Higher chylomicron remnants and LDL particle numbers associate with common *CD36* SNPs and DNA methylation sites that reduce *CD36* expression

Short title: *CD36* SNPs influence postprandial lipids and LDL

Latisha Love-Gregory[1], Aldi T. Kraja[2], Fiona Allum[3], Stella Aslibekyan[4], Åsa K. Hedman[5,6], Yanan Duan[2], Ingrid B. Borecki[2], Donna K. Arnett[4], Mark I. McCarthy[7,8,9], Panos Deloukas[10], Jose M. Ordovas[11], Paul N. Hopkins[12], Elin Grundberg[3], Nada A. Abumrad[1]

[1]Department of Medicine, Center for Human Nutrition and [2] Department of Genetics, Division of Statistical Genomics, Washington University School of Medicine, St. Louis, MO 63110 USA. [3]Department of Human Genetics, McGill University and Genome Quebec Innovation Centre, Montreal, Québec, Canada H3A 0G1. [4]Department of Epidemiology, University of Alabama, Birmingham, AL 35294 USA. [5]Department of Medical Sciences, Molecular Epidemiology, Uppsala University, Uppsala 75185, Sweden. [6]Science for Life Laboratory, Uppsala University, Uppsala 75185, Sweden. [7]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK; [8]Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, Churchill Hospital, Oxford OX3 7JU, UK. [9]Oxford National Institute for Health Research Biomedical Research Centre, Churchill Hospital, Oxford OX3 7JU, UK; [10]William Harvey Research Institute, Queen Mary University of London, EC1M 6BQ London, UK. [11]JM-USDA-Human Nutrition Research Center on Aging, Tufts University, Boston, MA, 02111 USA. [12]Cardiovascular Genetics Research, University of Utah, Salt Lake City, Utah 84132 USA.

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Correspondence: Latisha Love-Gregory, PhD
lgregory@dom.wustl.edu
Or
Nada A Abumrad, PhD
Nabumrad@wustl.edu
Department of Medicine
Washington University School of Medicine
Campus Box 8031
St Louis, MO 63110

Abbreviations: CD36- cluster of differentiation 36; GOLDN- Genetics of Lipid Lowering Drugs and Diet Network; CpG-cytosine phosphate guanine; NMR- Nuclear magnetic resonance; MAF- minor allele frequency

Abstract

CD36 variants influence fasting lipids and risk of metabolic syndrome, but their impact on postprandial lipids, an independent risk factor for cardiovascular disease, is unclear. We determined effects of SNPs within a ~410-kb region encompassing *CD36* and its proximal and distal promoters on chylomicron remnants (CM) and LDL particles at fasting, 3.5 and 6 hours following a high-fat meal (GOLDN study, n=1117). Five promoter variants associated with CM, four with delayed triglyceride clearance and five with LDL particle number. To assess mechanisms underlying the associations, we queried eQTL, DNA methylation and ChIP-seq datasets for adipose and heart, tissues that function in postprandial lipid clearance. Several SNPs that associated with higher serum lipids correlated with lower adipose and heart *CD36* mRNA and aligned to active motifs for PPARγ, a major CD36 regulator. The SNPs also associated with DNA methylation sites that related to reduced *CD36* mRNA and higher serum lipids but mixed-model analyses indicated that the SNPs and methylation independently influence *CD36* mRNA. The findings support contribution of *CD36* SNPs that reduce adipose and heart *CD36* RNA expression to inter-individual variability of postprandial lipid metabolism and document changes in *CD36* DNA methylation that influence both *CD36* expression and lipids.

Introduction

Elevated fasting lipid levels have traditionally been associated with increased risk of heart disease, diabetes and stroke. More recently, similar and independent associations were found with levels of non-fasting or postprandial lipids (1, 2). Non-fasting measurements incorporate those of chylomicrons (CM) produced in response to dietary fat ingestion. The triglycerides (TG) in CM particles are hydrolyzed by lipoprotein lipase (LPL) in adipose, skeletal muscle and heart vascular beds to deliver free fatty acids (FFA) for cellular uptake facilitated by membrane CD36 (3). These tissues quantitatively contribute to chylomicron clearance in humans (4-6) and express high levels of CD36 at the level of both endothelial and parenchymal cells (7, 8). Elevated concentrations of postprandial CM remnants after an oral fat load were reported in four subjects with complete CD36 deficiency (9) suggesting that low CD36 might impair CM clearance and result in sustained high levels of circulating CM remnants (10, 11). Studies support a heritable component in determining postprandial lipid metabolism (12, 13). A number of candidate genes were implicated including apolipoproteins and LPL, but together they account for a modest fraction of postprandial lipid variability (14).

CD36 is a highly conserved membrane protein expressed on metabolic and immune cells that functions in cellular recognition and uptake of many lipid-related molecules (15, 16) including dietary long chain fatty acids and native or oxidized lipoproteins (17). In addition, CD36 functions in transducing cell type specific intracellular signaling that serves to regulate lipid utilization (17-19) or the immune response (20). Variants in the *CD36* gene have been shown to influence fasting lipids (21, 22) and risk of metabolic syndrome (MetS) (22). Evidence from CD36 null mouse models and CD36 deficient humans suggests that the protein regulates postprandial triglycerides and cholesterol metabolism. In mice, CD36 deficiency increases

cholesterol levels, impairs chylomicron secretion into the lymph and delays postprandial TG clearance (9, 23-25). A similar phenotype is found in CD36 deficient subjects who were reported to have increased chylomicron remnants and cholesterol levels (9, 26, 27). Despite the established role of CD36 in lipid absorption and CM metabolism, there is no information on whether polymorphisms in the *CD36* gene influence variability in postprandial lipid metabolism in humans. Such information is important considering the documented link between CM remnants and cardiovascular disease.

In this study, we determined the effects of common *CD36* variants (minor allele frequency 5-45%) on lipoprotein levels before and after ingestion of a high fat shake in the family based study of 1117 Caucasian participants, the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) (28). We identified several SNPs that influence CM remnants, TG response and LDL. The most significant SNPs overlap those previously associated with the MetS. Examination of the potential mechanisms underlying the observed effects highlighted transcriptional and epigenetic regulatory regions of the gene.

Materials and Methods

Study Population: GOLDN participants of European ancestry were recruited from the NHLBI Family Heart Study. The data shown are from 1117 individuals (52% females); age: 48.4 ± 16.3, BMI: 28.3 ± 5.6 and blood pressure: systolic 115.3 ± 16.6, diastolic 68.1 ± 9.3 mmHg (Supplementary Table S1). Individuals ≥19 years old with fasting TG ≤1500 mg/dL, normal liver and kidney function were included while individuals with a history of pancreas, gallbladder or malabsorption diseases, use of lipid lowering drugs, insulin and warfarin were excluded, as

previously reported (29). Fasted (8 hours) participants ingested (within 15 minutes) a high fat milkshake (700 calories/m^2 body surface area, with 3% from protein, 14% from carbohydrate and 83% from fat). Blood was collected at fasting and after the meal at 3.5 and 6 hours for measuring lipid levels and lipoprotein profiling using nuclear magnetic resonance (NMR) (28). Blood collection at 3.5 and 6 hours is based on TG kinetics and designed to assess differences in lipid absorption and clearance, respectively (30-33).

SNP selection and analysis: Genotype data for 81 SNPs within a ~410-kb (chr7: 79,900,000-80,310,331) encompassing the *CD36* gene region met QC procedures and were used to impute 337 SNPs based on phased genotype data for HapMap CEU (release 22, Human Genome build 36) with minor allele frequencies (MAF) ≥ 5% (28). SNP genotyping was performed using the Affymetrix Human 6.0 platform (34). Linkage disequilibrium (LD) block structure was determined using the default method of Haploview for the Gabriel protocol (D' confidence interval 0.70-0.98) (35). Conventional and NMR derived lipid variables were transformed to render a normal distribution and adjusted for age, age^2 and sex within a stepwise regression. Lead SNPs were tested against lipid traits using the same linear mixed-effects model, accounting for familial relationship (36). TG uptake and clearance post meal were calculated from normalized growth curve models where uptake was based on time points 1 (fasting) and 2 (3.5 h post meal) and clearance on time point 3 (6 h post-meal) (28). Bonferroni corrections for multiple tests ($p \leq 0.006$) were based on the major variables LDL, TG and 7 LD blocks across the *CD36* region (HapMap CEU, Version 4.2).

Metabolic phenotype, Transcript level, DNA methylation and genotypes in the MuTHER cohort: The MuTHER (Multiple Tissue Human Expression Resource) cohort includes subcutaneous adipose tissue collected from 856 females of European descent recruited from the

TwinsUK adult registry (37, 38). Adipose RNA was used for expression profiling (Illumina Human HT-12 V3 BeadChips) (38) while DNA was bisulfite converted (EZ-96 DNA Methylation Kit, Zymo Research) and profiled on the Illumina HumanMethylation450 array (Illumina Inc.) (37). Genotypes were generated using a combination of Illumina arrays (HumanHap300, HumanHap610Q, 1M Duo and 1.2MDuo 1M) and imputed with IMPUTE (v2) using two reference panels, P0 (HapMap2, rel 22, combined CEU+YRI+ASN panels) and P1 (610k+, including the combined HumanHap610k and 1M array). The SNPs were filtered at MAF >5% and IMPUTE info value of >0.8.

The MuTHER study analyzed DNA methylation and transcript levels for 210,984 methylation and 18,818 expression probes situated on or 1500bp upstream of 13,532 genes. For this study, only sites localized to *CD36* promoter region (chr7: 80,060,000-80,095,000) were examined. Association of DNA methylation and transcript levels with probabilities of imputed genotypes (MAF >5%, info >0.8) were tested in samples of related individuals using a two-step statistical approach implemented in the GenABEL/ProbABEL packages. Age, beadchip, BS conversion efficiency and BS-treated DNA input were included as cofactors in the DNA methylation analysis (metQTL, N_{total}=603) (37) and age and experimental batch in the expression analysis (eQTL, N_{total}=776) (38), respectively. The *cis*-metQTL analysis was limited to SNPs within 100-kb either side of probe location whereas the *cis*-eQTL analysis considered SNPs located within 1MB either side of transcription start or end site or within the gene body. False discovery rate (FDR) were calculated using the qvalue package 30 implemented in R2.11 26 (37, 38) where 1% FDR in the *cis*-metQTL, *cis*-eQTL and methylation vs. expression analysis corresponded to p<8.6E-4, p<5.0E-5 and p<2.6E-4, respectively.

Associations between phenotypes or CD36 transcript levels with DNA methylation were tested with a linear mixed-effects model in R (39) using the lmer() function in the lme4 package (40) fitted by maximum-likelihood. The linear mixed-effects model was adjusted for both fixed (age, beadchip, BS conversion efficiency and BS-treated DNA input) and random effects (family relationship and zygosity). A likelihood ratio test was used to assess significance of the phenotype effect. The p-value of the phenotype effect in each model was calculated from the Chi-square distribution with 1 degree of freedom using -2log (likelihood ratio) as the test statistic.

Results

Common *CD36* SNPs influence chylomicron remnants and TG levels: The GOLDN cohort includes relatively healthy, overweight to obese individuals (as indicated in Methods). The plasma lipid profile for this cohort before and after the meal is in Supplementary Table S1. To comprehensively examine effects of common *CD36* variants on postprandial lipids, we tested SNPs across the *CD36* locus encompassing all six alternative first exons (1A-1F) (41, 42) and the two 3'UTRs (short and long) (Fig. 1). SNP associations with CM levels were identified (Table 1 and Fig. 1) at 3.5h and 6h post meal. At 3.5 h, independent common SNPs near the distal promoter (1D in Fig. 1A) tagged by rs10081383, rs2048474 and rs13230620 associated with lower CM levels while CM levels were higher for the less frequent rs37920 (7%) (Table 1). At 6 h post meal, the same relationships were maintained for SNPs rs10081383 and rs37920 (Fig. 1B, Table 1). The effect of rs37920 (C allele) was much more significant at 6h suggesting that the major impact of the allele is to reduce clearance, a more substantial contributor to CM

levels at 6h post the meal. The rs10081383 (A-allele) and rs12535593 (intronic ~60kb downstream of 1D) SNPs associated with lower CM at 6h implying either reduced CM input or enhanced CM clearance. In addition, there was an effect in the 3'UTR at rs7755 (G, 46%) on increasing CM remnants (Fig. 1B) but this association was not significant after Bonferroni correction.

We then tested SNP associations with changes in TG levels post meal, using slope of growth curve modeling (43), which distinguishes between TG appearance (0-3.5h) and clearance (3.5 - 6.0 h) phases. As shown in Table 2, three common SNPs near the distal first exon 1D (rs304763, rs304798, rs304802) associated with slower TG appearance (or negative β estimate) and three other SNPs with delayed TG removal (rs37920, rs13228738, rs3211842). Of interest, rs37920 associated with higher CM remnants (Table 1), which is consistent with slow TG removal (Table 2). These findings support a role for the distal 1D region of *CD36* in the regulation of postprandial lipid metabolism.

LDL: Associations with higher LDL particle numbers at fasting and at the post meal times (Fig. 2, Table 3, Supplemental Table 2) were identified for SNPs in the central (near first exon 1C) and proximal (near 1B) promoters and in the longer 3'UTR. The most significant observations were with tag rs1761665 (C, 44%) and rs6970109 (A, 10%). Two additional SNPs sharing modest LD with rs1761665 showed similar effects. In the 3'UTR, rs7755 (A, 46%) (Table 3) previously linked to risk of MetS and stroke (21, 44) associated with higher LDL. As noted earlier it also associated with higher CM remnants at 6h post-meal but this did not survive the Bonferroni correction. Of note the associations shown for fasting LDL remained significant at 3.5 and 6h post meal (Supplementary Table S2) as would be expected.

Lipid-associated SNPs influence *CD36* mRNA levels in adipose tissue and heart: To determine the potential functional relevance of the lipid-associated SNPs, we examined their influence on *CD36* level in adipose and heart, tissues that are highly active in lipid metabolism and where CD36 is abundant. Expression quantitative trait loci (eQTL) were available for adipose tissue from 776 healthy individuals of the MuTHER project (Multiple Tissue Human Expression Resource) (38). As shown in Table 4, analysis of the adipose tissue dataset documented strong influence of the SNPs on the most abundantly expressed *CD36* transcript (1B long 3'UTR, NM_001001548) (41, 42). Alleles that related to increased CM remnants or higher LDL associated with lower adipose tissue *CD36* mRNA (Table 4). Another independent genome-wide eQTL analysis performed with non-diseased human heart samples from 129 Western Europeans (45) showed similar negative relationships between the lipid-SNPs and *CD36* mRNA (Table 4). These findings document strong effects of the SNPs on *CD36* transcripts in heart and adipose tissue and suggest that expression in these tissues inversely relates to higher lipids consistent with the role of CD36 in enhancing lipid clearance and reducing circulating lipid levels.

SNPs align near sites for PPARγ and DNA methylation in adipose tissue *CD36*: Human adipocyte ChiP-seq data (46) showed that many of the associated promoter SNPs lie within close proximity of previously validated binding sites for PPARγ, a major *CD36* transcriptional regulator (Fig. 3). In addition, this region is characterized by active histone H3 lysine methylation and acetylation marks (46).

Previously, an adipose tissue epigenome study in MuTHER showed the most significant association with fasting TG levels at a CpG site (cg05917188) in the *CD36* promoter (47). In the

same dataset (37), we tested whether a significant relationship exists between the lipid-associated SNPs we identified and methylation using a multivariate linear regression model with age and BMI as covariates. Interestingly, the lipid-SNPs that negatively related to adipose *CD36* level associated with DNA methylation at several CpG sites (cg25783969, cg21055948, cg19096849 and cg18508525, cg26138637, cg06601993) (Table 5) within the central promoter region. Hypomethylation of four of these sites strongly related to *CD36* mRNA levels (Table 6). To gain a better perspective of the contribution of altered methylation to the SNP effects on *CD36* mRNA levels, a mixed-linear model for effects of the two most significant eQTLs, rs1761665 (promoter) or rs7755 (3'UTR) was adjusted for methylation (Supplementary Table S3). Comparison of the adjusted beta estimates shown in Supplementary Table S3 to those shown in Table 4, where associations are unadjusted demonstrates that adjustment for methylation does not significantly attenuate the associations between the SNPs and the most abundant CD36 transcript (1B with long 3'UTR). This indicated that the effect of the SNPs on *CD36* is independent of methylation at the above sites and is more likely to involve transcriptional regulation of the gene by PPARγ or posttranslational regulatory sites in the 3'UTR (rs7755). We then examined whether methylation also relates to higher lipid levels. As shown in Table 7, DNA methylation correlated with higher TG and LDL, suggesting that it independently impacts lipid metabolism through its influence on CD36 level (Table 6).

Discussion

Complex interactions between diet, lifestyle, and genetic factors can lead to sustained elevation of postprandial lipids, which independently increases the risk of cardiovascular disease

(1, 2). Earlier genetic studies linked *CD36* to fasting lipids (21, 48), MetS (22, 49-51) and stroke risk (44). However, information related to the role of the gene in postprandial lipid metabolism, which plays an important role in etiology of the MetS and cardiovascular disease remains limited. This study is the first comprehensive examination of the impact of CD36 variants on postprandial lipids. We present findings implicating common promoter SNPs in inter-individual variability of the CM remnant response after a fatty meal. We also identify SNPs that associate with LDL particle number at both fasting and two postprandial time points. We document that the identified lipid SNPs, which align close to validated PPARγ sites, associate with reduced adipose and heart CD36 and higher lipid traits supporting the role of CD36 in enhancing lipid clearance. In addition, we provide evidence that higher serum lipids associate with altered DNA methylation and reduced *CD36* mRNA.

The most significant promoter SNPs identified to influence CM levels and TG clearance (rs1931694, rs13228738, rs3211842) are in strong LD with variants previously associated with MetS or coronary artery disease, CAD (22, 48, 49). Similarly, the 3'UTR variant rs7755 identified to associate with LDL and to trend with higher CM remnants has been linked to diabetes-associated CAD (48) stroke (44) as well as MetS (22). Overall our findings support an important role of CD36 in the handling of dietary lipids in humans and are consistent with data in CD36 null mice showing defective CM metabolism and persistently high levels of CM remnants (9, 23-25). Elevated CM remnant levels were reported in small cohorts of CD36 deficient subjects (9, 26, 27). Our study indicates that common *CD36* alleles (5-45% frequency) contribute to variability of the postprandial response and possibly to risk of diet-induced metabolic abnormalities. GOLDN is the largest cohort to-date in which postprandial lipid measures are available and ideally our findings will need replication in other large studies as they

become available.

We identified five *CD36* SNPs to associate with higher LDL while previous GWAS did not link LDL to the CD36 locus. Several considerations might explain the discrepancy. The GOLDN family-based study consists of relatively healthy overweight to obese Caucasians who underwent a washout period to neutralize effects of lipid-lowering medications. Earlier GWAS included cohorts with a wide range of ages, metabolic disease traits, medications and BMI (52). We queried available Global Lipid Genetics (GLG) Consortium datasets using LocusZoom and found that most cohorts did not include sufficient SNP coverage of the *CD36* promoter region comparable to this study. In one of the GLG studies (53) with similar coverage and that included SNPs in LD with those we genotyped, we found modest raw associations ($p<0.05$) with LDL cholesterol. Several of the identified variants, rs799979, rs646722, rs6949840 rs2030711, and rs17154155, are in strong LD with rs1722507 and rs1761665 in this study. In addition, our study measured LDL particle number as opposed to LDL cholesterol obtained by the Friedwald calculation. While both measures predict cardiovascular risk, they do not always correlate (1, 54). In support of our findings, are the reports of higher cholesterol levels in partially (55) or completely CD36 deficient individuals (9) and the higher LDL levels and abnormal cholesterol absorption of CD36 null mice (24, 56). In addition, a recent linkage analysis of LDL particle response to fenofibrate treatment in GOLDN reported a peak encompassing the *CD36* locus (57). Thus, the LDL associations we identified and their potential relationship to disease risk warrant further investigation.

Our findings highlight regulatory regions in the *CD36* gene: SNPs near the distal promoter (designated 1D), which is almost exclusive to endothelial cells (42), associated with TG uptake/clearance and CM remnants. The central promoter region had signals for LDL and

TG clearance, and contained the validated PPARγ sites and the lipid-associated DNA methylation sites. Finally, the long 3'UTR had signals for LDL and contained predicted miRNA and RNA editing sites.

We showed that SNPs that associated with higher CM remnants and LDL reduced adipose and heart *CD36*. The rs1761665 SNP in particular is in strong LD with promoter SNPs previously shown to influence monocyte and platelet CD36 (58, 59). Several of the promoter SNPs we identified here, lie in close proximity to validated binding sites for PPARγ and to methylation sites that associate with *CD36* mRNA levels (Figure 3). Further analyses involving the two most significant eQTLs (rs1761665 and rs7755) for CD36 indicated that the effect of these SNPs is independent of methylation and is more likely to involve altered transcriptional regulation by PPARγ (rs1761665) or posttranscriptional effects involving the 3'UTR (rs7755). Although changes in methylation do not account for the SNP effect on CD36 level, our data suggest that they influence lipid levels. Methylation might contribute to the metabolic and dietary regulation of the *CD36* gene. For example, fatty acids were reported to alter global DNA methylation and to participate in shaping metabolic disease related methylomes (60).

In summary, our findings link common *CD36* SNPs that reduce adipose and heart *CD36* levels to higher CM remnants and LDL in humans. These SNPs would contribute to individual differences in the handling of dietary lipid and in susceptibility to diet induced metabolic abnormalities. Finally, factors that alter methylation of the *CD36* gene impact lipid levels and potentially disease risk.

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Disclosures

None declared.

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Table 1: *CD36* tag SNPs influence chylomicron levels.

SNP	Effect Allele	MAF	β(se)	*p-value
		3.5 h post-meal		
rs10081383	G/**A**	0.24	-0.18 (0.06)	0.0035
rs37920	T/**C**	0.07	0.40 (0.14)	0.0034
rs2048474	A/**G**	0.32	-0.19 (0.06)	0.0011
rs13230620	G/**C**	0.28	-0.18 (0.06)	0.0032
		6.0 h post-meal		
rs10081383	G/**A**	0.24	-0.18 (0.06)	0.0005
rs37920	T/**C**	0.07	0.57 (0.14)	0.00003
rs12535593	T/**C**	0.27	-0.26 (0.07)	0.0002

Shown are beta-estimates (β) ± standard errors and raw p-values for the most significant *CD36* tag SNP (within respective LD blocks) that associate with chylomicron levels. SNPs are listed based on genomic positions (see Fig. 1). *Bonferroni correction threshold $p<0.006$.

Table 2: Effect of *CD36* SNP on growth curve slopes for TG appearance (0 - 3.5 h) and clearance (3.5 - 6.0 h) after the meal.

SNP	Effect allele	MAF	β(se)	*p-value
		0 - 3.5 h		
rs304763	G/**A**	0.38	-0.15 (0.05)	0.0049
rs304798	A/**G**	0.21	-0.21 (0.07)	0.0019
rs304802	T/**C**	0.32	-0.20 (0.06)	0.0009
		3.5 - 6.0 h		
rs37920	T/**C**	0.07	0.44 (0.13)	0.0008
rs13228738	G/**A**	0.38	0.16 (0.05)	0.0032
rs1931694	G/**C**	0.47	0.15 (0.05)	0.0048
rs3211842	G/**A**	0.42	0.17 (0.05)	0.0013

Shown are beta-estimates (β) + standard errors and raw p-values for the most significant CD36 tag SNP (within respective LD blocks). For TG appearance negative β estimates indicate the allele reduces absorption. For clearance, negative β indicates enhanced TG removal and positive β reduced TG removal.*Bonferroni correction threshold $p<0.006$.

Table 3: *CD36* tag SNPs associate with higher LDL.

SNP	Effect Allele	MAF	β(se)	*p*-value
rs6970109	C/**A**	0.10	0.26 (0.09)	0.0027
rs1722507[b]	A/**G**	0.39	0.17 (0.05)	0.0011
rs1761665[a,b]	T/**C**	0.44	0.20 (0.05)	0.0001
rs3211842[a]	G/**A**	0.42	0.02 (0.05)	0.0017
rs7755[c]	G/**A**	0.46	0.16 (0.05)	0.0020

SNPs are listed by genomic location. Effect allele is bolded. Shown are beta-estimates (β) ± standard errors and raw p-values for most significant *CD36* tag SNP (within respective LD blocks).

*Bonferroni correction threshold p<0.006. LD between SNPs: [a]r^2>0.80, D'=0.93, [b]r^2=0.68, D'=1.0, [c]r^2>0.80, D'=0.96. Data shown are for fasting LDL, Supplementary Table S2 shows data for 3.5 and 6h post meal.

Table 4: Lipid-associated SNPs influence CD36 transcript level.

		Adipose Tissue		Heart[c]	
SNP	**Effect Allele**	**β(se)**	***p*-value**	**β(se)**	***p*-value**
rs1931694 (TGc)	G/**C**	-0.17 (0.03)	1.24×10^{-09}	---	
rs1722507[b] (TGc)	A/**G**	-0.25 (0.03)	5.58×10^{-17}	---	
rs1761665[a] (LDL)	T/**C**	-0.27 (0.03)	3.31×10^{-22}	-0.40 (0.08)	7.25×10^{-7}
rs13228738[b] (TGc)	G/**A**	-0.24 (0.03)	1.03×10^{-16}	---	
rs3211842[a] (TGc)	G/**A**	-0.28 (0.03)	2.27×10^{-22}	---	
rs7755 (LDL)	G/**A**	-0.44 (0.03)	1.12×10^{-50}	-0.53 (0.08)	4.32×10^{-11}

Correlations between alleles and *CD36* mRNA were determined using simple regression. SNPs are listed by effect size. Frequency of the effect allele is ≥40% for all listed SNPs. The lipid trait affected by the SNP is in brackets, TGc = TG clearance. SNPs in LD [a]r^2>0.80, D'>0.8), [b](r^2=0.93, D'>0.8). SNPs are listed based on genomic location. *CD36* mRNA levels (ILMN_1784863; Illumina HT-12 v3) in abdominal subcutaneous adipose tissue in the Multiple Tissues Human Expression Resource (MuTHER) were retrieved from GENEVAR database. [c]SNP effects on heart *CD36* expression (ILMN_1784863; Illumina HumanHT-12 v4) from a genome wide eQTL dataset in non-diseased tissue of 129 Western Europeans (45). --- indicates effects were not reported.

Table 5: Lipid- SNPs associate with DNA methylation in adipose tissue.

CpG ID	rs13228738	rs3211842	rs1722507	rs1761665	rs7755
cg18508525[a]	9.65×10^{-06}	3.83×10^{-04}	2.76×10^{-07}	3.20×10^{-05}	3.04×10^{-04}
cg25783969[a]	1.24×10^{-08}	---	7.23×10^{-11}	6.72×10^{-05}	9.26×10^{-05}
cg21055948[a]	1.57×10^{-11}	5.01×10^{-06}	2.37×10^{-13}	3.08×10^{-07}	6.14×10^{-06}
cg19096849	3.36×10^{-09}	5.87×10^{-06}	3.64×10^{-11}	1.40×10^{-07}	7.97×10^{-07}
cg26138637[a]	---	---	8.38×10^{-05}	---	---
cg06601993	---	---	6.75×10^{-04}	4.00×10^{-04}	---

Adipose tissue CpG methylation levels determined in the MuTHER study using the Infinium Human Methylation 450 platform. CpGs listed based on chromosomal position. P values are for CpG-SNP associations. [a]CpG associates with adipose *CD36* mRNA (Table 6).

Table 6: Hypomethylation of promoter CpG sites associates with adipose tissue CD36 expression levels.

CpG ID	*p*-value
cg18508525	2.40×10^{-05}
cg25783969	2.03×10^{-05}
cg21055948	3.06×10^{-05}
cg26138637	6.66×10^{-05}

CD36 gene expression (ILMN_1784863) was quantified in adipocyte nuclei from 776 individuals using Illumina whole genome expression array (Human HT-12 v3).

Table 7: Methylation at *CD36* expression-associated CpG sites correlates with TG and LDL concentrations.

CpG ID	TG		LDL	
	β	p-value	β	p-value
cg18508525[a]	0.24	1.36×10^{-06}	0.17	3.05×10^{-05}
cg25783969[a]	0.21	5.17×10^{-04}	0.11	1.49×10^{-02}
cg21055948[a]	0.22	3.99×10^{-04}	0.12	7.23×10^{-03}
cg26138637[a]	0.20	1.12×10^{-05}	0.13	2.82×10^{-03}

Adipose tissue CpG methylation levels were determined in the MuTHER study using the Infinium Human Methylation 450 platform. CpGs are listed based on chromosomal position. [a]CpG associates with adipose *CD36* mRNA (Table 6).

Figure 1.

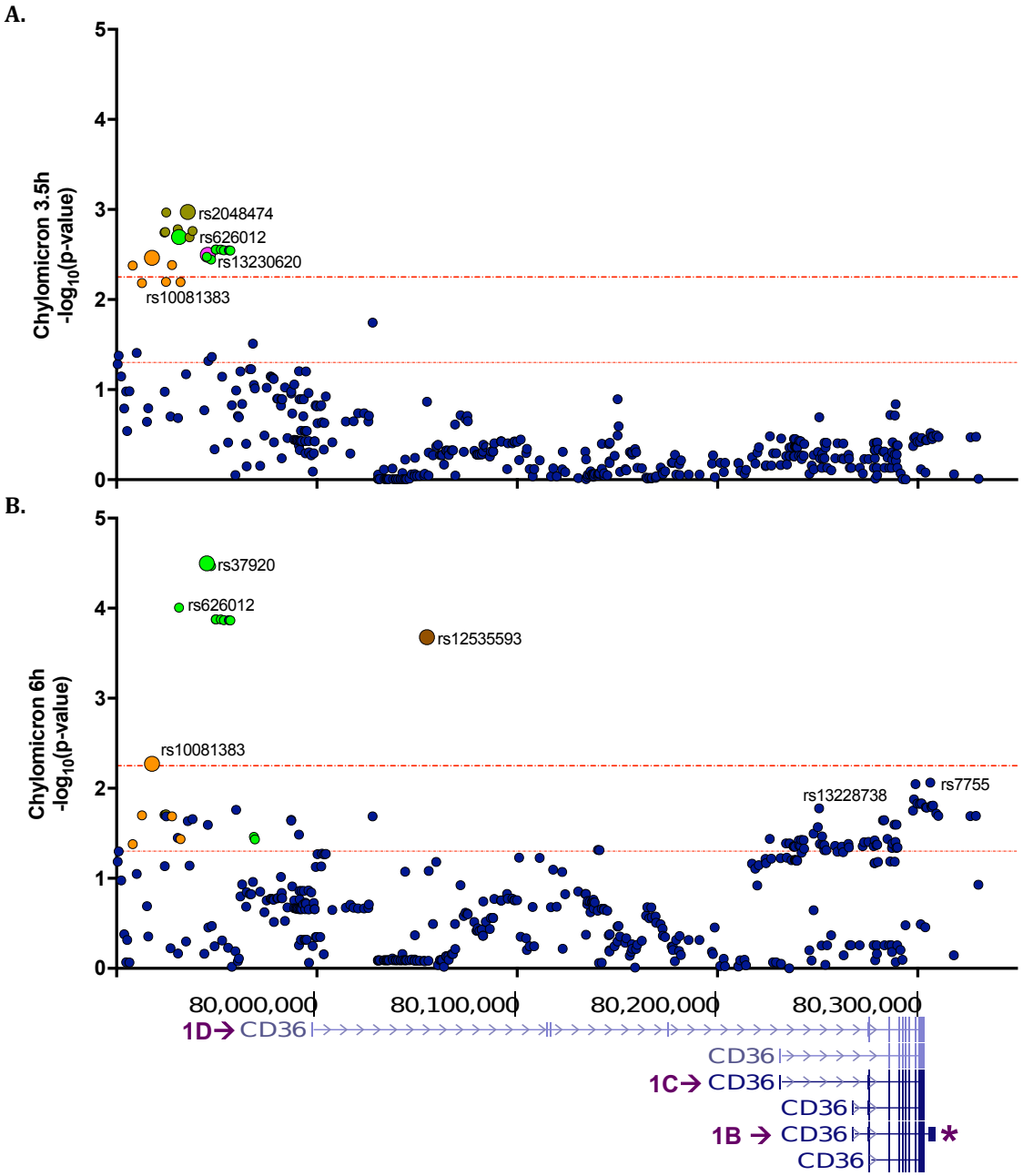

Figure 1: SNPs near distal first exon 1D of *CD36* associate with Chylomicron remnants. Regional plot of *CD36* SNPs that associate with chylomicron remnant concentration at **A)** 3.5h and **B)** 6 h after a the fat meal. P-values ($-\log_{10}$) are plotted based on chromosomal position (human genome GRCh37/hg19). Colors differentiate LD blocks ($r^2 \geq 0.8$). The most significant SNP (representative tag SNP) is identified by a larger symbol and the rs ID number. The overlay of the *CD36* gene from the UCSC genome browser illustrates the SNP position relative to alternative *CD36* promoters and transcripts (referred to as 1B, 1C, 1D) and the long 3'UTR (asterisk). Dotted red lines indicates p-value ≤ 0.05 and the Bonferroni-corrected threshold, $p<0.006$.

Figure 2.

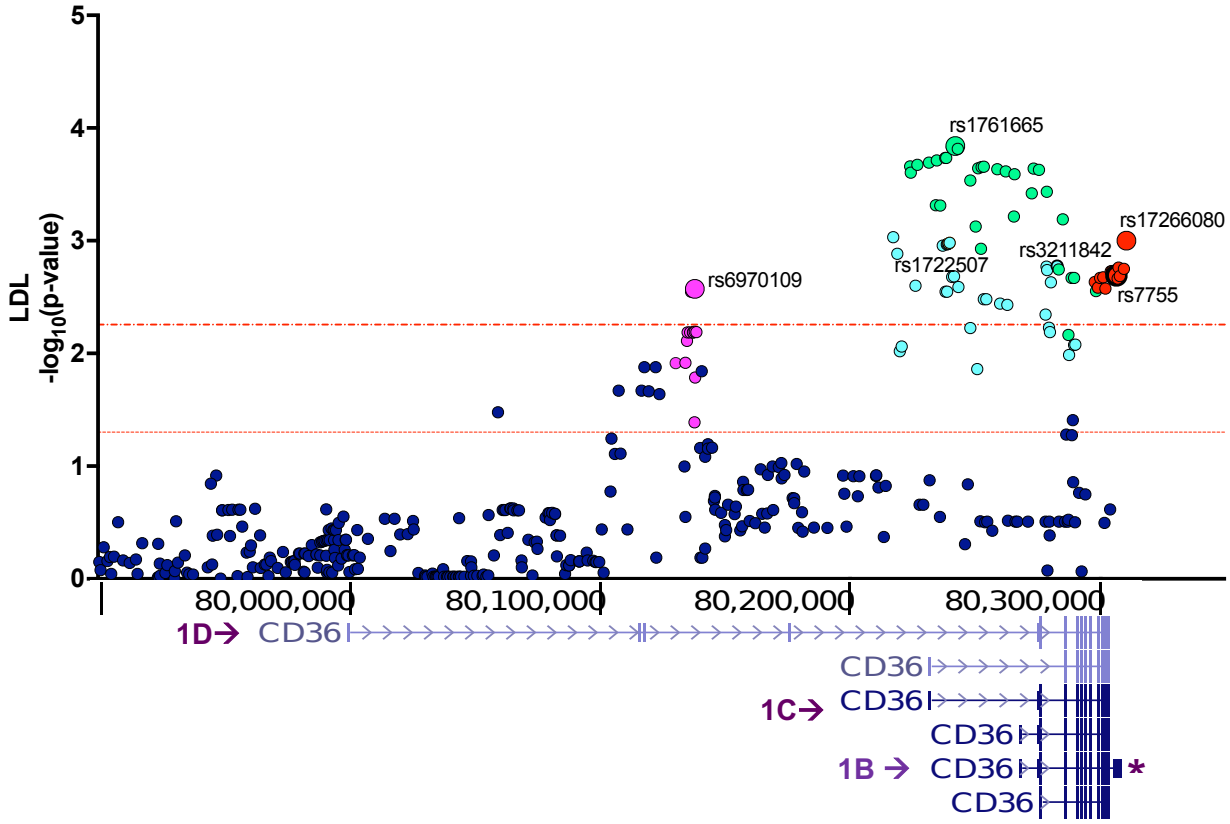

Figure 2: *CD36* SNPs in the proximal and central promoter regions associate with fasting LDL. P-values ($-\log_{10}$) are plotted based on chromosomal position (human genome GRCh37/hg19). Colors differentiate LD blocks ($r^2 \geq 0.8$) with the most significant SNP (representative tag) identified by a larger symbol and rs ID number. Dotted red lines indicates p-value ≤ 0.05 and Bonferroni threshold, p<0.006.

Figure 3.

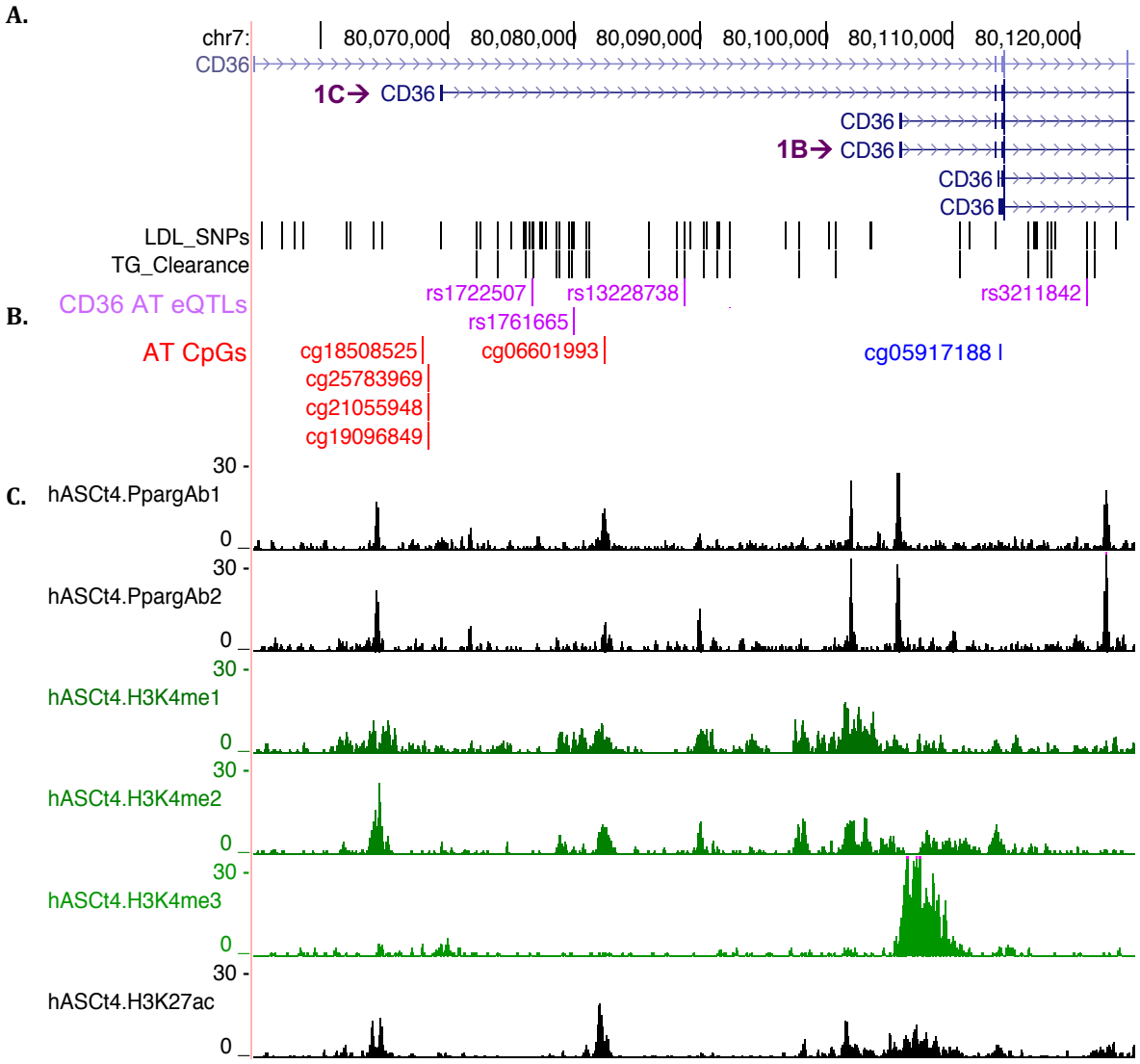

Figure 3: TG Clearance and LDL associated CD36 SNPs align in proximity to PPARγ motifs and to Cpg sites in the CD36 promoter. The central CD36 promoter region (chr7: 80,060,000-80,095,000) is tagged by rs1761665 which associates with methylation levels at CpG sites in adipose tissue near *CD36* alternative exon 1C that is specific to adipocytes (absent from immune cells and endothelial cells). **(A)** Purple text indicates SNPs that associate with both lipid levels and adipose tissue CD36 expression. **(B)** SNP-associated methylation sites (red text). The location of a previously published TG-associated CpG site is shown in blue text (47). **(C)** Chromatin state maps of the *CD36* locus from mature human adipocytes. Histograms of ChIP-Seq fragments aligned to the human reference genome (hg18) highlighting active PPARγ binding sites (peaks) and epigenetic signatures (H3K4me1, H3K4me2, H3K4me3, H3K27ac).