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Identifying the Lipidomic Effects of a Rare Loss-of-Function Deletion in *ANGPTL3*

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Abstract

Background: The identification and understanding of therapeutic targets for atherosclerotic cardiovascular disease (ASCVD) is of fundamental importance given its global health and economic burden. Inhibition of angiopoietin-like 3 (ANGPTL3) has demonstrated a cardioprotective effect, showing promise for ASCVD treatment, and is currently the focus of ongoing clinical trials. Here we assessed the genetic basis of variation in ANGPTL3 levels in the San Antonio Family Heart Study.

Methods: We assayed ANGPTL3 protein levels in ~1,000 Mexican Americans from extended pedigrees. By drawing upon existing plasma lipidome profiles and genomic data we conducted

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Supplemental Materials:
Supplemental Methods
Supplemental Tables I–IV
Supplemental Figures I–II
References[39–57]

analyses to understand the genetic basis to variation in ANGPTL3 protein levels, and accordingly the correlation with the plasma lipidome.

Results: In a variance components framework we identified that variation in ANGPTL3 was significantly heritable (h^2=0.33, P=1.31×10^{-16}). To explore the genetic basis of this heritability, we conducted a genome-wide linkage scan and identified significant linkage (LOD = 6.18) to a locus on chromosome 1 at 90 cM, corresponding to the *ANGPTL3* gene location. In the genomes of 23 individuals from a single pedigree, we identified a loss of function (LoF) variant, rs398122988 (N121Kfs*2), in *ANGPTL3*, that was significantly associated with lower ANGPTL3 levels (β=−1.69 SDU, P=3.367×10^{-13}), and accounted for the linkage signal at this locus. Given the known role of ANGPTL3 as an inhibitor of endothelial and lipoprotein lipase we explored the association of ANGPTL3 protein levels and rs398122988 with the plasma lipidome and related phenotypes, identifying novel associations with phosphatidylinositols.

Conclusions: Variation in ANGPTL3 protein levels is heritable and under significant genetic control. Both ANGPTL3 levels and LoF variants in *ANGPTL3* have significant associations with the plasma lipidome. These findings further our understanding of ANGPTL3 as a therapeutic target for ASCVD.

Journal Subject Terms

Biomarkers; Genetic, Association Studies; Genetics; Lipids and Cholesterol

Keywords

lipids; genetics, linkage analysis; genetics, association studies; family study; ANGPTL3

Introduction

The identification of therapeutic targets for the management and treatment of atherosclerotic cardiovascular disease (ASCVD) is a critical area of interest to the medical community. A central paradigm within the field is that cardiometabolic diseases (including ASCVD and type 2 diabetes) are intimately linked with abnormal levels of the classical lipid and lipoprotein variables of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol[1]. Increased levels of total cholesterol, LDL-C and TG, and decreased levels of HDL-C are all associated with cardiometabolic disease[2] and are in part determined by genetic factors.[3, 4] Each of these lipid parameters, as well as ASCVD risk, have been shown previously to have a significant genetic component.[5]

There has been continued interest in the identification of therapeutic targets that modulate these classical lipids toward a healthy or cardioprotective profile. One target that is being actively pursued is angiopoietin-like 3 (ANGPTL3).[6–8] Clinical trials targeting a cardioprotective decrease in ANGPTL3 protein levels using antibodies and antisense oligonucleotides are ongoing and to date have shown promise for the targeting of ANGPTL3 in ASCVD.[6, 8, 9] Recent topline reports of an ANGPTL3 siRNA, ARO-ANG3, in a Phase 1 clinical study demonstrate safety, durability and efficacy promoted by both single and

multiple injections.[10–12] Phase 2 and 3 trial results of evinacumab, a monoclonal antibody against ANGPTL3, and phase 2 trial results of vupanorsen, an antisense oligonucleotide, have shown them to be safe, and effective for the treatment of homozygous (phase 3) and heterozygous (phase 2) familial hypercholesterolemia (evinacumab)[13, 14] and for lowering triglycerides and atherogenic lipoproteins in patients with type 2 diabetes, hepatic steatosis and hypertriglyceridaemia (vupanorsen).[9]

In humans, ANGPTL3 is a 460-amino acid hepatokine that has established roles as an inhibitor of lipoprotein lipase (LPL) and endothelial lipase (EL).[15, 16] ANGPTL3 was first implicated in lipid metabolism in mice[17] and subsequently implicated in a common variant based genome-wide association study (GWAS) of human plasma lipids as loci associated with TG levels.[18, 19] In 2009, a study of unselected individuals from the Dallas Heart Study cohort identified rare loss-of-function (LoF) variants in *ANGPTL3* and other *ANGPTL* protein family members (*ANGPTL4* and *ANGPTL5*) that resulted in a lowering of the circulating levels of plasma TG in variant carriers.[20] Successively, multiple studies have shown that loss-of-function variants in *ANGPTL3* are causal for familial combined hypolipidemia (OMIM: 605019) identifying both homozygous and compound heterozygous LoF variant carriers with clinical characteristics.[21, 22] Through the inhibition of EL, ANGPTL3 regulates plasma HDL-C levels, whereas through the inhibition of LPL, ANGPTL3 regulates plasma TG levels. In *ANGPTL3* LoF variant carriers, the inhibitory effects of ANGPTL3 are reduced resulting in a decrease in plasma levels of HDL-C, LDL-C and TG.[20, 21, 23–25] Recent work now suggests that the mechanism by which LDL-C is decreased through ANGPTL3 inhibition occurs through the resulting derepression of EL.[26, 27] These pleiotropic effects across lipids and lipoproteins, together with other non-lipid related effects underlie the rationale for which ANGPTL3 is a target for ASCVD. In addition, a joint meta-analysis of multiple cohorts, including Mendelian randomization analytical approaches, showed that carriers of LoF variants have 34–39% reduction in major adverse cardiac event (MACE) incidence, underscoring the possible therapeutic utility of targeting ANGPTL3.[6, 28]

Using a well characterized cohort of over 1,000 Mexican Americans from extended pedigrees in the San Antonio Family Heart Study (SAFHS), we set out to identify the genetic basis of variation in ANGPTL3 levels. Using multipoint quantitative trait linkage analysis, we conducted a 1cM genome-wide linkage scan to identify genomic regions influencing levels of circulating ANGPTL3. Then, within the linkage region identified we focused on a clear positional candidate gene and used available whole genome sequence (WGS) to identify a rare genetic variant influencing ANGPTL3. We then used plasma lipidome profiles available for this cohort to assess the relationship between ANGPTL3 and levels of individual lipid species leading to the identification of previously unknown lipid associations with ANGPTL3. Further, we show that a genetic variant identified to influence ANGPTL3 levels also affects individual lipids in the plasma lipidome. This work provides a new, deeper understanding into ANGPTL3 biology which is important for advancing the development of this clinical target.

Methods

Data used in this paper are publicly available through dbGaP (accession numbers: phs000462.v2.p1, phs001215.v1.p1) except for the lipidome data and ANGPTL3 protein levels associated with the San Antonio Family Heart Study. Both the lipidome data and ANGPTL3 protein measurements for the San Antonio Family Heart Study can be made available to researchers from the corresponding author Dr. Joanne E. Curran (joanne.curran@utrgv.edu) via a material transfer agreement for work consistent with the informed consent.

Informed consent was obtained from all participants before sample collection. The study conformed to the Declaration of Helsinki and has been approved by the Institutional Review Boards of the University of Texas Health Sciences Center at San Antonio and the University of Texas Rio Grande Valley. The full methodology for this work is described in detail in the supplemental methods section.

Results

ANGPTL3 protein levels are heritable

ANGPTL3 protein levels were measured by ELISA in fasting plasma samples from 1,030 individuals in the San Antonio Family Heart Study. The mean concentration of ANGPTL3 protein was 218.64 ng/ml (median = 199.99, standard deviation = 101.79), with a measured minimum value of 24.59 ng/ml and a maximum of 811.53 ng/ml, in line with previously reported ranges.[29]

Through variance components modelling in SOLAR we assessed - for the first time to our knowledge - the heritability of ANGPTL3 plasma protein levels. In this fully adjusted model, we identified that ANGPTL3 levels were highly heritable, $h^2 = 0.325$ (SE = 0.05, $P=1.31\times10^{-16}$) indicating a significant genetic component to variation in levels of this protein.

Genome-wide linkage scan identifies significant linkage on chromosome 1

To genomically localize genetic factors underlying the heritability of ANGPTL3 we conducted a 1 centimorgan (cM) genome-wide linkage scan, in an empirical kinship framework. This identified a significant linkage for ANGPTL3 on chromosome 1 at 90 cM of LOD = 6.18 (Figure 1). This locus, spanning at chr1:62151202-63714422, contains a clear positional candidate for ANGPTL3 protein levels, the *ANGPTL3* structural gene itself.

Measured genotype association testing of variants in *ANGPTL3* identifies a rare variant associated with protein levels

Using available whole genome sequence data from individuals in the SAFHS, we conducted a multi-sample joint call of the *ANGPTL3* gene, chr1:63063191-63071984 (hg19 coordinates) using freebayes to identify exonic variation in this gene. There were eight exonic variants in *ANGPTL3* across the 1,222 SAFHS genomes including one 5bp deletion and seven SNVs. Among the 1,030 SAFHS individuals with available ANGPTL3

measurements, six of these variants were present. All SAFHS variant carriers were heterozygous. These variants are summarized in Supplemental Table II.

To identify variants associated with ANGPTL3 protein levels, we conducted a measured genotype association analysis of the six exonic variants. The frameshift deletion rs398122988 (N121Kfs*2), with 23 heterozygous carriers among the sample was identified as associated with a significant decrease in ANGPTL3 protein levels ($\beta = -1.69$ SDU, $P = 3.367 \times 10^{-13}$). This is a very large biological effect size. For carriers of rs398122988 the unadjusted mean ANGPTL3 protein concentration was 82.76 ng/ml (median = 86.36, standard deviation = 38.46), whereas for non-carriers the unadjusted mean ANGPTL3 protein concentration was 221.74 ng/ml (median = 203.34, standard deviation = 100.67). The remaining five exonic variants in *ANGPTL3* were not associated with ANGPTL3 protein levels, even after controlling for rs398122988. We were unable to assess the effect of two variants, rs767910330 (E98K) and rs1196457133 (R428R), as the carriers of these variants did not have ANGPTL3 protein level measurements.

The rs398122988 variant is a rare 5bp frameshift deletion, introducing an early STOP codon into the mRNA sequence. Across all gnomAD populations (v3.1) the minor allele frequency of this variant is 0.0003 and it is most frequent in non-Finnish Europeans (MAF=0.0005) and not present in Asian populations.[30] This variant is predicted to produce a truncated protein of 121 amino acids, instead of the normal 460 amino acid full length protein, and thus is classified as a loss of function variant. In our cohort this variant occurs in a single multigenerational family (Figure 2). Figure 3 shows the distribution of ANGPTL3 protein levels in carriers and non-carriers of rs398122988.

ANGPTL3 frameshift deletion variant rs398122988 accounts for the linkage signal detected on chromosome 1

To test whether the rs398122988 variant identified as associated with ANGPTL3 levels accounts for the chromosome 1 linkage signal we conducted a 1 cM genome-wide linkage scan including this variant as a covariate in the linkage model. When incorporated into the multipoint linkage model, the rs398122988 variant completely accounts for the linkage detected at the chr1 90 cM locus (Supplemental Figure I).

The rs398122988 shows significant associations with plasma lipidome species

Previously, we measured 319 lipid species in 23 classes of lipids in blood plasma from participants of the SAFHS. For 1,020 of these individuals, whole genome sequence data are available. This includes 22 carriers of the rs398122988 variant. Given the importance of ANGPTL3 in lipid metabolism, we set out to identify whether this functional variant was associated with individual lipid species.

After appropriate adjustment for the effective number of tests in our plasma lipidome analyses, the rs398122988 variant was associated with decreases in 7 individual lipid species and one combined total lipid class measure, as shown in Table 1. The strongest association was with phosphatidylinositol (PI) species PI(36:2), where carriers of the rs398122988 variant had a 1.36 SDU decrease (SE = 0.27, $P=2.77\times10^{-7}$).

Across the lipidome, 67 individual lipid species - in 14 classes - as well as six combined total lipid class measures had nominally significant (P<0.05) associations with rs398122988 indicating that this loss of function variant in *ANGPTL3* has a broad effect on lipid biology. Figure 4 shows the effect of the rs398122988 variant across the plasma lipidome. As the majority of the phosphatidylinositol class of lipids were associated, Figure 5 shows the specific effects of rs398122988 on this class. The full list of plasma lipidome associations of rs398122988 is summarized in Supplemental Table III.

To determine whether the lipidome changes were specific to the effect of the *ANGPTL3* variant or were secondary to the changes in the overall lipoprotein profile, we performed the same association analysis with PI(36:2) but corrected for traditional lipid and lipoprotein parameters. The significant effect of rs398122988 was retained ($P=2.51\times10^{-4}$), although the effect size was slightly reduced ($\beta=-0.86$). This shows that there is an independent effect of rs398122988 on PI that is not secondary to global changes in classical plasma lipids and proteins.

ANGPTL3 levels are associated with a broad range of plasma lipidome species

For 876 individuals with plasma lipidome profiles we have matching ANGPTL3 measures from the same visit. We considered whether ANGPTL3 protein levels associate with individual plasma lipidome species, or total lipidome class measures. After appropriate adjustment for the effective number of tests in our plasma lipidome analyses, associations with ANGPTL3 were detected in 15 classes of lipids. This included associations with 49 individual species, as well as five total lipidome class measures. Supplemental Figure II summarizes the association of ANGPTL3 protein levels across the lipidome, highlighting the additional 75 nominally significant associations and the broad relationship of ANGPTL3 with the plasma lipidome. The full list of plasma lipidome associations with ANGPTL3 is provided in Supplemental Table IV.

To determine whether the lipidomic effect of rs398122988 was due solely to its effect on ANGPTL3 protein levels, we conducted an analysis of the top associated plasma lipidome PI species from Table 1 conditional on ANGPTL3 protein levels. The association of rs398122988 with lipid species is retained, however significance and the effect size of the association are reduced ~0.1 to 0.3 SDU (depending on the lipid species). For example, the significance of the lead rs398122988 association with PI(36:2), after controlling for ANGPTL3 protein levels, decreases to $P=5.10\times10^{-4}$ and an effect size of $\beta=-1.19$. These conditional findings suggest that the effect of the variant on the lipidome is not solely a result of quantitative changes in ANGPTL3 protein levels and may reflect the influence of the structural alteration of the protein on downstream pathways.

Broader phenotypic associations of ANGPTL3 protein levels and rs398122988

Beyond the plasma lipidome, ANGPTL3 protein levels and the rs398122988 variant show nominal associations with multiple phenotypes in the SAFHS (Table 2 and Table 3 respectively). Individuals who experienced a MACE including non-fatal myocardial infarction, stroke and cardiovascular death, during 25 years of follow-up visits of the San Antonio Family Heart Study, had higher ANGPTL3 protein levels. ANGPTL3 protein levels

were higher for both individuals with a lifetime MACE event (including death), P=0.034 (β=0.18 SDU), and for individuals who died from MACE, P=0.004 (β=0.51 SDU). Participants who reported that they were cigarette smokers also showed increased ANGPTL3 levels (P=0.015, β=0.16 SDU).

Classical lipid phenotype associations were also observed; however, these were primarily with the rs398122988 variant. Variant carriers had lower cholesterol (total, calculated very-low-density lipoprotein cholesterol (VLDL-C), and HDL-C) and lower total TGs. A non-significant decrease in calculated LDL-C cholesterol was also observed in variant carriers (1026 individuals, P=0.28, β=−0.27 SDU). Both ANGPTL3 protein levels and rs398122988 were associated with cholesterol efflux measures, with a positive association observed between ANGPTL3 protein and total cholesterol efflux and cholesterol efflux with cAMP (Table 2), meanwhile rs398122988 variant carriers have lower cholesterol efflux with no cAMP (Table 3). Variant carriers also had nominally significant lower glucose levels after a fasting 2-hour oral glucose tolerance test challenge.

ANGPTL3 protein levels were positively associated with waist circumference and ApoE serum levels. Whereas negative associations were identified with ApoA-II serum levels (Table 2). Comparatively, the rs398122988 variant was associated with decreased ApoA-I serum levels (Table 3).

Discussion

Here we report the largest genomics and lipidomics based study of ANGPTL3.

Our investigation of the genetic factors underlying variation in ANGPTL3 protein levels in a Mexican American family-based study has measured for the first time the heritability of ANGPTL3, finding that around 33% of the total phenotypic variation in ANGPTL3 is due to genetic factors. We have conducted the first multipoint linkage analysis of ANGPTL3, identifying significant linkage to the *ANGPTL3* gene locus. Drawing upon WGS data from our cohort, we then identified a rare LoF frameshift deletion variant in *ANGPTL3*, rs398122988, that was significantly associated with lower ANGPTL3 levels in 23 variant carriers from a single family. This variant completely accounted for the identified linkage signal and suggests that at least in this cohort, the primary genetic driver of variation in ANGPTL3 occurs at the structural gene locus. The rs398122988 variant has previously been identified in individuals with familial combined hypolipidemia[23, 24], particularly as a homozygous variant, but has also been identified as a rare variant in multiple large population cohorts.[6, 30, 31]

Given the known biological role of ANGPTL3 in metabolism of the classical lipid parameters of HDL-C, LDL-C and TG and our plasma lipidome measurements for 319 lipid species available in 1,020 individuals with genotype information for the rs398122988 variant we assessed the effect of this variant on lipid profiles in this cohort. For the classical lipid parameters, rs398122988 shows nominally significant associations with total cholesterol, total HDL-C, and TG levels, with a decrease in each associated with this LoF variant. These were expected associations given the established biology of ANGPTL3. Unexpectedly,

LDL-C levels were not associated with this LoF variant, however a nominally significant decrease in calculated VLDL-C was observed. Recent elegant work with mouse knock-out models has shown that the reported LDL receptor (LDLR) independent decreases in LDL-C levels associated with ANGPTL3 inhibition are driven through the derepression of EL, specifically the upstream remodeling of VLDL-C particles.[26] Our nominal association with a decrease in calculated VLDL-C in LoF variant carriers is in line with this work. However, we infer from our findings that a partial loss of ANGPTL3 (as in heterozygous LoF variant carriers), due to the rs398122988 variant, is insufficient to drive enough derepression of EL to result in downstream decreases in LDL-C levels.

A particularly novel finding within our cohort has been the identification that *ANGPTL3* LoF variant carriers had significantly decreased levels of several phosphatidylinositol (PI) species. Shown in Figure 5, carriers had 0.31 – 1.36 SDU decreases in PIs, with the largest effect of a 1.36 SDU decrease seen for PI(36:2). This is a completely novel association for ANGPTL3 in humans and is of significant interest in light of the clinical potential of ANGPTL3 targeting. Phosphatidylinositol species associations were reported recently in a study of the plasma lipidome effects of pravastatin treatment[32], negative associations were shown with multiple species of PI and the lipid ratio PI(36:2)/PC(38:4) ratio was identified as predicting the efficacy of pravastatin therapy in secondary prevention of MACE. Together this raises the possibility that the cardioprotective effects of ANGPTL3 targeting may be mediated through the lowering of PI, in addition to the demonstrated effects on the clinical lipid parameters.

ANGPTL3 protein levels in this study showed positive associations with ApoE levels and negative associations with ApoA-II levels. ApoE is enriched in triglyceride-rich lipoprotein particles (TRLP) such as VLDL-C and in remnant lipoprotein particles, and it mediates clearance of these particles in the liver via LRP1 and LDL receptors.[33] The positive relationship between ApoE and ANGPTL3 levels may be explained by decreased LPL-mediated catabolism and clearance of TRLPs when ANGPTL3 levels are elevated. ApoA-II is the second most abundant protein associated with HDL-C and interacts with ABCA1 to promote ABCA1-mediated cholesterol efflux.[34] The inverse relationship between ApoA-II and ANGPTL3 protein levels is somewhat surprising since ANGPTL3 represses EL activity that mediates HDL-C catabolism. However, when studying the lipoprotein metabolism in a hypobetalipoproteinemia family harboring compound heterozygous *ANGPTL3* LoF variants, the observed low HDL-C and ApoA-I levels were caused by higher ApoA-I fractional catabolic rates without significant decreases in ApoA-I production rates.[35] Thus, HDL-C, and possibly ApoA-II, is likely produced at normal levels with the same or more potential to mediate peripheral cholesterol efflux and is cleared more rapidly in *ANGPTL3* LoF variant carriers.

We also observed that increased ANGPTL3 protein levels were associated with increased waist circumference, and that LoF variant carriers had a nominal association with lower plasma glucose levels 2 hours after an oral glucose tolerance test. This observation, together with previous findings that a deficiency of ANGPTL3 is associated with an increased insulin sensitivity and TG lowering in mutation carriers, supports an extended role for ANGPTL3 targeting in cardiometabolic disease and diabetes.[36]

The recent work of Adam et al.[26] with LDLR and EL knockout mouse models provides a great deal of understanding as to the lipidomic differences observed here between carriers and non-carriers of the rs398122988 LoF variant. Adam et al. established that several lipidomic changes occur as a result of the derepression of EL from ANGPTL3 inhibition using evinacumab. Further, as shown previously in mouse models where EL has been overexpressed, there is a reduction in cholesterol efflux as a result of HDL-C lowering[37], which is in line with the decrease in cholesterol efflux observed in rs398122988 variant carriers and positive correlations between ANGPTL3 protein levels and cholesterol efflux. Together, these observations corroborate that the decreases in PI and phosphatidylcholine species observed here might be explained by the derepression of EL leading to increased phospholipid hydrolysis from HDL-C, whereas the observed decreases in cholesteryl esters are primarily a result of increased LPL activity. The decrease in PI may be important given that it provides a source of phosphatidylinositol phosphate (PIPs), signaling molecules that are themselves associated with multiple aspects of ASCVD.[38] However, at this stage we do not know if the plasma levels of PI also reflect the cellular levels in relevant tissues and so further studies are required to define the potential protection imparted by PI lowering.

The pursuit of disease modifying and disease eradicating therapeutics for ASCVD is a priority for human medical research. ANGPTL3 has been identified and confirmed as a viable target for ASCVD therapy with clinical trials actively underway. Our findings here provide evidence that the effect of ANGPTL3 targeting extends beyond traditional lipid and lipoprotein lowering to the effect on specific species of the plasma lipidome. By joining a pedigree-based study and classical genetic analyses with whole genome sequencing and lipidome profiling, we identified a LoF ANGPTL3 variant segregating in a single Mexican American family. This facilitated direct profiling of the effect of the variant on the plasma lipidome and identified novel associations with phosphatidylinositol species, further advancing our biological understanding of ANGPTL3 in ASCVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

ABCA1	ATP binding cassette subfamily A member 1
ApoA	apolipoprotein A
ApoE	apolipoprotein E
ASCVD	atherosclerotic cardiovascular disease
ANGPTL3	angiopoietin-like 3
β	beta coefficient
cAMP	cyclic adenosine monophosphate
chr	chromosome
cM	centimorgan
dbGaP	database of genotypes and phenotypes
EL	endothelial lipase
ELISA	enzyme-linked immunosorbent assay
GWAS	genome-wide association study
h^2	narrow-sense heritability
LDLR	low-density lipoprotein receptor
LOD	logarithm of odds
LoF	loss of function
LPL	lipoprotein lipase
LRP1	low-density lipoprotein receptor related protein 1
MACE	major adverse cardiac event
MAF	minor allele frequency
OMIM	Online Mendelian Inheritance in Man
PC	phosphatidylcholine
PI	phosphatidylinositol
PIP	phosphatidylinositol phosphate
SAFHS	San Antonio Family Heart Study
SDU	standard deviation units
SE	standard error

siRNA	short interfering ribonucleic acid
SNV	single nucleotide variant
SOLAR	Sequential Oligogenetic Linkage Analysis Routines software
WGS	whole genome sequence

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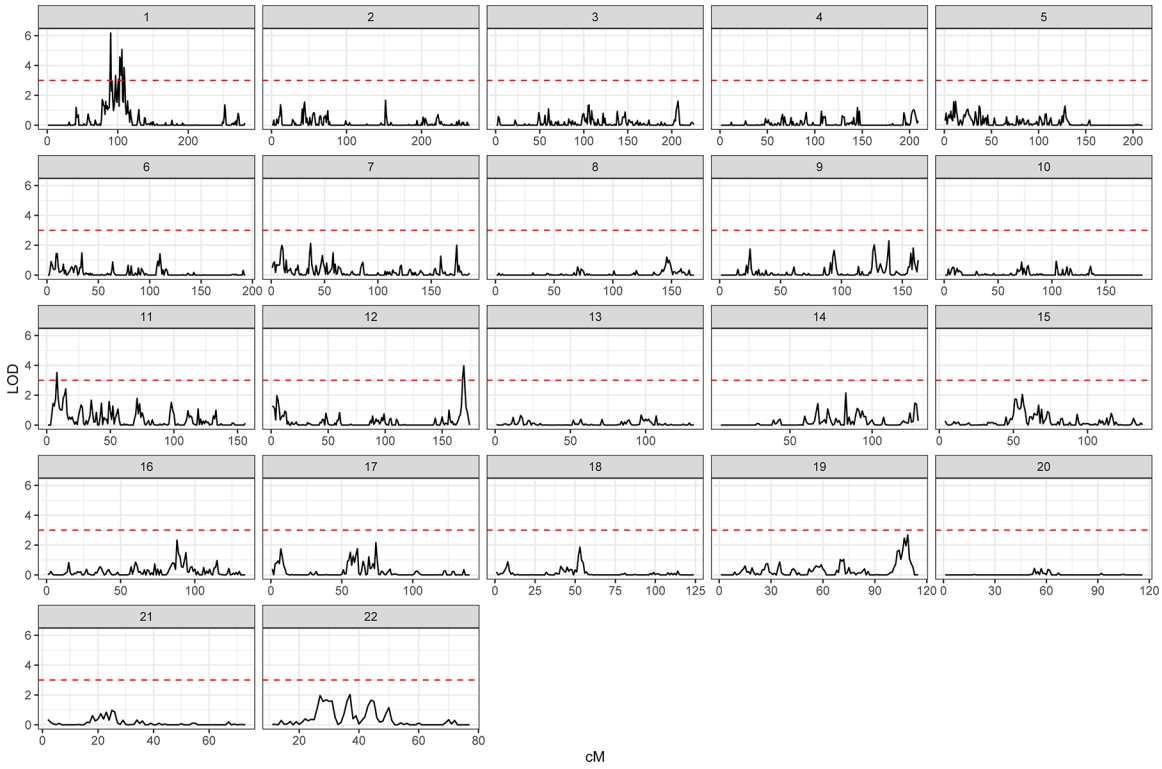

Figure 1.

A 1 cM genome-wide linkage scan for ANGPTL3 identifies a significant linkage on chromosome 1 at 90 cM with LOD = 6.18. Dashed red line indicates the LOD=3 linkage significance threshold.

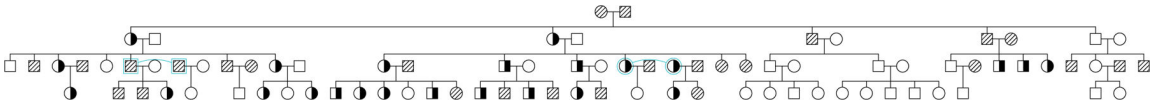

Figure 2.

Subset of the SAFHS pedigree displaying inheritance of 23 copies of the *ANGPTL3* 5bp deletion variant rs398122988. This rare loss of function frameshift deletion variant was only detected in this single family. Half-filled symbols are heterozygous carriers of the rs398122988 variant, cross-hatched symbols are individuals for whom WGS data is not available, empty white symbols are individuals who do not carry the variant. Blue highlights indicate individuals with multiple spouses.

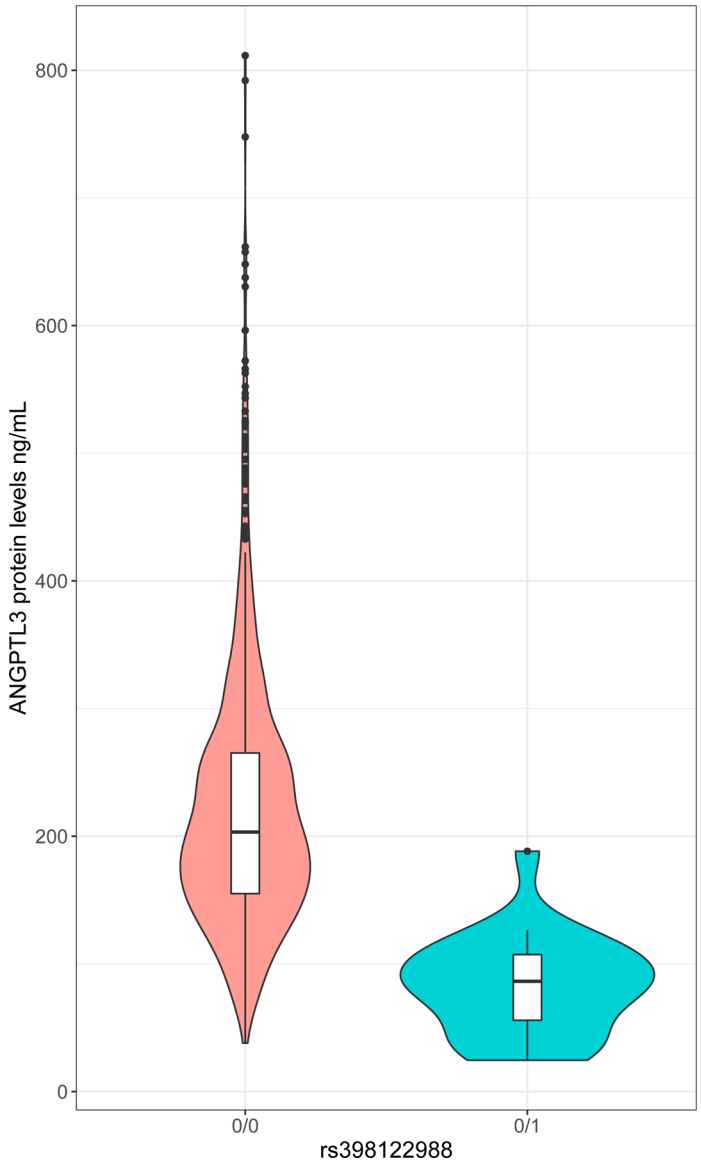

Figure 3.

Distribution of ANGPTL3 protein levels in rs398122988 carriers (0/1, n=23) and non-carriers (0/0, n=1007), shown as violin plots with internal box plots. The unadjusted mean ANGPTL3 protein concentration was 82.76 ng/ml (median = 86.36, standard deviation = 38.46) for carriers of rs398122988 and 221.74 ng/ml (median = 203.34, standard deviation = 100.67) in non-carriers.

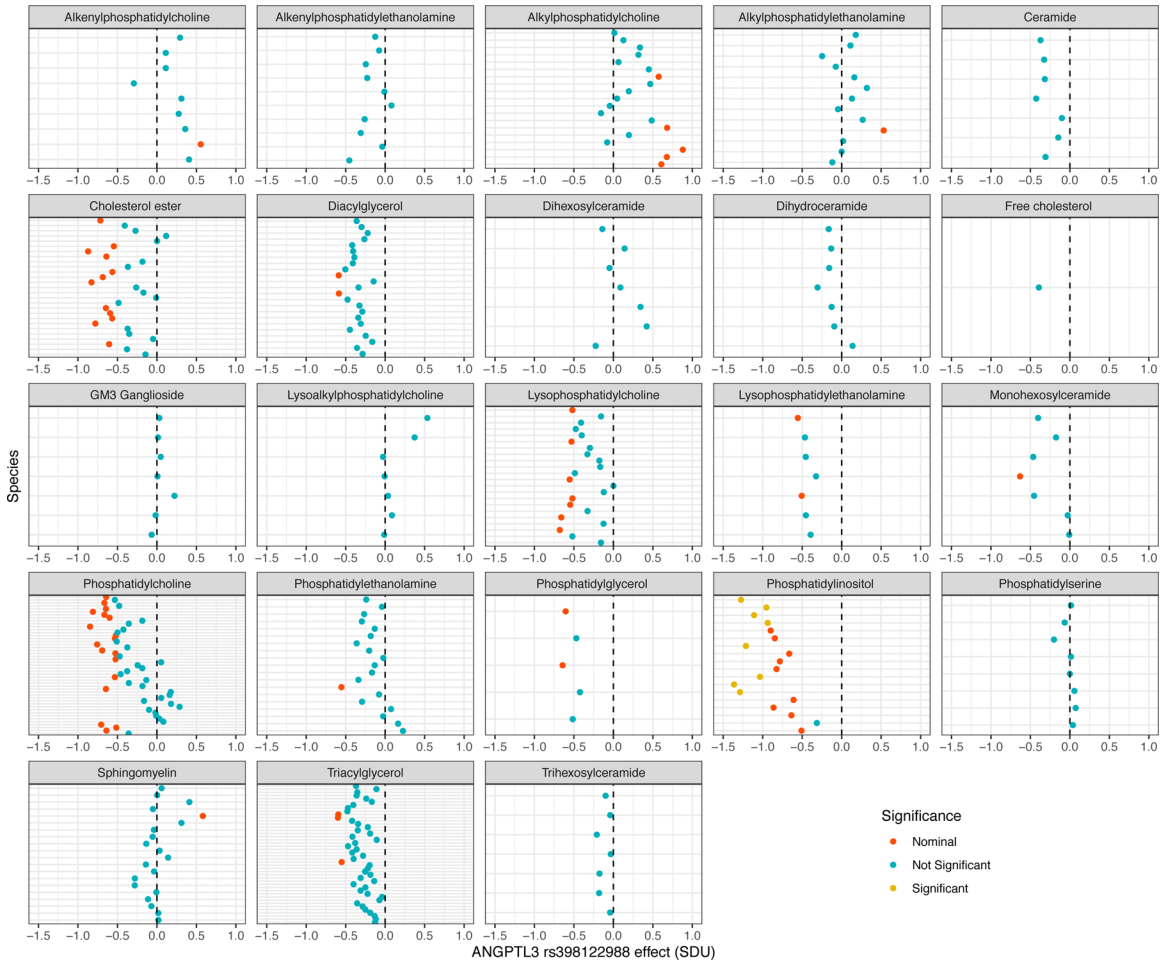

Figure 4.

Plasma lipidome associations with the *ANGPTL3* 5bp deletion variant rs398122988. Significant decreases in phosphatidylinositols are observed with nominally significant increases and decreases in other lipid classes.

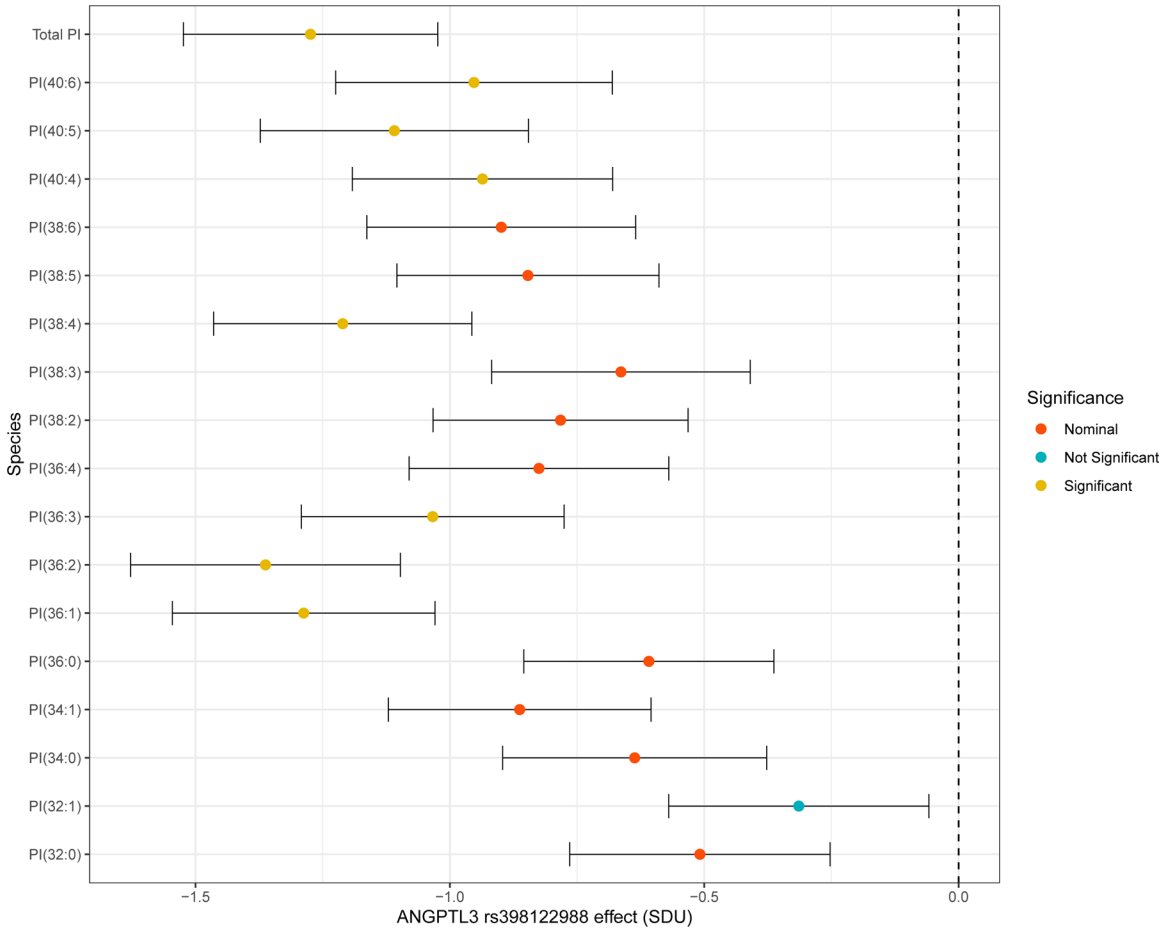

Figure 5.

Specific phosphatidylinositol lipid associations with the *ANGPTL3* 5bp deletion variant rs398122988. Error bars indicate standard error of beta coefficients.

Table 1.

Significant lipidome associations with rs398122988

Lipid species*	p(SNP)	β (SDU)
PI(36:2)	2.77×10^{-7}	−1.36
Total PI	3.49×10^{-7}	−1.27
PI(36:1)	6.15×10^{-7}	−1.29
PI(38:4)	1.85×10^{-6}	−1.21
PI(40:5)	2.60×10^{-5}	−1.11
PI(36:3)	6.20×10^{-5}	−1.03
PI(40:4)	2.55×10^{-4}	−0.94
PI(40:6)	4.58×10^{-4}	−0.95

* PI = phosphatidylinositol

Table 2.

Phenotypic associations with ANGPTL3 protein levels

Phenotype	Individuals	ANGPTL3 P	ANGPTL3 β (SDU)
ApoE (mg/dL)	992	3.00×10^{-6}	0.14
ApoA-II (mg/dL)	994	0.001	−0.09
Cholesterol Efflux (with cAMP)	1001	0.001	0.10
Total Cholesterol Efflux	1001	0.003	0.09
MACE Death	1030	0.004	0.51
Waist Circumference (mm)	1028	0.009	0.17
Smoking Status	1018	0.015	0.16
MACE Event (including death)	1030	0.034	0.18

Table 3.

rs398122988 variant associations with SAFHS phenotypes

Phenotype	Individuals	P	β (SDU)	MAC
VLDL-C (calculated)	1026	0.002	−0.78	23
Total Triglycerides	1191	0.003	−0.77	23
ApoA-I (mg/dL)	1156	0.004	−0.85	18
Total Serum Cholesterol (mg/dL)	1191	0.005	−0.71	23
Cholesterol Efflux (no cAMP)	1023	0.014	−0.66	23
Total HDL-C Levels	1190	0.023	−0.60	23
2 hour OGTT Glucose Levels (mg/dL)	1151	0.043	−0.30	23