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Consistency between cross-sectional and longitudinal SNP: blood lipid associations

Michael C. Costanza · Sigrid Beer-Borst · Richard W. James · Jean-Michel Gaspoz · Alfredo Morabia

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Abstract Various studies have linked different genetic single nucleotide polymorphisms (SNPs) to different blood lipids (BL), but whether these "connections" were identified using cross-sectional or longitudinal (i.e., changes over time) designs has received little attention. Cross-sectional and longitudinal assessments of BL [total, high-, low-density lipoprotein cholesterol (TC, HDL, LDL), triglycerides (TG)] and non-genetic factors (body mass index, smoking, alcohol intake) were measured for 2,002 Geneva, Switzerland, adults during 1999–2008 (two measurements, median 6 years apart), and 20 SNPs in 13 BL metabolism-related genes. Fixed and mixed effects repeated measures linear regression models, respectively, were employed to identify cross-sectional and longitudinal SNP:BL associations among the 1,516 (76%) study participants who reported not being treated for hypercholesterolemia at either measurement time. One-third more (12 vs. 9) longitudinal than cross-sectional associations were found [Bonferroni-adjusted two-tailed $p < 0.00125$ (=0.05/2)/20) for each of the four ensembles of 20 SNP:individual BL associations tested under the two study designs]. There was moderate consistency between the cross-sectional and longitudinal findings, with eight SNP:BL associations consistently identified across both study designs: [APOE.2 and APOE.4 (rs7412 and rs429358)]:TC; HL/LIPC (rs2070895):HDL; [APOB (rs1367117), APOE.2 and APOE.4 (rs7412 and rs429358)]:LDL; [APOA5 (rs2072560) and APOC III (rs5128)]:TG. The results suggest that cross-sectional studies, which include most genome-wide

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M. C. Costanza
Department of Community Medicine and Primary Care, University Hospitals of Geneva, University of Geneva, Geneva, Switzerland

M. C. Costanza (✉)
University of Vermont, Burlington, VT, USA
e-mail: michael.c.costanza@uvm.edu

M. C. Costanza
Rushden, Northamptonshire, UK

S. Beer-Borst
Section of Health, Applied R&D in Nutrition and Dietetics, Department of Business and Administration, Health, and Social Work, Bern University of Applied Sciences, Bern, Switzerland
e-mail: sigrid.beer@bfh.ch

R. W. James
Lipid Laboratory, Clinical Diabetes Unit, University Hospitals of Geneva, Geneva, Switzerland
e-mail: james-richard@diogenes.hcuge.ch

J.-M. Gaspoz
Department of Community Medicine and Primary Care, University Hospitals of Geneva, University of Geneva, Geneva, Switzerland
e-mail: gaspoz-jean-michel@diogenes.hcuge.ch

A. Morabia
Center for the Biology of Natural Systems, Queens College, City University of New York, New York, NY, USA
e-mail: alfredo.morabia@qc.cuny.edu

A. Morabia
Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA

association studies (GWAS), can assess the large majority of SNP:BL associations. In the present analysis, which was much less powered than a GWAS, the cross-sectional study was around 2/3 (67%) as efficient as the longitudinal study.

Keywords Association · Blood cholesterol · Cross-sectional design · Genetic marker · Longitudinal design · Single nucleotide polymorphism

Introduction

Concentrations of human blood lipids (BL) are, in part, under genetic control (e.g., [1–6]). The evidence has mainly been derived from cross-sectional associations, which include most genome-wide association studies (e.g., [2–5]), that estimate how much of the between-individual variability in BL is explained by the absence versus presence of specific alleles. The cross-sectional study design rationale is that the BL concentrations of two groups of subjects, assumed to be exchangeable except for an allelic variant of interest, should differ only because of their genetic backgrounds. This may happen either if specific genes have an absolute impact on BL levels, or, more likely, if they modify BL concentration changes over time. From this perspective, cohort studies that relate genetic traits to BL at a single point in time capture the same type of associations as cross-sectional studies. This second type of genetic effect may be missed in cross-sectional studies because longitudinal differences may not necessarily translate into differences in baseline concentrations. For example, Chasman et al. [7] found no association between two intronic HMG-CoA-reductase single nucleotide polymorphisms (SNPs) and lipid levels at baseline but an association with lipid changes following treatment with statins. Statins are an intervention, but BL vary with age.

The present work was designed to investigate the consistency of cross-sectional versus longitudinal assessments of the associations of 20 candidate SNPs with specific BL [total, high, and low density lipoprotein cholesterol, and triglycerides (TC, HDL, LDL, TG)] concentrations. These analyses were performed in a population-based, longitudinal study of adult residents of Geneva, Switzerland who were not receiving any hypolipemic treatment.

Methods

Study sample

The study was authorized by the Institutional Review Board of the University of Geneva, Switzerland to re-examine 2,002 (62%) out of 3,250 randomly selected, primarily French-speaking Geneva residents from 35 to 74 years of age. All had baseline data collected between 1999 through 2004 (Time.1) and agreed to participate in a follow-up study conducted between 2005 and 2008 (Time.2). The sampling and recruitment methodologies were identical to those reported elsewhere [6].

Exclusions of study participants treated for hypercholesterolemia

Hypolipemic treatment was assessed at both points in time by asking the study participants whether "a doctor had prescribed a treatment to lower blood cholesterol". Only those study participants who reported not being treated for hypercholesterolemia at either time point and who had no missing data on all BL measures and non-genetic variables at both time points were eligible to be included in the present cross-sectional and longitudinal analyses ($n = 1{,}516$ (76% of 2,002) before exclusions (range: 1.3–4.3%) for missing genetic data).

Blood lipid assays

TC, HDL, and TG (mmol/L) were assayed in fasting blood (Bayer Technicon Diagnostics, Brussels, Belgium, with monthly quality controls performed by the Swiss Center for Quality Control in Clinical Chemistry and Hematology). LDL (for TG <4.52 mmol/L) was calculated as (TC-HDL—TG/2.2) [8], and was considered missing otherwise. To reduce (right) skewness, log(TG) was employed in most of the subsequent statistical analyses, with corresponding back-transformation to geometric means (GM) and various ratios thereof in estimates and confidence intervals (exceptions: see Table 2); for ease of presentation, the log(TG) results are still referred to under the rubric "TG" below.

Genetic factors

The following rationale for selecting the genes and SNPs was employed: 10 of the SNPs were chosen because they belonged to seven genes involved in the reverse cholesterol transport pathway (*ABCA1*, *APOE*, *HL/LIPC*, *LDLR*, *LPL*, *PLTP*, *SRB1*), which were previously reported to be independently associated with BL over and beyond the very strong associations of the non-genetic factors (e.g., body mass index, smoking, alcohol intake) [6]. In addition to the 10 SNPs of the working model, 12 more SNPs which were identified in the interim after the latter report were selected as good candidates: (a) apoliprotein B (APOB) (Thr71Ile, Ala618Val, Asn4311Ser), apolipoprotein E (APOE) (Prom −219 g/t), cholesteryl-ester transfer protein (CETP) (Prom −971 g/a) and apolipoprotein A5 (APOA5) (ser19Trp,

rs2072560 G/A), which were found to be strongly related to HDL and the HDL/LDL ratio in the study of Knoblauch et al. [9]; (b) HMG-CoA reductase SNPs 12 and 29 from Chasman et al. [7]; (c) peroxisome proliferator-activated receptor γ (Pro12Ala) from the study of Altshuler et al. [10]; (d) apolipoprotein-CIII (APOCIII) (rs5128 3′UTR Sst-1, rs2854116, promotor −455 C/T), which may play a role at the interface of the metabolism of lipids, insulin, and glucose [11–14].

All blood samples collected from 2005 through 2008 had total genomic DNA extracted from EDTA blood using the Gentra PUREGENE® DNA purification kit (BioConcept, Allschwil, Switzerland).

All samples were genotyped for the following 20 SNPs from 13 genes [SNP acronyms (rs no)]: ATP-binding cassette protein 1 [ABCA1 (rs2777801)]; apolipoprotein A5 [APOA5 (rs2072560)]; apoliprotein B [APOB.1/APOB. 2/APOB. 3 (rs679899/rs1042034/rs1367117)]; apolipoprotein C III [APOC3.1/APOC3.2 (rs2854116/rs5128)]; apolipoprotein E [APOE.prom/APOE.2/APOE.4 (rs405509/rs7412/429358)]; cholesteryl-ester transfer protein [CETP (rs4783961)]; hepatic lipase (HL)/LIPC [HL.prom/HL.2 (rs2070895/rs12909325)]; HMG-CoA (3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase) [HMG.1/HMG.2 (rs17238540/rs17244841)]; low density lipoprotein receptor [LDLR (rs2228671)]; lipoprotein lipase [LPL (rs328)]; phospholipid transfer protein [PLTP (rs2294213)]; peroxisome proliferator-activated receptor γ [PPARg (rs1801282)]; scavenger receptor class B type I [SRB1 (rs5888)]. The allelic frequencies of these SNPs in the untreated study participants are shown in Table 1.

Nine of these 20 SNPs (ABCA1, APOE.2, APOE.4, HL.prom, HL.2, LDLR, LPL, PLTP, SRB1) were assayed twice in over n = 1,000 study participants (range: 1,036–1,077) [6, 15], with excellent reliability in terms of gamma (range: 0.96–0.99) and kappa [simple (range: 0.82–0.95) and weighted (range: 0.83–0.95)] measures of agreement (Supplementary Table 1).

Non-genetic factors

The five non-genetic factors (acronym) were: (SEX), (AGE) (yrs), measured (BMI) (kg/m^2), self-reported current cigarette smoking (CSMOKE) (yes/no), and sex-specific [men/women] alcohol intake (two yes/no variables) derived from a food frequency questionnaire [16] and categorized using approximate gender-specific risk criteria for daily consumption (g alcohol/day) [17]): (ALCO.L) (low [1–40/1–20]); (ALCO.MH) (medium + high [41+/21+]; implied reference group (0 (none)) at the Time.1 and Time.2 surveys.

Physical examinations included: (a) medical scale-measured weight (precision 0.1 kg) with participants lightly dressed and without shoes (1 kg deducted across seasons to allow for clothing); (b) medical gauge-measured standing height without shoes (precision 0.5 cm); (c) a fasting blood

Table 1 Allelic frequencies of the 20 SNPs assayed at the 2005–2008 (Time.2) survey for study participants not being treated for hypercholesterolemia and who had no missing data on all BL measures and non-genetic variables at either time point

Geneva, Switzerland (n = 1,516*)

* Before exclusions for missing genetic data (range: 1.3–4.3% with missing data on individual SNPs)

SNP.name (rs no)	Nucleotide call	n (%)	SNP.name (rs no)	Nucleotide call	n (%)
ABCA1 (rs2777801)	TT/TG/GG	1,163/300/25 (78.2/20.2/1.7)	CETP (rs4783961)	GG/GA/AA	407/731/352 (27.3/49.1/23.6)
APOA5 (rs2072560)	CC/CT/TT	1,305/175/4 (87.9/11.8/0.3)	HL.prom (rs2070895)	GG/GA/AA	878/504/100 (59.2/34.0/6.8)
APOB.1 (rs679899)	GG/GA/AA	393/722/336 (27.1/49.8/23.2)	HL.2 (rs12909325)	GG/GA/AA	507/696/279 (34.2/47.0/18.8)
APOB.2 (rs1042034)	AA/AG/GG	882/519/71 (59.2/35.3/4.8)	HMG.1 (rs17238540)	TT/TG/GG	1,399/94/3 (93.5/6.3/0.2)
APOB.3 (rs1367117)	GG/GA/AA	744/590/120 (51.2/40.6/8.3)	HMG.2 (rs17244841)	AA/AT/TT	1,372/86/6 (93.7/5.9/0.4)
APOC3.1 (rs2854116)	GG/GC/CC	572/704/215 (38.4/47.2/14.4)	LDLR (rs2228671)	CC/CT/TT	1,147/319/27 (76.8/21.4/1.8)
APOC3.2 (rs5128)	AA/AG/GG	1,225/255/8 (82.3/17.1/0.5)	LPL (rs328)	CC/CG/GG	1,126/327/28 (76.0/22.1/1.9)
APOE.prom (rs405509)	CC/CA/AA	445/738/305 (29.9/49.6/20.5)	PLTP (rs2294213)	CC/CG/GG	1,210/260/16 (81.4/17.5/1.1)
APOE.2 (rs7412)	CC/CT/TT	1,270/217/7 (85.0/14.5/0.5)	PPARG (rs1801282)	CC/CG/GG	1,170/305/21 (78.2/20.4/1.4)
APOE.4 (rs429358)	TT/TC/CC	1,172/281/18 (79.7/19.1/1.2)	SRB1 (rs5888)	CC/CT/TT	421/719/348 (28.3/48.3/23.4)

sample (with informed consent). Further details on the survey measurements can be found elsewhere [6].

Statistical analyses

For assessing statistical significance of simple (unadjusted) (Time.1–Time.2) changes in the continuous measurements BMI and BL, two-tailed paired Student's t tests were used (SAS PROC TTEST [18]); for the dichotomous current smoking and (essentially) trichotomous [none/low/(medium + high)] alcohol variables, respectively, McNemar's and Bowker's χ^2 tests of symmetry were used (SAS PROC FREQ [18]).

In the main statistical analyses fixed (cross-sectional study) and mixed (longitudinal study) effects multiple linear regression models were employed to assess the genetic association of each SNP on the four BL outcome variables. Each SNP was coded 0 if the rare allelic variant was absent, or 1 if it was present (as heterozygote/homozygote). This coding assumes that the rare allele effect is dominant. The rare allele homozygote frequencies for 12 of the 20 SNPs investigated were too small (<5%, see Table 1) to be meaningfully analyzed assuming an additive [0-1-2 coding for rare allele(s)] model. For the other eight SNPs with rare allele homozygote frequencies of 100 or more (>5%, see Table 1) individuals, additive models were also analyzed to provide some idea of agreement with the dominant model results.

All measurements at the Time.1 and Time.2 surveys are identified by the suffixes ".1" and ".2". Separate analyses were run using: (a) SAS PROC GLM [18] for fixed effects cross-sectional linear models using the BL.2 measurements as the outcome variables regressed ($\sim$) as:

$$\mathrm{BL.2} \sim \mathrm{SEX.2} + \mathrm{AGE.2} + \mathrm{BMI.2} + \mathrm{CSMOKE.2} + \mathrm{ALCO.L.2} + \mathrm{ALCO.MH.2} + \mathrm{SNP};$$

and (b) SAS PROC MIXED [18] for mixed effects longitudinal repeated measures (Time = 1,2) linear models using the paired (BL.1, BL.2) (="BL" below, with similar notation for the pairs of other variable) measurements as the outcome variables regressed as:

$$\mathrm{BL} \sim \mathrm{SEX} + \mathrm{AGE} + \mathrm{BMI} + \mathrm{CSMOKE} + \mathrm{ALCO.L} + \mathrm{ALCO.MH} + \mathrm{SNP} + \mathrm{Time} + \{\mathrm{SNP} \times \mathrm{Time}\}.$$

To account (approximately) for multiple comparisons in the above models, Bonferroni-adjusted, simultaneous $\alpha = 0.05$ statistical significance for the presence of any of the 20 individual SNP associations with each of the four individual BL as determined from the corresponding 20 cross-sectional model, or 20 longitudinal model, ensembles of tests was claimed only when the SNP:BL association $p < (0.05/2)/20 = 0.00125$. Correspondingly, 99.75% (Bonferroni-adjusted simultaneous 95%) confidence intervals (CI) were employed for estimation of LSMEANS (least squares means) of SNP absent and/vs. SNP present subgroups adjusted for all the other non-genetic variables in the various cross-sectional/longitudinal statistical models using SAS PROC GLM [18] and SAS PROC MIXED [18].

Results

Descriptive

Summary statistics (unadjusted) for the non-genetic factors and the blood lipids at the cross-sectional Time.2 survey and the mean longitudinal changes from the Time.1 to the Time.2 surveys among the untreated study participants are shown in Table 2. Almost all the mean longitudinal changes examined, including those for all four BL, were statistically significant (individual nominal $p < 0.05$), even though they were sometimes small.

SNP:BL associations

There were nine SNP:BL associations identified in the cross-sectional models, versus 12 (33% more) identified in the longitudinal models (Table 3). In the longitudinal models there was little indication of any (SNP × Time) interaction effects; in other words, on average the $\mathrm{SNP}_{\mathrm{absent}}$ and $\mathrm{SNP}_{\mathrm{present}}$ subgroups were very similar in terms of within-individual BL changes (some results shown in Table 4).

One association was observed only in the cross-sectional study (APOB.2:TG), while four associations were found only longitudinally: APOE.prom:TC, (CETP:HDL, LPL:HDL), and APOE.prom:LDL.

Eight SNP:BL associations were consistently identified across both models (Table 3): [APOE.2 and APOE.4 (rs7412 and rs429358):TC]; [HL (rs2070895):HDL]; [APOB.3 (rs1367117), APOE.2 and APOE.4 (rs7412 and rs429358):LDL]; [APOA5 (rs2072560) and APOC3.2 (rs5128):TG].

Further results on the magnitudes and directions of the eight SNP:BL associations consistently identified across both study designs are provided by the point estimates with 99.75% (Bonferroni-adjusted simultaneous 95%) CIs shown in Table 4. All six of the adjusted longitudinal (BL.1–BL.2) differences for TC, HDL, and LDL in the $\mathrm{SNP}_{\mathrm{absent}}$ subgroup (Table 4) were significantly different; that only two of those differences were significantly different for the (much) smaller (exceptions: APOB.3, HL.prom) $\mathrm{SNP}_{\mathrm{present}}$ subgroup (Table 4) was likely due to the smaller sample size (e.g., all six differences were in the same direction as those of the $\mathrm{SNP}_{\mathrm{absent}}$ subgroup). To the

Table 2 Summaries of non-genetic factors and blood lipid (BL) concentrations at the 2005–2008 (Time.2) survey and changes from the 1999–2004 (Time.1) survey for study participants not being treated for hypercholesterolemia and who had no missing data on any of the BL measures and non-genetic variables at either time point

Non-genetic factor	Cross-sectional study (Time.2)			Longitudinal study changes (Time.1–Time.2)		
	Mean	(SD)	[Range]	Mean	(SD)	[Range]
Age (years)	56.3	(10.3)	[39,81]			
Follow-up time (years)				5.9	(0.8)	[2.3,8.0]

	Subgroup	n	(%)	% change Time.1 ⇒ Time.2	p[a]
Current smoker	No (N)	1,252	(82.6)	N ⇒ Y: 2.1	<0.0001
	Yes (Y)	264	(17.4)	Y ⇒ N: 68.3	
Alcohol	None (N)	198	(13.1)	N ⇒ L: 34.8; N ⇒ MH: 0	0.053
	Low (L)	1,179	(77.8)	L ⇒ N: 6.6; L ⇒ MH: 5.4	
	Med + Hi (MH)	139	(9.2)	MH ⇒ N: 1.8; MH ⇒ L: 51.8	

	Mean	(SD)	[P25, P75]	Mean	(SD)	[P25, P75]	p[b]
BMI, kg/m^2)	25.0	(4.1)	[22.1,27.3]	0.59	(1.59)	[−0.21,1.32]	<0.0001
Blood lipid[b] (mmol/L)							
TC	5.62	(0.91)	[4.98, 6.23]	0.04	(0.72)	[−0.41, 0.49]	0.026
HDL	1.49	(0.38)	[1.21, 1.72]	0.09	(0.26)	[−0.06, 0.24]	<0.0001
LDL	3.62	(0.83)	[3.06, 4.16]	−0.05	(0.63)	[−0.45, 0.34]	0.001
TG	1.01[c]	(0.42)[c]	[0.75, 1.30]	0.01	(0.48)	[−0.19, 0.24]	0.015

Geneva, Switzerland [n = 1,516 (men: n = 691 (45.6%); women: n = 825 (54.4%)]

TC total serum cholesterol; *HDL* high density lipoprotein cholesterol; *LDL* low density lipoprotein cholesterol; *TG* triglycerides

[a] McNemar (Current smoker) or Bowker (Alcohol) omnidirectional p value for testing the null hypothesis of off-diagonal symmetry of proportions

[b] Two-tailed p value for testing the null hypothesis that the population mean = 0 with paired Student's t test (based on log-transformed differences for TG)

[c] Geometric mean; SD of log-transformed TG at Time.2

contrary, that the only longitudinal (BL.1/BL.2) geometric mean ratio for TG (for APOA5) that differed significantly from 1.0 occurred in the much smaller $SNP_{present}$ subgroup (Table 4) was likely due more to violations of distributional symmetry, even after log transformation, than to sample size issues.

All of the cross-sectional ($SNP_{absent}-SNP_{present}$) differences and the ($SNP_{absent}/SNP_{present}$) ratios (Table 4) were significantly different from the respective null values 0 and 1.0. On the other hand, neither any of the longitudinal differences of ($SNP_{absent}-SNP_{present}$) differences, nor any of the longitudinal ratios of ($SNP_{absent}/SNP_{present}$) ratios, differed significantly from the respective null values 0 and 1.0, consistent with the finding mentioned above that there was little evidence for ant (SNP × Time) interaction effects.

Dominant versus additive models

There were eight SNPs, (APOB.1, APOB. 3, APOC3.1, APOE.prom, CETP, HL.prom, HL.2, SRB1), for which it was feasible (rare allelic homozygotes 100 or more (>5%), see Table 1) to compare the use of additive models (0-1-2 SNP coding) in the SNP:BL analyses instead of the dominant model (0-1 SNP coding) results reported above and in Table 3. In the cross-sectional dominant model results (Table 3), only two of the latter eight SNPs, (APOB. 3, HL.prom), were found to have one or more statistically significant SNP:BL associations. On the other hand, in the dominant model longitudinal results (Table 3), four of those SNPs, (APOB. 3, APOE.prom, CETP, HL.prom), had one or more statistically significant SNP:BL associations.

When, instead, additive models were employed in the otherwise analogous cross-sectional analyses, the dominant and additive model results were in perfect agreement in terms of identifying statistically significant SNP:BL associations. When additive models were employed in the otherwise analogous longitudinal analyses, the dominant and additive model results were almost in perfect agreement, with just one very close exception for the APOB.3:LDL associations (dominant model $p = 0.0011 < 0.00125$; additive model $p = 0.0029 > 0.00125$).

Table 3 SNP:blood lipid associations with $p < 0.00125$ [=(0.05/2)/20] identified in the cross-sectional (2005–2008), longitudinal [(1999–2004) ⇒ (2005–2008)], and both models

Geneva, Switzerland, 2005–2008 (n = 1,516)

Before exclusions for missing genetic data (range: 1.3–4.3% with missing data on individual SNPs)

TC total serum cholesterol; *HDL* high density lipoprotein cholesterol; *LDL* low density lipoprotein cholesterol; *TG* triglycerides (log(TG) employed in models)

For definitions of SNP acronyms see Table 1

Blood lipid	SNP effect(s) identified in:		
	Cross-sectional models	Longitudinal models	Both models
TC	2 associations:	3 associations:	2 associations:
	APOE.2, APOE.4	APOE.prom, APOE.2, APOE.4	APOE.2, APOE.4
HDL	1 association:	3 associations:	1 association:
		CETP	
	HL.prom	HL.prom	HL.prom
		LPL	
LDL	3 associations:	4 associations:	3 associations:
	APOB.3	APOB.3	APOB.3
	APOE.2, APOE.4	APOE.prom, APOE.2, APOE.4	APOE.2, APOE.4
TG	3 associations:	2 associations:	2 associations
	APOA5	APOA5	APOA5
	APOB.2		
	APOC3.2	APOC3.2	APOC3.2
Total	9 associations	12 associations	8 associations

Table 4 SNP:blood lipid associations identified consistently across both the cross-sectional and longitudinal study designs

Blood Lipid (mmol/L)	Adjusted least squares means [99.75% (Bonferroni-adjusted 95%) CI]				
	SNP (rs n^o)	Study design	SNP absent	SNP present	Difference ($SNP_{absent} - SNP_{present}$) [TG: Ratio ($SNP_{absent}/SNP_{present}$)]
TC.2	APOE.2 (rs7412)	Cross-sectional	5.68 (5.60, 5.75)	5.33 (5.15, 5.50)	0.35 (0.16, 0.54)
TC.1-TC.2		Longitudinal	0.11 (0.04, 0.18)	0.01 (−0.14, 0.16)	0.10 (−0.07, −0.26)
TC.2	APOE.4 (rs429358)	Cross-sectional	5.58 (5.50, 5.65)	5.81 (5.65, 5.96)	−0.23 (−0.40, −0.06)
TC.1-TC.2		Longitudinal	0.10 (0.03, 0.17)	0.03 (−0.10, 0.15)	0.08 (−0.07, 0.22)
HDL.2	HL.prom (rs2070895)	Cross-sectional	1.45 (1.42, 1.49)	1.54 (1.50, 1.58)	−0.08 (−0.13, −0.03)
HDL.1-HDL.2		Longitudinal	−0.07 (−0.10, −0.04)	−0.11 (−0.14, −0.07)	0.04 (−0.04, 0. 11)
LDL.2	APOB.3 (rs1367117)	Cross-sectional	3.55 (3.46, 3.64)	3.72 (3.63, 3.81)	−0.17 (−0.30, −0.04)
LDL.1-LDL.2		Longitudinal	0.20 (0.12, 0.28)	0.10 (0.02, 0.18)	0.10 (−0.01, 0.21)
LDL.2	APOE.2 (rs7412)	Cross-sectional	3.69 (3.62, 3.76)	3.27 (3.11, 3.43)	0.42 (0.25. 0.60)
LDL.1-LDL.2		Longitudinal	0.18 (0.12, 0.24)	0.06 (−0.07, 0.19)	0.12 (−0.02, 0.27)
LDL.2	APOE.4 (rs429358)	Cross-sectional	3.57 (3.50, 3.54)	3.83 (3.69, 3.97)	−0.26 (−0.42. −0.10)
LDL.1-LDL.2		Longitudinal	0.17 (0.11, 0.23)	0.10 (−0.02, 0.21)	0.07 (−0.06, 0.20)
TG.2	APOA5 (rs2072560)	Cross-sectional	1.00 (0.97, 1.04)	1.12 (1.03, 1.23)	0.89 (0.81 0.98)
TG.1-TG.2		Longitudinal	1.02 (0.98, 1.06)	1.04 (0.96, 1.13)	0.98 (0.88, 1.08)
TG.2	APOC3.2 (rs5128)	Cross-sectional	1.00 (0.97, 1.03)	1.10 (1.03, 1.18)	0.91 (0.84, 0.98)
TG.1-TG.2		Longitudinal	1.00 (0.99, 1.06)	1.01 (0.95, 1.08)	1.01 (0.94, 1.09)

Adjusted least squares means, mean differences, and differences of mean differences (For Triglycerides, replace "means" by "geometric means (GM)", "mean differences" by "GM ratios", and "differences of mean differences" by "ratios of GM ratios" in the previous phrase). Geneva, Switzerland, 2005–2008

Cross-sectional: adjusted for (SEX, AGE.2, BMI.2, CSMOKE.2, ALCO.LO.2, ALCO.MH.2)

Longitudinal : adjusted for (SEX, AGE, AGE, BMI, CSMOKE, ALCO.LO, ALCO.MH)

TC total serum cholesterol; *HDL* high density lipoprotein cholesterol; *LDL* low density lipoprotein cholesterol; *TG*: log(TG) models and GM used in estimates

Discussion

The two study designs focus on assessing genetic associations with different types of outcome variables: between-individual differences at single timepoint(s) in cross-sectional studies versus within-individual changes at several timepoints in longitudinal studies (as well as further associations with or adjustments for changes in non-genetic covariates).

After adjusting for multiple comparisons using a Bonferroni procedure, there was moderate consistency in terms of identifying potentially important SNP:BL associations between the cross-sectional study (nine associations) and the longitudinal study (12 (33% more) associations) findings. These results did not appear to depend on whether dominant (0-1 SNP coding) or additive (0-1-2 SNP coding) models were employed in the statistical analyses. Because eight SNP:BL associations were consistently identified across both study designs, the cross-sectional study was around 2/3 (8/12 = 67%) as efficient as the longitudinal study for identifying SNP:BL associations.

It is noteworthy that the associations detected by both designs involved well-established candidate genes such as *APOE*, *HL*, *APOB* and the complex including *APOA5* and *APOC III* [1–6]. Other associations may require more statistical power to be detected. Teslovich et al. [5] more than doubled (from 36 to 95) the number of identified loci for BL when they studied >100,000 individuals instead of ~20,000. Thus, it is reasonable to expect that the consistency between the two study designs would have been even larger had our study been based on a much larger sample size.

In general, we expected both types of associations to capture the same underlying biologic phenomena and be consistent. We can only speculate on the reasons why some associations failed to be reproduced in both designs. Longitudinal studies, in which BL are measured in the same individuals at several points in time, have the advantage over cross-sectional studies of facilitating direct assessment of genetic associations on changes in BL concentrations. With a cross-sectional design it is not possible to determine whether differences between individuals are due directly to differences in absolute concentrations of the BL, or indirectly to differences in BL in response to aging or behavioral changes, such as changes in weight, alcohol intake, or smoking.

However, we cannot rule out the possibility that the differences between the two study designs have purely statistical causes. Firstly, longitudinal studies may have a comparative statistical power advantage over cross-sectional designs because within-individual differences over time, though usually much smaller than between-individual differences at a single time point, also tend to be much less variable. This advantage may be attenuated by the concomitant disadvantages of having to deal with measurement errors at several points in time instead of just one. Secondly, four of the eight SNP:BL associations that were identified by both designs were in opposite directions, which may reflect statistical instability of the estimated regression coefficients.

The present study indicates that interpretations of "connections" between specific SNPs and BL concentrations may not differ substantially whether evaluated cross-sectionally or longitudinally. Any differences between the two design approaches may be even less in GWAS, which usually have tremendous statistical power to detect associations in cross-sectional designs. A formal confirmation of this conclusion in other populations is warranted.

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