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Meta-analysis of genome-wide association studies identifies three novel loci for saturated fatty acids in East Asians

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Abstract

Purpose—We aimed to characterize common genetic variants that influence saturated fatty acid concentrations in East Asians.

Methods—Meta-analysis of genome-wide association studies for circulating SFAs was conducted in two population-based cohorts comprising 3,521 participants of Chinese ancestry.

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Conflict of interest

The authors declare no conflict of interest.

Ethical standards

Both NHAPC and MESA cohorts have been approved by ethics committees and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants in both studies.

Results—We identified two novel 14:0-associated loci at *LMX1A* (LIM homeobox transcription factor 1) and *AMPD3* (AMP deaminase 3) (P=5.08×10^{-9} and P=4.33×10^{-8}, respectively), and a novel 20:0-associated locus at *CERS4*(ceramide synthase 4) (P=1.73×10^{-10}). We also confirmed the previously reported association of *FADS1*/2-rs102275 with 18:0 (P=1.115×10^{-5}). In addition, the A alleles of rs11042834 in *AMPD3* and rs17159388 in *CERS4* also exhibited evidence of associations with high density lipoprotein cholesterol (P=0.0162 and P=0.0161, respectively).

Conclusions—To our knowledge, this is the first GWAS analysis to examine SFA concentrations in East Asian populations. Our findings provide novel evidence that genetic variations of several genes from multiple pathways are associated with SFA concentrations in human body.

Keywords

myristic acid; arachidic acid; genome-wide association study; Chinese; lipids

Introduction

Dietary saturated fatty acids (SFAs) have long been considered independently as major risk factors for coronary heart disease (CHD) [1]. Moreover, plasma or erythrocyte SFAs, as surrogates of their intakes, have also been shown to be associated with risks of CHD [2, 3], type 2 diabetes [4], heart failure [5], ischemic stroke [6]and Alzheimer's disease [7]. However, individual SFAs seem to have distinctive pathophysiological effects. For example, plasma phospholipid fatty acids 16:0 and 18:0 are associated with increased risk of CHD [3], but no association between 14:0 and cardiovascular diseases is observed [3, 8], and effects of long-chain SFAs, 20:0, 22:0, and 24:0, on type 2 diabetes and CHD remain to be known.

Circulating SFA concentrations are determined by dietary intake, absorption and endogenous synthesis. Therefore, genetic variants that alter uptake, absorption or synthesis of fatty acids may all contribute to the variation in blood SFA concentrations. So far, there is only one genome-wide association study (GWAS) in European ancestry populations identified three novel loci that are associated with circulating concentrations of 16:0 and 18:0 [9], and no studies have investigated the associations between genetic variants and circulating concentration of other SFAs, including 14:0 and long-chain SFAs. Moreover, compared with Europeans, East Asian populations have different dietary pattern and genetic background. A recent genetic adaptation analyses also demonstrated that the effect of *fatty acid desaturase* (*FADS*) haplotype on the efficiency of long-chain fatty acids synthesis ability differed between European and African descent, due to a shift in diet polyunsaturated fatty acids intake [10]. Therefore, it is of interest to investigate the transferability of reported genetic associations for *de novo* lipogenesis fatty acids to other ethnic populations. In the current analysis, we aimed to identify novel genetic variants for individual SFAs, and to examine whether previously identified loci are also associated with circulating concentrations of 16:0 and 18:0 in East Asians from the Nutrition and Health of Aging Population in China (NHAPC) and the Multi-Ethnic Study of Atherosclerosis (MESA), two cohort studies from the Cohorts from Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

Subjects and methods

Study cohorts

All data for this study were obtained from two cohort studies in the CHARGE Consortium, the NHAPC and MESA study. The NHAPC study is a population-based cohort study among 3,210 Chinese Hans, who aged 50 to 70 years during recruitments in Beijing and Shanghai. The study design, methods and measurements of this cohort study have been described in detail elsewhere [11]. Briefly, data on demographic variables, health status and physical activity was collected using a standardized questionnaire and anthropometric measurements and overnight fasting blood samples were collected using a standardized protocol. Total cholesterol, LDL-c, HDL-c and triglyceride concentrations were measured enzymatically on an automatic analyzer (Hitachi7080, Japan) with reagents purchased from WakoPure Chemical Industries (Osaka, Japan). The MESA Study is a study of the characteristics of subclinical cardiovascular disease and risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease [12]. MESA study is a diverse, population-based sample of 6,814 asymptomatic men and women aged 45 to 84 years, with about 12 percent Asians, predominantly of Chinese descent. Written informed consent was obtained from all participants in both studies.

Fatty acid measurements

The total erythrocyte fatty acids were measured in NHAPC samples, while plasma phospholipid fatty acids were measured in the MESA samples. The method for erythrocyte fatty acid measurement in the NHAPC study has been previously described [13]. After being extracted by hexane and iso-propanol, erythrocyte fatty acids were incubated with a mixture of methanol and sulfuric acid for fatty acid methyl esters (FAMEs). FAMEs were then separated and identified by gas chromatography coupled with positive chemical ionization(Agilent 6890 GC-5975B) using methane as reagent gas. Fatty acids were obtained for a subset of 712 Chinese with genotypes available through MESA SHARe. Fatty acids of plasma collected with EDTA and frozen at −70°C, were measured using methods previously described by Cao et al [14]. Lipids are extracted from the plasma using a chloroform/methanol extraction method, and cholesterol esters, triglycerides, phospholipids and free fatty acids are separated by thin layer chromatography. The fatty acid methyl esters are obtained from the phospholipids and are detected by gas chromatography flame ionization. In both studies, relative amount of each fatty acid was calculated as the percentage of total fatty acids. Among the individual SFAs, 14:0, 16:0, 18:0, 20:0, and 22:0 were available in both cohorts and 24:0 was available only in the NHAPC study.

Genotyping and quality control

Details on genotyping and imputation for each cohort are provided in Supplemental Table 1. Briefly, sampleswere genotyped using high-density single nucleotide polymorphism (SNP) marker platforms, Illumina Human660W (Illumina, Inc., San Diego, California) in NHPAC and Affymetrix Genome-Wide Human SNP Array 6.0 in MESA (Santa Clara, California). Samples with call rate< 97% (NHAPC)or ≤ 95% (MESA) were excluded. The samples in the NHAPC study that passed all QC criteria were then used to impute for the ungenotyped or missing SNPs from the HapMap phase II CHB+JPT (release #22- NCBI Build 36)

reference panel, and samples in MESA SHARe Asian were imputed from the HapMap Phase I and II CEU+YRI+CHB+JPT (release #22- NCBI Build 36) reference panel using IMPUTE software [15]. We removed all SNPs with MAF <5% (after meta-analysis), HWE $P<10^{-6}$, or poor imputation quality, defined as the info measure ≤ 0.5 (NHAPC) or an observed divided by expected variance ≤ 0.8 (MESA).

Statistical analysis

Genome-wide association analysis for each individual fatty acid was conducted separately in NHAPC and MESA, and the summary statistics were then combined by inverse-variance weighted meta-analysis using METAL software (www.sph.umich.edu/csg/abecasis.metal). In cohort-specific GWAS, linear regression analysis was applied to examine the association of each genotyped and imputed SNP with individual SFA level under an additive genetic model using ProbABEL(version 0.4.5) [16]. All analyses were adjusted for age, sex, site of recruitment, and first two principal components and used robust standard errors. *P* values were adjusted for genomic control inflation factor (λ_{GC}) [17]. Genomic control inflation factors in NHAPC and MESA were 1.043and 1.017 for 14:0, 1.011and 1.056 for 16:0, 1.013and 1.013 for 18:0, 1.001 and 1.023 for 20:0, and 1.047 and1.026 for 22:0, respectively, while inflation factor was 1.046 for 24:0 in NHAPC, suggesting minimal population stratification for each cohort. We then combined summary statistics of the associations from NHAPC and MESA cohorts using fixed-effect meta-analyses and tested for the heterogeneity between these two studies. *P*-values for heterogeneity and I^2 were estimated. *P*-values less than 5×10^{-8} were considered significant. Generalized linear regression was applied to examine associations between SNPs with $P\leq5\times10^{-8}$and plasma lipid concentrations in NHAPC study, adjusting for age, sex, recruiting site, and first two principal components under an additive genetic model using R software (version 2.15). All *P* values were two-sided, and $P< 0.05$ was considered to be statistically significant.

Results

The characteristics of two populations are shown in Table 1. Mean concentrations of circulating SFAs are 0.38% and 0.23% for 14:0, 21.91% and 25.71% for 16:0, 14.53% and 13.14% for 18:0, 0.32% and 0.25% for 20:0, and 1.29% and 0.62% for 22:0 for NHAPC and MESA samples, respectively. Mean concentration of SFA 24:0 is 4.21% of total fatty acids for NHAPC samples.

As shown in Fig. 1 and Table 2, multiple SNPs at *LMX1A*, *AMPD3* and *CERS4* loci reached genome-wide significance (*P* values$<5\times10^{-8}$) for associations with SFA (Table 2). Supplemental Tables 2–7 presented the associations of top SNPs ($P<5\times10^{-6}$) with each SFAs in overall individuals and Supplemental Table 8 presented the genome-wide significant SNPs in cohort-specific GWASs.

Each copy of the minor T-allele of SNP rs11589386, near *LMX1A* gene, was associated with 0.034 lower percentage of 14:0 ($P=5.08\times10^{-9}$) (Fig. 2A). *AMPD3*(at 11p15) is another locus (rs11042834 is the index SNP) that showed genome-wide significance for association with 14:0 ($P=4.33\times10^{-8}$), and each copy of minor allele of this SNP was associated with 0.031 percent decreased in 14:0 (Fig. 2B).

Several SNPs at the *CERS4* locus were associated with erythrocyte fatty acid 20:0 in the NHAPC study, and rs17159388 is the most significant SNP (P=1.73×10^{-10}, Table 2 and Fig. 3). Moreover, SNP rs651821 in *APOA5*and rs168622 in *SPTLC3* also showed suggestive evidence for association with erythrocyte fatty acid 20:0 (P≤6.48×10^{-7}) (Supplemental Table 5), whereasrs9349666 in *ELOVL5* gene that involves in fatty acid metabolism showed suggestive evidence for association with fatty acid 24:0 (P=2.10×10^{-7})in the NHAPC study (Supplemental Table 7).

For loci that have been previously reported to be associated with SFA (16:0 and 18:0), the association between 16:0 and *ALG14*-rs6675668 (P=0.035), in high linkage disequilibrium with rs2391388 (r^2=0.90), was confirmed (number of nominal significant SNPs=10; Supplemental Table 8), and multiple SNPs in *FADS1/2* also exhibited significant associations with 18:0 (i.e. P=1.115×10^{-5} for rs102275; number of nominal significant SNPs=36)(Table 1 and Supplemental Table 9) in this study.

We also examined the three novel genome-wide significant loci for associations with total cholesterol, low density lipoprotein cholesterol(LDL-c), high density lipoprotein cholesterol(HDL-c) and triglycerides in 2,865 participants from NHAPC study (Supplemental Table 10). Minor alleles of *AMPD3*-rs11042834 and *CERS4*-rs17159388 were significantly associated with lower HDL-c concentrations (P=0.0162 and 0.0161, respectively), after adjustment of age, gender, region and the first two principle components. Further controlling for 14:0 did not attenuate the effect of minor G-allele of *AMPD3*-rs11042834 on HDL-c, while adjustment for 20:0 abolished the association between minor A-allele of *CERS4*-rs17159388and HDL-c.

Discussion

In this study of 3,521 individuals of Chinese descent from two cohorts, three novel variants showed associations with blood SFA concentrations at genome-wide significance. Genetic variants in/or near *AMPD3* and *LMX1A* genes were significantly associated with concentrations of 14:0, while genetic variants in *CERS4* gene were significantly associated with concentrations of 20:0. *AMPD3*-rs11042834 and *CERS4*-rs17159388 were also nominally associated with plasma HDL-c concentration.

The strongest signal for association with 14:0is rs11589386-C, which is near*LMX1A* gene (160kb) encoding a homeodomain and LIM-domain containing protein [18]. *LMX1A*is widely expressed in pancreas, skeletal muscle, adipose tissue, kidney, brain, and pituitary [19]. Previous studies have suggested that LIM-homeodomain proteinLmx1 acts as a positive regulator of insulin gene transcription by cooperating with the basic helix-loop-helix (bHLH) protein E47/Pan-1 [20], and existing evidence also shown positive correlation between insulin sensitivity and 14:0 in cross-sectional studies [21, 22]. However, the mechanism underlying the association between *LMX1*-rs11589386 and 14:0 remains unknown.

Another genetic variant associated with 14:0 is rs11042834 in *AMPD3* gene, which encodes erythrocyte adenosine monophosphate deaminase. The same variant is also associated with

HDL-c, independent of erythrocyte 14:0 concentration. Consistent with our findings, a prior GWAS observed inverse association of rs2923084-A (not in linkage disequilibrium with rs11042834) near *AMPD3* with HDL-c in populations of European origin (beta:−0.41mg/dL; equals to −0.0106 mmol/L) [23]. The protein AMPD3 is an erythrocyte-specific enzyme that catalyzes the hydrolytic deamination of adenosine monophosphate to inosine monophosphate, in the adenylate catabolic pathway [24], and is widely expressed in tissues, including those of brain. Lanaspa *et al.* reported that the activation of AMP deaminase led to increased production of uric acid and generation of mitochondrial oxidative stress, which further stimulated *de novo* lipogenesis and activated ATP-citrate lyase and long chain saturated fatty acids syntheses [25]. Further studies are needed to investigate whether genetic variation of *AMP* gene influence *de novo* synthesis of 14:0.

We found variants in *CERS4* were associated with 20:0 concentrations at genome-wide significance concentration, with the most significant SNP being rs17159388. The saturated fatty acid 20:0 is an important component of sphingolipids. Our observation is consistent with prior GWAS in European participants which showed that several noncoding genetic variants in *CERS4*, although having non-linkage disequilibrium with rs17159388 (r^2<0.05), were associated with circulating plasma concentrations of sphingomyelins species 18:0, 18:1, 20:0, 20:1, ceramides 20:0, and ratios of sphingolipids [26, 27]. More recently, genetic variants in *CERS4* associated with 20:0 were also reported in European populations [28]. The most significant SNP rs2100944 was not in LD with SNP rs17159388 identified in the current GWAS (r^2=0.012) and the *P* value of rs2100944 in Chinese populations was 0.040. *CERS4* is expressed in most tissues including those of adipose and liver and involved in sphingolipid synthesis, especially longer chain ceramides [29]. The 20:0-increasing allele of *CERS4*-rs17159388 was also associated with higher HDL-c. But the association was abolished after further adjustment for 20:0, suggesting that the effect of *CERS4* on HDL-c is likely to be mediated through an increased concentration of 20:0.

In cohort-specific GWAS, nine loci reached genome-wide significance: six (*FECHP*, *FAM110B*, *A1CF*, *JAM3*, *RASSF8* and *MACROD2*) for 14:0 and one (*CERS4*) for 20:0 in the NHAPC cohort, and one (*LOC100128956*) for 14:0 and one (*ST8SIA5*) for 22:0 in the MESA cohort (Supplemental Table 8). To avoid false positive results from single GWAS, we conducted meta-analyses of GWAS study with larger sample size and only reported genome-wide significant SNPs in meta-analyses. SNPs at *CERS4* remained significant after meta-analysis and was presented in Table 2. Other SNPs showed suggestive significance after meta-analyses. Furthermore, we applied genomic control corrections in each study before meta-analysis to minimize potential confounding by population stratification[17].

The current study highlights the strength of pooling GWAS data from different cohorts for identification of additional novel loci associated with SFA levels. Potential limitations should also be considered. Environmental factors, including dietary intake and lifestyles, may exert confounding effects on the SNP-SFA associations. However, we did not observe any heterogeneity between these two studied cohorts for the significant SNPs identified in the meta-analyses. Future research focusing on the interactions between genetic variants and environmental factors on SFA levels are needed.

In conclusion, we identified three novel loci that were genome-wide significantly associated with saturated fatty acids, mainly with 14:0 and 20:0. These loci also affected concentrations of HDL-c and LDL-c. Our findings expand our knowledge of genes involved in the determination of saturated fatty acids concentrations and provide new insights for future research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Menotti A, Puddu PE. Coronary heart disease differences across Europe: a contribution from the Seven Countries Study. J Cardiovasc Med. 2013; 14:767–72. DOI: 10.2459/JCM.0b013e3283628dff
2. Wang L, Folsom AR, Eckfeldt JH. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Nutr Metab Cardiovasc Dis: NMCD. 2003; 13:256–66. DOI: 10.1016/S0939-4753(03)80029-7 [PubMed: 14717057]
3. Khaw KT, Friesen MD, Riboli E, Luben R, Wareham N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. PLoS Med. 2012; 9:e1001255.doi: 10.1371/journal.pmed.1001255 [PubMed: 22802735]
4. Kroger J, Zietemann V, Enzenbach C, Weikert C, Jansen EH, Doring F, Joost HG, Boeing H, Schulze MB. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Am J Clin Nutr. 2011; 93:127–42. DOI: 10.3945/ajcn.110.005447 [PubMed: 20980488]
5. Yamagishi K, Nettleton JA, Folsom AR. Investigators AS. Plasma fatty acid composition and incident heart failure in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am Heart J. 2008; 156:965–74. DOI: 10.1016/j.ahj.2008.06.017 [PubMed: 19061714]
6. Yaemsiri S, Sen S, Tinker LF, Robinson WR, Evans RW, Rosamond W, Wasserthiel-Smoller S, He K. Serum fatty acids and incidence of ischemic stroke among postmenopausal women. Stroke. 2013; 44:2710–7. DOI: 10.1161/STROKEAHA.111.000834 [PubMed: 23899914]
7. Wang DC, Sun CH, Liu LY, Sun XH, Jin XW, Song WL, Liu XQ, Wan XL. Serum fatty acid profiles using GC-MS and multivariate statistical analysis: potential biomarkers of Alzheimer's disease. Neurobiol Aging. 2012; 33:1057–66. DOI: 10.1016/j.neurobiolaging.2010.09.013 [PubMed: 20980076]

8. de Oliveira Otto MC, Nettleton JA, Lemaitre RN, Steffen LM, Kromhout D, Rich SS, Tsai MY, Jacobs DR, Mozaffarian D. Biomarkers of dairy fatty acids and risk of cardiovascular disease in the multi-ethnic study of atherosclerosis. J Am Heart Assoc. 2013; 2:e000092.doi: 10.1161/JAHA.113.000092 [PubMed: 23868191]

9. Wu JH, Lemaitre RN, Manichaikul A, Guan W, Tanaka T, Foy M, Kabagambe EK, Djousse L, Siscovick D, Fretts AM, et al. Genome-wide association study identifies novel loci associated with concentrations of four plasma phospholipid fatty acids in the de novo lipogenesis pathway: results from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. Circ Cardiovasc Genet. 2013; 6:171–83. DOI: 10.1161/CIRCGENETICS.112.964619 [PubMed: 23362303]

10. Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, Johansson AC, Rivas MA, Daly MJ, Schmitz G, Hicks AA, et al. Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. Am JHumGenet. 2012; 90:809–20. DOI: 10.1016/j.ajhg.2012.03.014 [PubMed: 22503634]

11. Ye X, Yu Z, Li H, Franco OH, Liu Y, Lin X. Distributions of C-reactive protein and its association with metabolic syndrome in middle-aged and older Chinese people. J Am Coll Cardiol. 2007; 49:1798–805. DOI: 10.1016/j.jacc.2007.01.065 [PubMed: 17466231]

12. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr, Kronmal R, Liu K, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol. 2002; 156:871–81. DOI: 10.1093/aje/kwf113 [PubMed: 12397006]

13. Zong G, Ye X, Sun L, Li H, Yu Z, Hu FB, Sun Q, Lin X. Associations of erythrocyte palmitoleic acid with adipokines, inflammatory markers, and the metabolic syndrome in middle-aged and older Chinese. Am J Clin Nutr. 2012; 96:970–6. DOI: 10.3945/ajcn.112.040204 [PubMed: 23015321]

14. Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin Chem. 2006; 52:2265–72. DOI: 10.1373/clinchem.2006.072322 [PubMed: 17053155]

15. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet. 2007; 39:906–13. DOI: 10.1038/ng2088 [PubMed: 17572673]

16. Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. BMC Bioinformatics. 2010; 11:134.doi: 10.1186/1471-2105-11-134 [PubMed: 20233392]

17. Devlin B, Roeder K. Genomic control for association studies. Biometrics. 1999; 55:997–1004. [PubMed: 11315092]

18. German MS, Wang J, Fernald AA, Espinosa R 3rd, Le Beau MM, Bell GI. Localization of the genes encoding two transcription factors, LMX1 and CDX3, regulating insulin gene expression to human chromosomes 1 and 13. Genomics. 1994; 24:403–4. DOI: 10.1006/geno.1994.1639 [PubMed: 7698771]

19. Thameem F, Wolford JK, Wang J, German MS, Bogardus C, Prochazka M. Cloning, expression and genomic structure of human LMX1A, and variant screening in Pima Indians. Gene. 2002; 290:217–25. DOI: 10.1016/S0378-1119(02)00582-6 [PubMed: 12062816]

20. Johnson JD, Zhang W, Rudnick A, Rutter WJ, German MS. Transcriptional synergy between LIM-homeodomain proteins and basic helix-loop-helix proteins: the LIM2 domain determines specificity. Mol Cell Biol. 1997; 17:3488–96. [PubMed: 9199284]

21. Roberts R, Hodson L, Dennis AL, Neville MJ, Humphreys SM, Harnden KE, Micklem KJ, Frayn KN. Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. Diabetologia. 2009; 52:882–90. DOI: 10.1007/s00125-009-1300-4 [PubMed: 19252892]

22. Ebbesson SO, Tejero ME, Lopez-Alvarenga JC, Harris WS, Ebbesson LO, Devereux RB, MacCluer JW, Wenger C, Laston S, Fabsitz RR, et al. Individual saturated fatty acids are associated with different components of insulin resistance and glucose metabolism: the GOCADAN study. Int J Circumpolar Health. 2010; 69:344–51. 1038. [PubMed: 20719107]

23. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 2010; 466:707–13. DOI: 10.1038/nature09270 [PubMed: 20686565]

24. Sims B, Mahnke-Zizelman DK, Profit AA, Prestwich GD, Sabina RL, Theibert AB. Regulation of AMP deaminase by phosphoinositides. J Biol Chem. 1999; 274:25701–7. DOI: 10.1074/jbc.274.36.25701 [PubMed: 10464307]

25. Lanaspa MA, Sanchez-Lozada LG, Choi YJ, Cicerchi C, Kanbay M, Roncal-Jimenez CA, Ishimoto T, Li N, Marek G, Duranay M, et al. Uric acid induces hepatic steatosis by generation of mitochondrial oxidative stress: potential role in fructose-dependent and -independent fatty liver. J Biol Chem. 2012; 287:40732–44. DOI: 10.1074/jbc.M112.399899 [PubMed: 23035112]

26. Hicks AA, Pramstaller PP, Johansson A, Vitart V, Rudan I, Ugocsai P, Aulchenko Y, Franklin CS, Liebisch G, Erdmann J, et al. Genetic determinants of circulating sphingolipid concentrations in European populations. PLoS Genet. 2009; 5:e1000672.doi: 10.1371/journal.pgen.1000672 [PubMed: 19798445]

27. Demirkan A, van Duijn CM, Ugocsai P, Isaacs A, Pramstaller PP, Liebisch G, Wilson JF, Johansson A, Rudan I, Aulchenko YS, et al. Genome-wide association study identifies novel loci associated with circulating phospho- and sphingolipid concentrations. PLoS Genet. 2012; 8:e1002490.doi: 10.1371/journal.pgen.1002490 [PubMed: 22359512]

28. Lemaitre RN, King IB, Kabagambe EK, Wu JH, McKnight B, Manichaikul A, Guan W, Sun Q, Chasman DI, Foy M, et al. Genetic loci associated with circulating levels of very long-chain saturated fatty acids. JLipid Res. 2015; 56:176–84. DOI: 10.1194/jlr.M052456 [PubMed: 25378659]

29. Mizutani Y, Kihara A, Igarashi Y. Mammalian Lass6 and its related family members regulate synthesis of specific ceramides. Biochem J. 2005; 390:263–71. DOI: 10.1042/BJ20050291 [PubMed: 15823095]

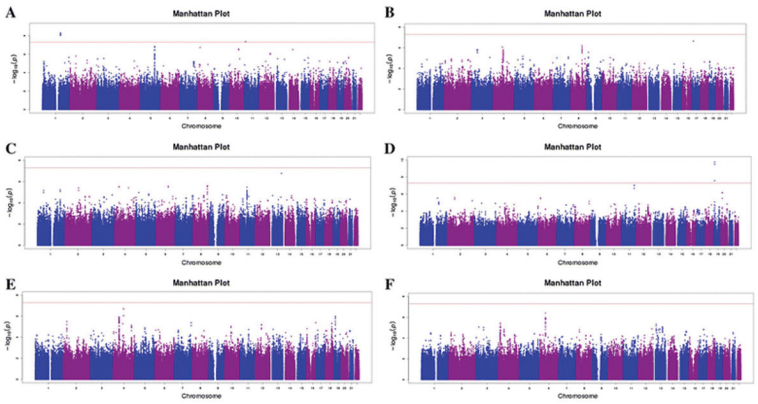

Fig. 1.

Manhattan plot for meta-analysis of genome-wide associations with saturated fatty acids. The $-\log_{10}P$ values from pooled analysis adjusted for age, gender, region and the first two principle components are presented in the figure.

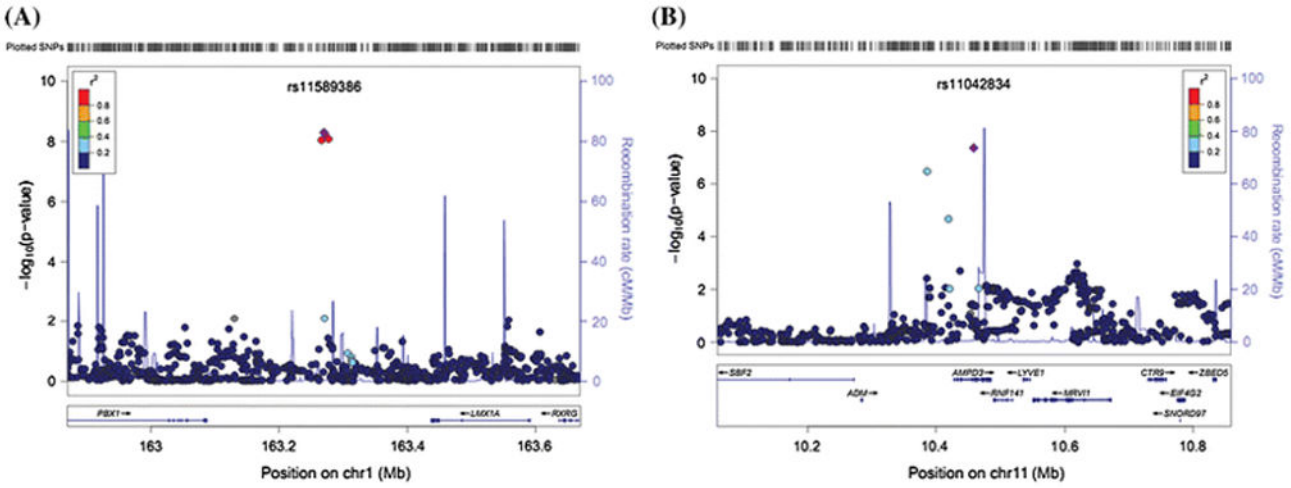

Fig. 2.

Regional plots of two novel loci for 14:0. Imputed SNPs were estimated by MACH software (see URLs) using LD information from 194 Asians (including 68 CHB, 25 CHS, 84 JPT and 17 MXL) in 1000 Genome 2010.08 release as references. P values were from meta-analysis adjusting for age, gender, region and the first two principle components. The regional plots for the 400kb region centered on index SNPs were generated by using LocusZoom (see URLs). The –log10 P values of SNPs were plotted against their genomic position (NCBI Build 37). The positions of genes were annotated from the UCSC Genome Browser by using GRCh37 assembly. The Index SNPs are purple colored. Linkage disequilibrium (LD) is indicated by color scale in relationship to the Index SNPs, with red for strong LD ($r^2 \geq 0.8$) and blue for lower LD.

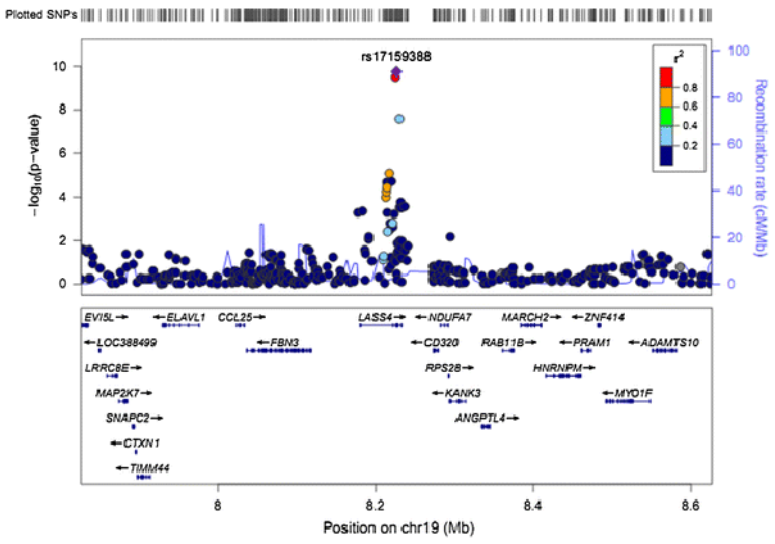

Fig. 3.

Regional plots of novel locus for 20:0. Imputed SNPs were estimated by MACH software (see URLs) using LD information from 194 Asians (including 68 CHB, 25 CHS, 84 JPT and 17 MXL) in 1000 Genome 2010.08 release as references. P values were from meta-analysis adjusting for age, gender, region and the first two principle components. The regional plots for the 400kb region centered on index SNP was generated by using LocusZoom (see URLs). The –log10 P values of SNPs were plotted against their genomic position (NCBI Build 37). The positions of genes were annotated from the UCSC Genome Browser by using GRCh37 assembly. The Index SNP is purple colored. Linkage disequilibrium (LD) is indicated by color scale in relationship to the Index SNP, with red for strong LD ($r^2 \geq 0.8$) and blue for lower LD.

Table 1

CHARGE cohort characteristics

	NHAPC	MESA
Country	China	USA
Ethnicity	Chinese Hans	Chinese
Study design	Population-based	Population-based
Sample (n)	2865	656
Females (%)	56.8%	51.4%
Age (yrs)	58.6±6.0	62.5 ±10.3
Saturated fatty acids (%)[a]		
14:0	0.38 ±0.35	0.23 ±0.07
16:0	21.91 ±2.57	25.71 ±1.47
18:0	14.53 ±1.71	13.14 ±1.62
20:0	0.32±0.05	0.25 ±0.08
22:0	1.29 ±0.28	0.61 ±0.08
24:0	4.21 ±1.78	-
Number of significant SNPs ($P \lesssim 5\times10^{-8}$)		
14:0	11	3
16:0	0	0
18:0	0	0
20:0	1	0
22:0	0	5
24:0	0	-

Data are mean ±SD except where indicated otherwise.

NHAPC Nutritional Health and Aging Population of Chinese, *MESA* Multi-Ethnic Study of Atherosclerosis

[a]Fatty acids were measured in erythrocyte (NHAPC)and plasma phospholipid (MESA)

Table 2

SNPs reaching genome-wide significance in the meta-analysis

Fatty acid	Nearest Gene	SNP	CHR	Position	Coded allele/non-coded allele	CAF	NHAPC			MESA			Combined		I^2	P_{heter}
							CAF	beta (SE)	*P*	CAF	beta (SE)	*P*	beta (SE)	*P*		
14:0	*LMX1A*	rs11589386	1	163270086	C/T	0.948	0.930	0.042 (0.013)	1.52E-03	0.952	0.032 (0.006)	4.78E-07	0.034 (0.006)	5.08E-09	0	0.52
14:0	*AMPD3*	rs11042834	11	10458998	A/G	0.914	0.912	0.027 (0.012)	2.46E-02	0.914	0.032 (0.006)	3.92E-07	0.031 (0.006)	4.33E-08	0	0.69
20:0	*CERS4*	rs17159388	19	8226555	A/G	0.191	0.191	−0.011 (0.002)	1.76E-10	NA	NA	NA	−0.011 (0.002)	1.76E-10	NA	NA
Previously reported loci																
16:0	*ALG14*	rs2391388	1	95258413	C/A	0.820	0.823	0.127 (0.086)	0.140	0.816	0.137 (0.112)	0.218	0.131 (0.069)	0.058	0	0.94
18:0	*ALG14*	rs6675668	1	95288225	T/G	0.818	0.820	−0.007 (0.058)	0.905	0.809	−0.113 (0.106)	0.289	−0.031 (0.051)	0.543	0	0.38
18:0	*LPGAT1*	rs11119805	1	209984867	A/T	0.145	0.137	−0.071 (0.062)	0.248	0.173	−0.002 (0.112)	0.984	−0.055 (0.054)	0.310	0	0.59
18:0	*FADS1/2*	rs102275	11	61314379	C/T	0.406	0.358	−0.159 (0.048)	1.01E-03	0.596	−0.316 (0.096)	9.79E-04	−0.191 (0.043)	1.12E-05	0.53	0.15

SNP single nucleotide polymorphism, *CHR* chromosome, *CAF* coded allele frequency, *NA* not available, I^2 I square of heterogeneity, P_{heter} heterogeneity of effect sizes between NHAPC and MESA studies