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Multi-ancestry study of blood lipid levels identifies four loci interacting with physical activity

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Many genetic loci affect circulating lipid levels, but it remains unknown whether lifestyle factors, such as physical activity, modify these genetic effects. To identify lipid loci interacting with physical activity, we performed genome-wide analyses of circulating HDL cholesterol, LDL cholesterol, and triglyceride levels in up to 120,979 individuals of European, African, Asian, Hispanic, and Brazilian ancestry, with follow-up of suggestive associations in an additional 131,012 individuals. We find four loci, in/near *CLASP1*, *LHX1*, *SNTA1*, and *CNTNAP2*, that are associated with circulating lipid levels through interaction with physical activity; higher levels of physical activity enhance the HDL cholesterol-increasing effects of the *CLASP1*, *LHX1*, and *SNTA1* loci and attenuate the LDL cholesterol-increasing effect of the *CNTNAP2* locus. The *CLASP1*, *LHX1*, and *SNTA1* regions harbor genes linked to muscle function and lipid metabolism. Our results elucidate the role of physical activity interactions in the genetic contribution to blood lipid levels.

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Circulating levels of blood lipids are strongly linked to the risk of atherosclerotic cardiovascular disease. Regular physical activity (PA) improves blood lipid profile by increasing the levels of high-density lipoprotein cholesterol (HDL-C) and decreasing the levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG)¹. However, there is individual variation in the response of blood lipids to PA, and twin studies suggest that some of this variation may be due to genetic differences². The genes responsible for this variability remain unknown.

More than 500 genetic loci have been found to be associated with blood levels of HDL-C, LDL-C, or TG in published genome-wide association studies (GWAS)^{3–12}. At present, it is not known whether any of these main effect associations are modified by PA. Understanding whether the impact of lipid loci can be modified by PA is important because it may give additional insight into biological mechanisms and identify subpopulations in whom PA is particularly beneficial.

Here, we report results from a genome-wide meta-analysis of gene–PA interactions on blood lipid levels in up to 120,979 adults of European, African, Asian, Hispanic, or Brazilian ancestry, with follow-up of suggestive associations in an additional 131,012 individuals. We show that four loci, in/near *CLASPI*, *LHX1*, *SNTA1*, and *CNTNAP2*, are associated with circulating lipid levels through interaction with PA. None of these four loci have been identified in published main effect GWAS of lipid levels. The *CLASPI*, *LHX1*, and *SNTA1* regions harbor genes linked to muscle function and lipid metabolism. Our results elucidate the role of PA interactions in the genetic contribution to blood lipid levels.

Results

Genome-wide interaction analyses in up to 250,564 individuals. We assessed effects of gene–PA interactions on serum HDL-C, LDL-C, and TG levels in 86 cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Gene-Lifestyle Interactions Working Group¹³. PA was harmonized across participating studies by categorizing it into a dichotomous variable. The participants were defined as inactive if their reported weekly energy expenditure in moderate-to-vigorous intensity leisure-time or commuting PA was less than 225 metabolic equivalent (MET) minutes per week (corresponding to approximately 1 h of moderate-intensity PA), while all other participants were defined as physically active (Supplementary Data 1).

The analyses were performed in two stages. Stage 1 consisted of genome-wide meta-analyses of linear regression results from 42 cohorts, including 120,979 individuals of European [$n = 84,902$], African [$n = 20,487$], Asian [$n = 6403$], Hispanic [$n = 4749$], or Brazilian [$n = 4438$] ancestry (Supplementary Tables 1 and 2; Supplementary Data 2; Supplementary Note 1). All variants that reached two-sided $P < 1 \times 10^{-6}$ in the Stage 1 multi-ancestry meta-analyses or ancestry-specific meta-analyses were taken forward to linear regression analyses in Stage 2, which included 44 cohorts and 131,012 individuals of European [$n = 107,617$], African [$n = 5384$], Asian [$n = 6590$], or Hispanic [$n = 11,421$] ancestry (Supplementary Tables 3 and 4; Supplementary Data 3; Supplementary Note 2). The summary statistics from Stage 1 and Stage 2 were subsequently meta-analyzed to identify lipid loci whose effects are modified by PA.

We identified lipid loci interacting with PA by three different approaches applied to the meta-analysis of Stage 1 and Stage 2: (i) we screened for genome-wide significant SNP \times PA-interaction effects ($P_{\text{INT}} < 5 \times 10^{-8}$); (ii) we screened for genome-wide significant 2 degree of freedom (2df) joint test of SNP main

effect and SNP \times PA interaction¹⁴ ($P_{\text{JOINT}} < 5 \times 10^{-8}$); and (iii) we screened all previously known lipid loci^{3–12} for significant SNP \times PA-interaction effects, Bonferroni-correcting for the number of independent variants tested ($r^2 < 0.1$ within 1 Mb distance; $P_{\text{INT}} = 0.05/501 = 1.0 \times 10^{-4}$).

PA modifies the effect of four loci on lipid levels. Three novel loci (>1 Mb distance and $r^2 < 0.1$ with any previously identified lipid locus) were identified: in *CLASPI* (rs2862183, $P_{\text{INT}} = 8 \times 10^{-9}$), near *LHX1* (rs295849, $P_{\text{INT}} = 3 \times 10^{-8}$), and in *SNTA1* (rs141588480, $P_{\text{INT}} = 2 \times 10^{-8}$), which showed a genome-wide significant SNP \times PA interaction on HDL-C in all ancestries combined (Table 1, Figs. 1–4). Higher levels of PA enhanced the HDL cholesterol-increasing effects of the *CLASPI*, *LHX1*, and *SNTA1* loci. A novel locus in *CNTNAP2* (rs190748049) was genome-wide significant in the joint test of SNP main effect and SNP \times PA interaction ($P_{\text{JOINT}} = 4 \times 10^{-8}$) and showed moderate evidence of SNP \times PA interaction ($P_{\text{INT}} = 2 \times 10^{-6}$) in the meta-analysis of LDL-C in all ancestries combined (Table 1, Fig. 5). The LDL-C-increasing effect of the *CNTNAP2* locus was attenuated in the physically active group as compared to the inactive group. None of these four loci have been identified in previous main effect GWAS of lipid levels.

No interaction between known main effect lipid loci and PA.

Of the previously known 260 main effect loci for HDL-C, 202 for LDL-C, and 185 for TG^{3–12}, none reached the Bonferroni-corrected threshold (two-sided $P_{\text{INT}} = 1.0 \times 10^{-4}$) for SNP \times PA interaction alone (Supplementary Data 4–6). We also found no significant interaction between a combined score of all published European-ancestry loci for HDL-C, LDL-C, or TG with PA (Supplementary Datas 7–9) using our European-ancestry summary results (two-sided $P_{\text{HDL-C}} = 0.14$, $P_{\text{LDL-C}} = 0.77$, and $P_{\text{TG}} = 0.86$, respectively), suggesting that the beneficial effect of PA on lipid levels may be independent of genetic risk¹⁵.

Potential functional roles of the loci interacting with PA.

While the mechanisms underlying the beneficial effect of PA on circulating lipid levels are not fully understood, it is thought that the changes in plasma lipid levels are primarily due to an improvement in the ability of skeletal muscle to utilize lipids for energy due to enhanced enzymatic activities in the muscle^{16,17}. Of the four loci we found to interact with PA, three, in *CLASPI*, near *LHX1*, and in *SNTA1*, harbor genes that may play a role in muscle function^{18,19} and lipid metabolism^{20,21}.

The lead variant rs2862183 (minor allele frequency (MAF) 22%) in the *CLASPI* locus which interacts with PA on HDL-C levels is an intronic SNP in *CLASPI* that encodes a microtubule-associated protein (Fig. 2). The rs2862183 SNP is associated with *CLASPI* expression in *esophagus muscularis* ($P = 3 \times 10^{-5}$) and is in strong linkage disequilibrium ($r^2 > 0.79$) with rs13403769 variant that shows the strongest association with *CLASPI* expression in the region ($P = 7 \times 10^{-7}$). Another potent causal candidate gene in this locus is the nearby *GLI2* gene which has been found to play a role in skeletal myogenesis¹⁸ and the conversion of glucose to lipids in mouse adipose tissue²⁰ by inhibiting hedgehog signaling.

The rs295849 (MAF 38%) variant near *LHX1* interacts with PA on HDL-C levels. However, the more likely causal candidate gene in this locus is acetyl-CoA carboxylase (*ACACA*), which plays a crucial role in fatty acid metabolism²¹ (Fig. 3). Rare acetyl-CoA carboxylase deficiency has been linked to hypotonic myopathy, severe brain damage, and poor growth²².

The lead variant in the *SNTA1* locus (rs141588480) interacts with PA on HDL-C and is an insertion only found in individuals

Table 1 Lipid loci identified through interaction with physical activity ($P_{INT} < 5 \times 10^{-8}$) or through joint test for SNP main effect and SNP \times physical activity interaction ($P_{JOINT} < 5 \times 10^{-8}$)

Trait	SNP	Chr:Pos	Gene	EA/OA	EAF	N inactive	N active	Beta _{INT}	se _{INT}	P _{INT}	P _{JOINT}
<i>Loci identified through interaction with physical activity</i>											
HDL-C	rs2862183	2:122415398	CLASP1	T/C	0.22	76,674	154,118	0.014	0.003	7.5E ⁻⁹	3.6E ⁻⁷
HDL-C	rs295849	17:35161748	LHX1	T/G	0.38	78,288	160,924	0.009	0.002	2.7E ⁻⁸	6.8E ⁻⁷
HDL-C	rs141588480	20:32013913	SNTA1	Ins/Del	0.95	8,694	18,585	0.054	0.010	2.0E ⁻⁸	6.1E ⁻⁷
<i>Loci identified through joint test for SNP main effect and SNP \times physical activity interaction</i>											
LDL-C	rs190748049	7:146418260	CNTNAP2	C/T	0.95	14,912	28,715	-7.2	1.5	1.6E ⁻⁶	4.2E ⁻⁸

All loci were identified in the meta-analyses of all ancestries combined. HDL-C was natural logarithmically transformed, whereas LDL-C was not transformed. The P values are two-sided and were obtained using a meta-analysis of linear regression model results. EA effect allele, EAF effect allele frequency, OA other allele, beta_{INT} effect size for interaction with physical activity (=the change in logarithmically transformed HDL-C or untransformed LDL-C levels in the active group as compared to the inactive group per each effect allele), se_{INT} standard error for interaction with physical activity

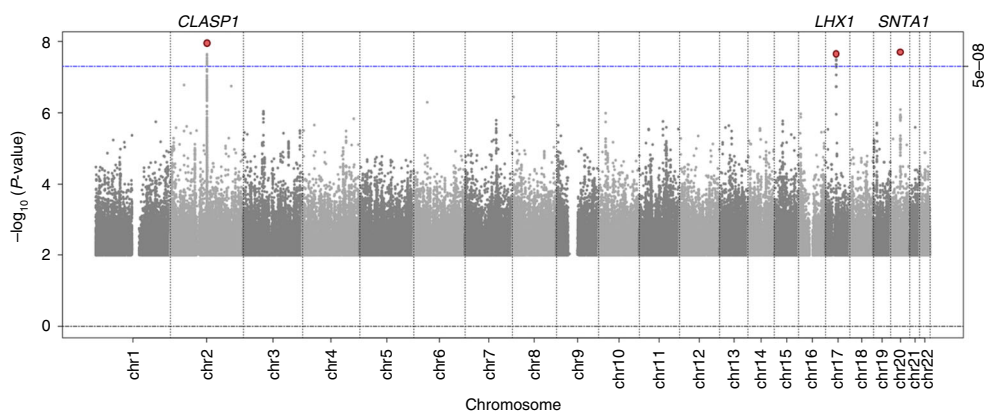


Fig. 1 Genome-wide results for interaction with physical activity on HDL cholesterol levels. The P values are two-sided and were obtained by a meta-analysis of linear regression model results (n up to 250,564). Three loci, in/near *CLASP1*, *LHX1*, and *SNTA1*, reached genome-wide significance ($P < 5 \times 10^{-8}$) as indicated in the plot

of African (MAF 6%) or Hispanic (MAF 1%) ancestry. The rs141588480 insertion is in the *SNTA1* gene that encodes the syntrophin alpha 1 protein, located at the neuromuscular junction and altering intracellular calcium ion levels in muscle tissue (Fig. 4). *Snta1*-null mice exhibit differences in muscle regeneration after a cardiotoxin injection¹⁹. Two weeks following the injection into mouse tibialis anterior, the muscle showed hypertrophy, decreased contractile force, and neuromuscular junction dysfunction. Furthermore, exercise endurance of the mice was impaired in the early phase of muscle regeneration¹⁹. In humans, *SNTA1* mutations have been linked to the long-QT syndrome²³.

The fourth locus interacting with PA is *CNTNAP2*, with the lead variant (rs190748049) intronic and no other genes nearby (Fig. 5). The rs190748049 variant is most common in African-ancestry (MAF 8%), less frequent in European-ancestry (MAF 2%), and absent in Asian- and Hispanic-ancestry populations. The protein coded by the *CNTNAP2* gene, contactin-associated protein like-2, is a member of the neurexin protein family. The protein is located at the juxtaparanodes of myelinated axons where it may have an important role in the differentiation of the axon into specific functional subdomains. Mice with a *Cntnap2* knockout are used as an animal model of autism and show altered phasic inhibition and a decreased number of interneurons²⁴. Human *CNTNAP2* variants have been associated with risk of autism and related behavioral disorders²⁵.

Joint test of SNP main effect and SNP \times PA interaction. We found 101 additional loci that reached genome-wide significance in the 2df joint test of SNP main effect and SNP \times PA interaction

on HDL-C, LDL-C, or TG. However, none of these loci showed evidence of SNP \times PA interaction ($P_{INT} > 0.001$) (Supplementary Data 10). All 101 main effect-driven loci have been identified in previous GWAS of lipid levels³⁻¹².

Discussion

In this genome-wide study of up to 250,564 adults from diverse ancestries, we found evidence of interaction with PA for four loci, in/near *CLASP1*, *LHX1*, *SNTA1*, and *CNTNAP2*. Higher levels of PA enhanced the HDL cholesterol-increasing effects of *CLASP1*, *LHX1*, and *SNTA1* loci and attenuated the LDL cholesterol-increasing effect of the *CNTNAP2* locus. None of these four loci have been identified in previous main effect GWAS for lipid levels³⁻¹².

The loci in/near *CLASP1*, *LHX1*, and *SNTA1* harbor genes linked to muscle function^{18,19} and lipid metabolism^{20,21}. More specifically, the *GLI2* gene within the *CLASP1* locus has been found to play a role in myogenesis¹⁸ as well as in the conversion of glucose to lipids in adipose tissue²⁰; the *ACACA* gene within the *LHX1* locus plays a crucial role in fatty acid metabolism²¹ and has been connected to hypotonic myopathy²²; and the *SNTA1* gene is linked to muscle regeneration¹⁹. These functions may relate to differences in the ability of skeletal muscle to use lipids as an energy source, which may modify the beneficial impact of PA on blood lipid levels^{16,17}.

The inclusion of diverse ancestries in the present meta-analyses allowed us to identify two loci that would have been missed in meta-analyses of European-ancestry individuals alone. In particular, the lead variant (rs141588480) in the *SNTA1* locus is only polymorphic in African and Hispanic ancestries, and the lead

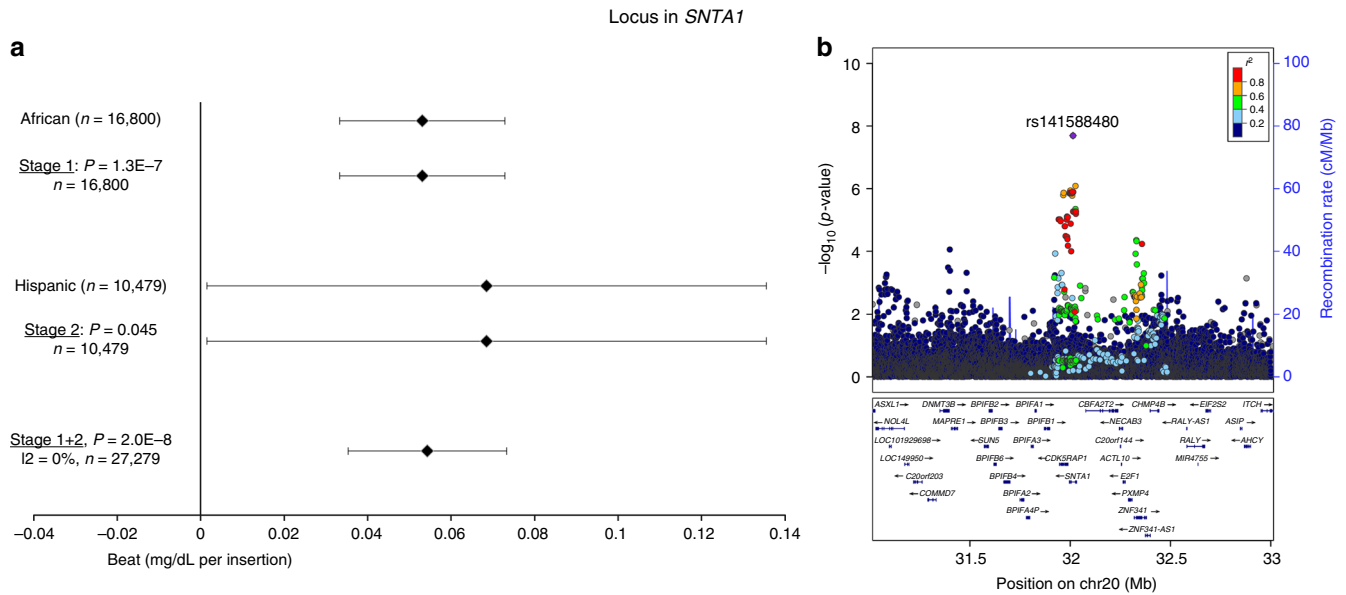


Fig. 4 Interaction of rs141588480 in *SNTA1* with physical activity on HDL cholesterol levels. The beta and 95% confidence intervals in the forest plot (a) is shown for the rs141588480 × physical activity interaction term, i.e., it indicates the increase in logarithmically transformed HDL cholesterol levels in the active group as compared to the inactive group per each insertion of rs141588480. The $-\log_{10}(p\text{-value})$ in the association plot (b) is also shown for the rs141588480 × physical activity interaction term. While the rs141588480 variant was identified in African-ancestry individuals in Stage 1, the variant did not pass QC filters in the Stage 2 African-ancestry cohorts, due to insufficient sample sizes of these cohorts. The P values are two-sided and were obtained by a meta-analysis of linear regression model results. The figure was generated using LocusZoom (<http://locuszoom.org>)

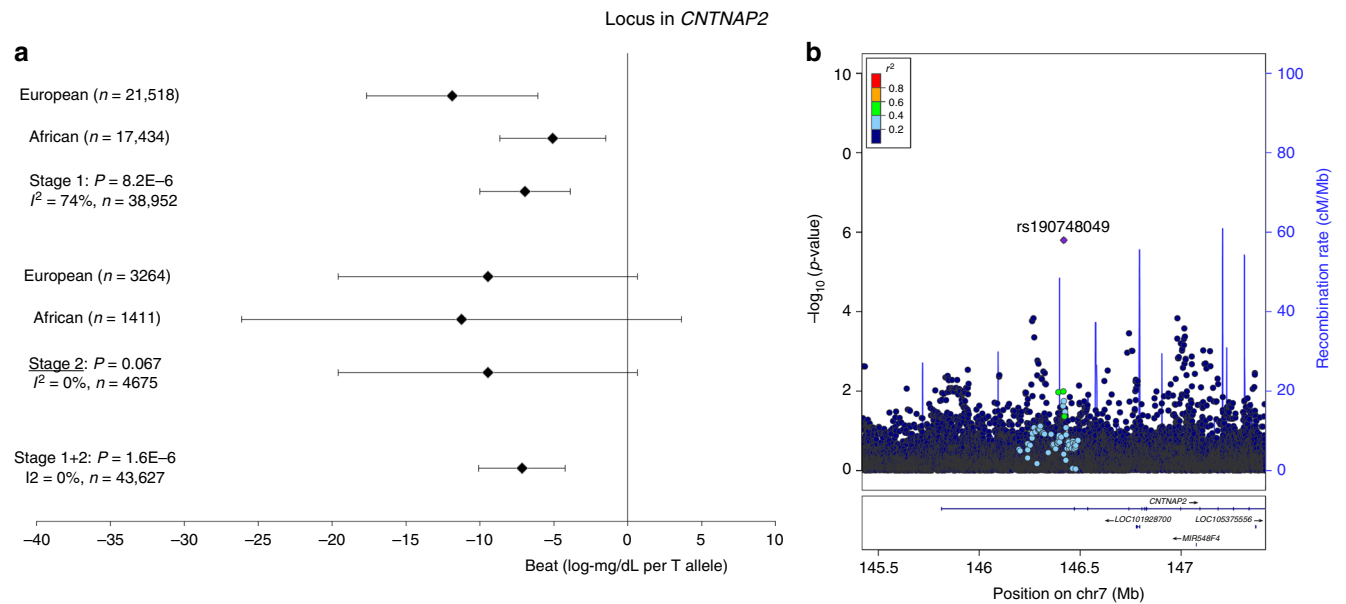


Fig. 5 Interaction of rs190748049 variant in *CNTNAP2* with physical activity on LDL cholesterol levels. The rs190748049 variant was genome-wide significant in the joint test for SNP main effect and SNP × physical activity interaction and reached $P = 2 \times 10^{-6}$ for the SNP × physical activity interaction term alone. The beta and 95% confidence intervals in the forest plot (a) is shown for the SNP × physical activity interaction term, i.e., it indicates the decrease in LDL cholesterol levels in the active group as compared to the inactive group per each T allele of rs190748049. The $-\log_{10}(P\text{-value})$ in the association plot (b) is also for the SNP × physical activity interaction term. The P values are two-sided and were obtained using a meta-analysis of linear regression model results. The figure was generated using LocusZoom (<http://locuszoom.org>)

showing the strongest SNP × PA interaction on lipid levels are not the same loci that show a strong main effect on lipid levels.

In summary, we identified four loci containing SNPs that enhance the beneficial effect of PA on lipid levels. The identification of the *SNTA1* and *CNTNAP2* loci interacting with PA was

made possible by the inclusion of diverse ancestries in the analyses. The gene regions that harbor loci interacting with PA involve pathways targeting muscle function and lipid metabolism. Our findings elucidate the role and underlying mechanisms of PA interactions in the genetic regulation of blood lipid levels.

Methods

Study design. The present study collected summary data from 86 participating cohorts and no individual-level data were exchanged. For each of the participating cohorts, the appropriate ethics review board approved the data collection and all participants provided informed consent.

We included men and women 18–80 years of age and of European, African, Asian, Hispanic, or Brazilian ancestry. The meta-analyses were performed in two stages¹³. Stage 1 meta-analyses included 42 studies with a total of 120,979 individuals of European ($n = 84,902$), African ($n = 20,487$), Asian ($n = 6,403$), Hispanic ($n = 4,749$), or Brazilian ancestry ($n = 4,438$) (Supplementary Table 1; Supplementary Data 2; Supplementary Note 1). Stage 2 meta-analyses included 44 studies with a total of 131,012 individuals of European ($n = 107,617$), African ($n = 5,384$), Asian ($n = 6,590$), or Hispanic ($n = 11,421$) ancestry (Supplementary Table 3; Supplementary Data 3; Supplementary Note 2). Studies participating in Stage 1 meta-analyses carried out genome-wide analyses, whereas studies participating in Stage 2 only performed analyses for 17,711 variants that reached $P < 10^{-6}$ in the Stage 1 meta-analyses and were observed in at least two different Stage 1 studies with a pooled sample size > 4000 . The Stage 1 and Stage 2 meta-analyses were performed in all ancestries combined and in each ancestry separately.

Outcome traits: LDL-C, HDL-C, and TG. The levels of LDL-C were either directly assayed or derived using the Friedewald equation (if $TG \leq 400$ mg dl⁻¹ and fasting ≥ 8 h). We adjusted LDL-C levels for lipid-lowering drug use if statin use was reported or if unspecified lipid-lowering drug use was listed after 1994, when statin use became common. For directly assayed LDL-C, we divided the LDL-C value by 0.7. If LDL-C was derived using the Friedewald equation, we first adjusted total cholesterol for statin use (total cholesterol divided by 0.8) before the usual calculation. If study samples were from individuals who were nonfasting, we did not include either TG or calculated LDL-C in the present analyses. The HDL-C and TG variables were natural log-transformed, while LDL-C was not transformed.

PA variable. The participating studies used a variety of ways to assess and quantify PA (Supplementary Data 1). To harmonize the PA variable across all participating studies, we coded a dichotomous variable, inactive vs. active, that could be applied in a relatively uniform way in all studies, and that would be congruent with previous findings on SNP \times PA interactions^{26–28} and the relationship between PA and disease outcomes²⁹. Inactive individuals were defined as those with < 225 MET-min per week of moderate-to-vigorous leisure-time or commuting PA ($n = 84,495$; 34% of all participants) (Supplementary Data 1). We considered all other participants as physically active. In studies where MET-min per week measures of PA were not available, we defined inactive individuals as those engaging in ≤ 1 h/week of moderate-intensity leisure-time PA or commuting PA. In studies with PA measures that were not comparable to either MET-min or hours/week of PA, we defined the inactive group using a percentage cut-off, where individuals in the lowest 25% of PA levels were defined as inactive and all other individuals as active.

Genotyping and imputation. Genotyping was performed by each participating study using Illumina or Affymetrix arrays. Imputation was conducted on the cosmopolitan reference panel from the 1000 Genomes Project Phase I Integrated Release Version 3 Haplotypes (2010–2011 data freeze, 2012-03-14 haplotypes). Only autosomal variants were considered. Specific details of each participating study's genotyping platform and imputation software are described in Supplementary Tables 2 and 4.

Quality control. The participating studies excluded variants with $MAF < 1\%$. We performed QC for all study-specific results using the EasyQC package in R³⁰. For each study-specific results file, we filtered out genetic variants for which the product of minor allele count (MAC) in the inactive and active strata and imputation quality [$\min(MAC_{INACTIVE}, MAC_{ACTIVE}) \times$ imputation quality] did not reach 20. This removed unstable study-specific results that reflected small sample size, low MAC, or low-imputation quality. In addition, we excluded all variants with imputation quality measure < 0.5 . To identify issues with relatedness, we examined QQ plots and genomic control inflation lambdas in each study-specific results file as well as in the meta-analysis results files. To identify issues with allele frequencies, we compared the allele frequencies in each study file against ancestry-specific allele frequencies in the 1000 Genomes reference panel. To identify issues with trait transformation, we plotted the median standard error against the maximal sample size in each study. The summary statistics for all beta-coefficients, standard errors, and P values were visually compared to observe discrepancies. Any issues that were found during the QC were resolved by contacting the analysts from the participating studies. Additional details about QC in the context of interactions, including examples, may be found elsewhere¹³.

Analysis methods. All participating studies used the following model to test for interaction:

$$E[Y] = \beta_0 + \beta_E * PA + \beta_G * G + \beta_{INT} * G * PA + \beta_C * C,$$

where Y is the HDL-C, LDL-C, or TG value, PA is the PA variable with 0 or 1

coding for active or inactive group, and G is the dosage of the imputed genetic variant coded additively from 0 to 2. The C is the vector of covariates which included age, sex, study center (for multi-center studies), and genome-wide principal components. From this model, the studies provided the estimated genetic main effect (β_G), estimated interaction effect (β_{GE}), and a robust estimate of the covariance between β_G and β_{GE} . Using these estimates, we performed inverse variance-weighted meta-analyses for the SNP \times PA interaction term alone, and 2df joint meta-analyses of the SNP effect and SNP \times PA interaction combined by the method of Manning et al.¹⁴, using the METAL meta-analysis software. We applied genomic control correction twice in Stage 1, first for study-specific GWAS results and again for meta-analysis results, whereas genomic control correction was not applied to the Stage 2 results as interaction testing was only performed at select variants. We considered a variant that reached two-sided $P < 5 \times 10^{-8}$ in the meta-analysis for the interaction term alone or in the joint test of SNP main effect and SNP \times PA interaction, either in the ancestry-specific analyses or in all ancestries combined, as genome-wide significant. The loci were defined as independent if the distance between the lead variants was > 1 Mb.

Combined PA-interaction effect of all known lipid loci. To identify all published SNPs associated with HDL-C, LDL-C, or TG, we extended the previous curated list of lipid loci by Davis et al.⁴ by searching PubMed and Google Scholar databases and screening the GWAS Catalog. After LD pruning by $r^2 < 0.1$ in the 1000 Genomes European-ancestry reference panel, 260 independent loci remained associated with HDL cholesterol, 202 with LDL cholesterol, and 185 with TG (Supplementary Datas 7–9). To approximate the combined PA interaction of all known European-ancestry loci associated with HDL-C, LDL-C, or TG, we calculated their combined interaction effect as the weighted sum of the individual SNP coefficients in our genome-wide summary results for European-ancestry. This approach has been described previously in detail by Dastani et al.³¹ and incorporated in the package “gtx” in R. We did not weigh the loci by their main effect estimates from the discovery GWAS data.

Examining the functional roles of loci interacting with PA. We examined published associations of the identified lipid loci with other complex traits in genome-wide association studies by using the GWAS Catalog of the European Bioinformatics Institute and the National Human Genome Research Institute. We extracted all published genetic associations with $r^2 > 0.5$ and distance < 500 kb from the identified lipid-associated lead SNPs³². We also studied the *cis*-associations of the lead SNPs with all genes within ± 1 Mb distance using the GTEx portal³³. We excluded findings where our lead SNP was not in strong LD ($r^2 > 0.5$) with the peak SNP associated with the same gene transcript.

Data availability

The meta-analysis summary results are available for download on the CHARGE dbGaP website under accession [phs000930](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=phs000930).

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T.O.K., K. Schwander, D.C.R., and R.J.F.L. conceived and designed the study. The members of the writing group were T.O.K., A.R.B., R.N., Y.J.S., K.Schwander, T. Winkler, H.J., D.I.C., A. Manning, I.N., B.M.P., K.R., P.B.M., M.F., L.A.C., C.N.R., A. C.M., D.C.R., and R.J.F.L. The genome-wide association results were provided by A.R.B.,

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Additional information

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Lifelines Cohort Study

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