the selected cohort having been followed up on average four times over 21 years with multiple metabolic measures, and thus was well-suited to addressing the aim of this study.

Our cross-sectional association analyses confirmed previous findings of associations between the *GCK* rs1799884 variant and raised fasting glucose levels [9, 22], the *APOA5* rs662799 and rs3135506 variants and raised triacylglycerol levels [16, 17, 34], and the *LPL* rs328 variant and reduced triacylglycerol levels and raised HDL-C levels [20, 21].

Our graphical presentation of the longitudinal glucose and lipid data with age (Figs 2, 3) show previously reported general trends towards higher fasting glucose and

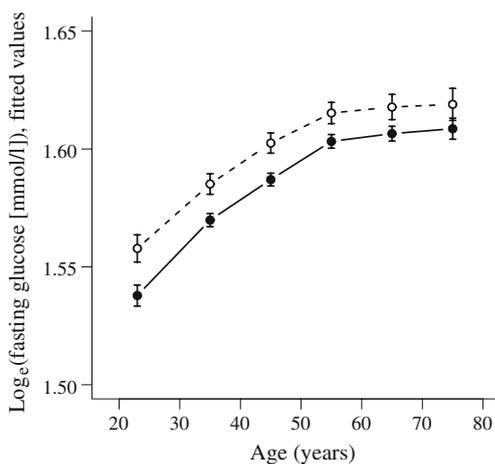


Fig. 2 Variation with age of adjusted natural log-transformed fasting glucose values by rs1799884 genotype (black circle, homozygous wild-type; white circle, heterozygote and homozygous mutant) in unrelated, non-diabetic participants aged 18–80 years. Values were fitted using full covariate, dominant genetic models

triacylglycerol levels with increasing age [11, 12], which were present for all SNP genotype groups. Though HDL-C has been reported previously to decline with age [14], no strong trend in HDL-C level was observed in this study.

When we examined the genotype–phenotype relationships in longitudinal analyses, we found that the association of the rs328 variant with raised HDL-C level increased with age. An increasing trend in the association between the rs328 mutant allele and HDL-C level is clearly visible across multiple age groups in the plot of fitted HDL-C values in Fig. 3f. This observation is also supported by the HDL-C level geometric means comparison for consecutive age groups in Table 5. Together, these results suggest that while the rs328 mutant allele does not significantly alter HDL-C levels in younger individuals in the 18–40 years age group, its association increases with age until the final 70–80 years age group at which the observation drops off. These results suggest that carriers of one or two 447X alleles are protected from decline in HDL-C, at least in middle to early old age. Further longitudinal studies are needed to confirm if and how the association between HDL-C and rs328 alters in the same individuals as they age.

We found no significant evidence of an interaction between age and rs1799884 on fasting glucose level, between age and either rs328, rs662799 or rs3135506 on triacylglycerol level, or between age and either rs662799 or rs3135506 on HDL-C level, and hence, no evidence that these SNPs are involved in age-related changes in the traits examined. These results are illustrated in the relevant plots of Figs 2 and 3, in which trait values vary with age at similar rates for the two genotype groups, irrespective of shifts related to overall variant associations. In this study, age was not a significant modifier of associations for the majority of variants.

We found no significant evidence that variant associations changed over time, independent of age-related changes, in all but one case. The triacylglycerol-raising association of the rs3135506 variant, which changes a serine residue to tryptophan (S19W), increased over time, indicating that rs3135506 may interact with environmental factors that have changed over the 20 year time period examined, to modify triacylglycerol levels. Such environmental factors could include lifestyle factors such as diet, physical activity and stress level.

Further studies are now required to assess the validity of these findings. One consideration is power. One of the strengths of this study was its ability to consider the same individuals at different ages using longitudinal data. However, with a mean follow-up time of 17.4 years, the study did not follow individuals from youth through to old age. Moreover, though power calculations showed that the dominant model used was adequately powered, there was insufficient power to detect recessive associations. A repli-

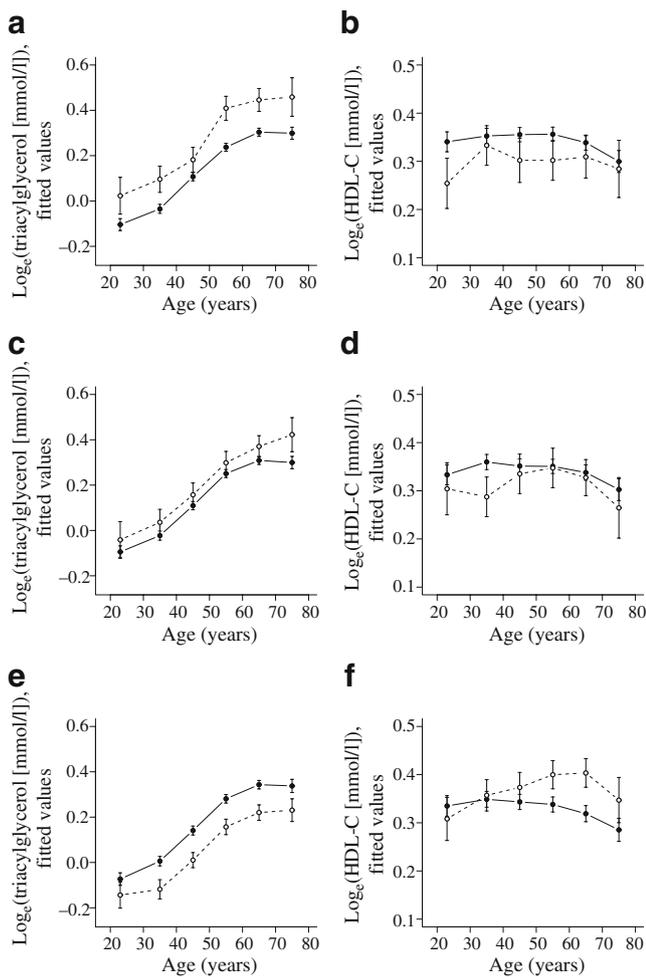


Fig. 3 Variation with age of adjusted natural log-transformed triacylglycerol and HDL-C values by genotype (black circle, homozygous wild-type; white circle, heterozygote and homozygous mutant), in unrelated participants aged 18–80 years, for three SNPs: **a, b** rs662799; **c, d** rs135506; **e, f** rs328. Values were fitted using full covariate, dominant genetic models

cation study in a larger cohort would have greater power to detect subtle interactions and would also enable investigation of whether a recessive model of inheritance would give the best fit.

As these findings are restricted to individuals of European ancestry, the results presented here are not necessarily generalisable to other ethnic groups. Replication in other ethnic groups may reveal how the SNPs studied here influence metabolic outcomes in other populations. Furthermore, this study considered the influence of only four variants in three genes involved in type 2 diabetes, though many common variants have been identified to influence metabolic traits and diabetes risk. Additional studies of the interactions between such variants and age would improve understanding of age-related genetic associations in metabolic disorders. However, replication studies will be limited by the available epidemiological databases, few of which

contain the multiple metabolic measures collected longitudinally for the BHS.

In conclusion, the results of this study are consistent with previous reports of the effects of *GCK*, *APOA5* and *LPL* variants on glucose and lipid traits. The effects of established gene variants with lipid and glucose traits do not tend to alter with age, although there is some evidence that the HDL-C-raising effect of the 447X allele of rs328 significantly increases with age.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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