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The Association Between *LRP-1* Variants and Chylomicron Uptake After a High Fat Meal

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Abstract

Background and aims—In vitro studies suggest that low density lipoprotein receptor-related protein 1 (LRP1) plays a role in the secondary uptake of chylomicrons. In addition, in vivo studies using *LRP-1* knockout mice show these animals exhibit delayed chylomicron clearance. Whether this is true in humans is unknown. We aimed to determine whether genetic variants in *LRP-1* are associated with postprandial chylomicron uptake in humans given an oral fat challenge.

Methods and Results—817 Men and women (mean age $\pm$ standard deviation = 48.4 $\pm$ 16.4 years) forming the study population for the Genetics of Lipid Lowering Drugs Network (GOLDN) study ingested an oral fat load of 700 kilocalories per m² of body surface area at 83% fat, after an 8-hour fast. Chylomicrons were measured by nuclear resonance spectroscopy (NMR) at fasting, and 3.5 and 6 hours after the meal. 26 Single nucleotide polymorphisms (SNPs) in the *LRP-1* gene were genotyped on the Affymetrix 6.0 array. Chylomicrons were, as expected, zero at fasting. Mixed linear models adjusted for age, sex, study site and pedigree tested for associations

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between *LRP-1* SNPs and changes in chylomicron concentrations 3.5–6 hours. A gene-based test across all 26 SNPs was conducted which corrected for the linkage disequilibrium (LD) between SNPs. 11 *LRP-1* SNPs were significantly associated with the change in chylomicron concentration correction for multiple testing ($Q < .05$). The subsequent gene-based test, was also significant ($P = .01$).

Conclusion—These results require replication but strongly indicate the role of *LRP1* in postprandial lipoprotein uptake and/or clearance.

Keywords

candidate gene; LRP-1; postprandial; uptake; clearance; GOLDN

INTRODUCTION

Evidence suggests that patients find the use of genetic information alongside dietary strategies in the treatment of cardiovascular risk factor such as obesity more understandable and more useful than general dietary advice alone¹. But, as yet, variants that associate with individual responses to specific dietary factors in humans have yet to be conclusively identified. In vitro studies suggest that the low density lipoprotein receptor-related protein 1 (LRP1) plays a role in the secondary uptake of chylomicrons, in addition to the low density lipoprotein (LDL) receptor^{2–4}. Thus, in vitro *LRP-1* elimination is associated with lipid depleted cells⁵, and in vivo animal studies have associated *LRP-1* knockout mice with Changes in fat mass^{5–8}. thought to occur, in part, through the reduced catabolism and clearance of triglyceride-rich lipoproteins (TGRL)⁶. However the role of *LRP-1* variants in human postprandial TGRL uptake remains largely unstudied.

Chylomicrons are secreted by the small intestine in the postprandial state; when they are responsible for the transport of exogenous lipids into the tissue. Once uptake – the removal of triglyceride lipid into the tissue – occurs, the chylomicron is a considered a chylomicron remnant. Given the role of LRP-1 in postprandial lipoprotein metabolism in animal studies, and in vitro supporting evidence, we hypothesized that variants in the *LRP-1* gene will be associated with the uptake of TGRL after a high-fat meal challenge, and so be associated with the ratio of excreted chylomicrons to those remaining after uptake, at 3.5 hours and 6 hours after the meal respectively.

METHODS

Study Population

GOLDN is part of the PROGENI (PROgram for GENetic Interaction) Network, a group of family intervention studies focusing on gene-environment interactions. The participants in the GOLDN study were mainly re-recruited from two NHLBI Family Heart Study (FHS) field centers: Minneapolis, MN, and Salt Lake City, UT. All subjects were of European ancestry. Eligibility criteria were: 1) 18 years of age; 2) fasting TGs < 1500 mg/dL; 3) willing to participate in the study and attend the scheduled clinic exams; 4) member of a family with at least two members in a sibship; 5) AST and ALT results within normal range; and 6) creatinine 2.0 mg/dL. Exclusion criteria were: 1) history of liver, kidney, pancreas, gall bladder disease, or malabsorption; 2) current pregnancy; 3) insulin use; 4) use of lipid lowering drugs (including prescription, OTC and nutraceuticals; volunteers taking these agents were withdrawn from them at least four weeks prior to the study with physician's approval); 5) use of warfarin; 6) women of childbearing potential not using an acceptable form of contraception; 7) known hypersensitivity to fenofibrate; and 8) history of pancreatitis within 12 months prior to enrollment. Previous data on these conditions were

available from the parent study, and individuals not fulfilling inclusion criteria were not invited to participate. A medication questionnaire was administered on the first visit, which confirmed eligibility for inclusion. A previous study demonstrated that Caucasians in UT and MN were homogeneous and pooling data across centers would not threaten the validity of this study.

Protocol—After granting informed consent, participants underwent a baseline screening visit, during which age, demographic and smoking (current/non) and alcohol (g/day) were collected by questionnaire⁹ (Figure 1). At a subsequent visit, a day later, participants were asked to fast (8-hour fast) and before the clinical visit, and then ingest 700 kilocalories per m² of body surface area, composed of 83% calories from fat, 14% from carbohydrates and 3% from protein at the beginning of the visit. Participants had 15 minutes to ingest the meal. Blood was drawn pre-meal (following the overnight fast) and at 3.5 and 6 hours after the meal.

Biochemical measures

Chylomicrons were isolated by nuclear resonance spectroscopy (NMR). The NMR measures were initially validated against chylomicrons isolated by salt density gradient ultracentrifugation¹⁰. All samples were analyzed for lipoprotein profiles once all collections were made in the study.

Smoking and alcohol intake

Trained study personnel collected smoking (current / non) and alcohol (g/day) data using the National Cancer Institute Diet History Questionnaire (NCI DHQ⁹) at visit 0 (Figure 1).

Genotyping

SNPs were genotyped using the Affymetrix Human 6.0 array and the Birdseed calling algorithm and described elsewhere¹¹. We used MACH software (Version 1.0.16) to impute untyped SNPs using Human Genome Build 36, CEU population, as the reference. All *LRP-1* SNPs passed our quality control measures including: exclusion of monomorphic SNPs or those with a call rate <96%; Mendelian errors as follows: for minor allele frequency (MAF)

20%, removed if errors were present in >3 families; for 20%>MAF 10%, removed if errors were present in >2 families; for 10%>MAF 5%, removed if errors were present in >1 family; for MAF<5%, removed if any errors were present. In families with remaining errors, no *LRP-1* SNPs exhibited Mendelian errors. All *LRP-1* SNPs showed Hardy-Weinberg equilibrium (HWE) $P<10^{-6}$.

From an original sample of 1,328 participants, data from the Affymetrix 6.0 array were only available for those who agreed to undergo both the high-fat meal protocol and a second part of the GOLDN study: a 3-week fenofibrate trial (n=817). Note that analyses here draw on data collected from the high fat meal challenge but before fenofibrate treatment.

Statistical Methods

Uptake was defined as the ratio of chylomicron concentration at 3.5 hours to the concentration at 6 hours. A ratio > 1 indicates a higher value at 6 hours postprandial than 3.5 hours i.e. poor or delayed uptake, for ratios < 1 the reverse is indicated. To approximate a normal distribution, values ± 3 SD from the mean were excluded and the remaining values log-transformed, resulting in a final sample size of 744. The associations of chylomicron uptake with smoking and alcohol were assessed using linear mixed models, adjusted for sex, age, pedigree and study center as fixed effects. Similar models were used for the SNP-phenotype associations, but pedigree was adjusted for using a kinship matrix (kinship

package, R software¹²). We assumed an additive genetic model for genotyped SNPs and imputed SNPs were continuous variables. LD were assessed using Haploview¹³ and LocusZoom¹⁴ (Supplementary Figures 1 and 2). To correct for multiple testing, False Discovery Rate corrected Q values are presented alongside P-values¹⁵. A gene-based test of association was conducted using Versatile Gene Based Association Study (VEGAS) which calculates a combined p-value for all SNPs within a gene corrected for the LD structure¹⁶.

RESULTS

The GOLDN study population characteristics are presented in Table 1. Neither smoking ($P=.55$) nor alcohol ($P=.67$) were associated with chylomicron uptake. SNP associations with chylomicron uptake within *LRP-1* are presented in Table 2. Out of twenty-six tested SNPs, eleven showed significant associations with chylomicron uptake ($Q<.05$), although many were in significant LD $r^2>.08$ (Supplementary Figures 1 & 2). The gene-based p-value for *LRP-1* was significant ($P=.01$).

DISCUSSION

This is the first study to our knowledge to examine associations between variants in the *LRP-1* gene and postprandial chylomicron uptake in humans. We report 11 significant SNP-phenotype associations but due to their novelty, these results should be treated as preliminary until replicated by independent groups.

The finding that 11 *LRP-1* variants, and *LRP-1* as a whole, are associated with changes in TGRL uptake and clearance is supported by several studies from *LRP-1* knockout mice⁶⁻⁸, and by a previous association between *LRP-1* SNPs and atherosclerosis¹⁷. In our analysis, minor alleles were associated with increased uptake of chylomicrons, which we may expect from animal studies to be associated with changes in fat mass⁶. However, the previously reported association between an *LRP-1* variant and higher BMI in humans was with rs715948, and rs715948 was not associated with chylomicron uptake in our analyses ($P=.32$), nor with SNPs in LD $r>.8$, making the overall effect of chylomicron metabolism on other phenotypes than chylomicron metabolism open to investigation.

In addition to requiring replication, our results are limited in generalizability to individuals of European descent. Our coverage of the *LRP-1* gene was not complete, and many of the SNPs significantly associated with chylomicron uptake were in high LD. Taken together, this makes it difficult to ascertain if our analysis captured the causal variant(s) in chylomicron uptake.

Nonetheless, through the first human post-prandial lipemia study, we provide initial evidence that *LRP-1* may be associated with chylomicron uptake. If replicated, this could eventually enhance our understanding of pathways from genes, to diet responses to atherosclerosis and obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Acronyms

FDR	false discovery rate
GOLDN	Genetics of Lipid Lowering Drugs Network
HWE	Hardy-Weinberg equilibrium
LD	linkage disequilibrium
LDL	Low-density lipoprotein
LRP-1	low density lipoprotein receptor-related protein 1
MAF	minor allele frequency
NCI DHQ	National Cancer Institute Diet History Questionnaire
NMR	nuclear resonance spectroscopy
SNP	single nucleotide polymorphism
TGRL	triglyceride-rich lipoproteins
VEGAS	Versatile Gene Based Association Study

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Figure 1.
Details of the GOLDN study design
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Table 1

Means (standard deviations) for demographic, anthropometric and chylomicron characteristics of the GOLDN study population

Variable	Mean (SD)
Age, y	48.4 (16.4)
Gender, % male	48.3
BMI, kg/m ²	28.3 (5.6)
Chylomicrons, mg/dL	
Fasting	4.80 (4.86)
3.5 hours postprandial	58.00 (50.65)
6 hours postprandial	49.82 (56.87)
Ratio 3.5–6 hours postprandial	.95 (.67)

Table 2

Associations between LRP-1 SNPs with postprandial chylomicron uptake

SNP	Position	Minor/Major allele	MAF	HWE ¹	Imputation quality score	(SE) ²	P	Q ³
rs10876966 ⁴	55829839	T/C	.23	>.99		.01(.04)	.79	
rs111172113	55813550	C/T	.41		.91	.07(.04)	.08	
rs12368582	55868814	A/G	.02		.43	-.32(.23)	.16	
rs12369345	55845835	T/C	.02		.43	.33(.23)	.16	
rs12814239	55855745	T/C	.09		.46	-.17(.10)	.09	
rs1385526	55819016	C/G	.36		<.99	.04(.04)	.31	
rs1466535	55820737	A/G	.36	.43		.04(.04)	.29	
rs17547610	55816608	A/C	.14	-	.98	.07(.05)	.18	
rs1799986	55821533	T/C	.24	>.99		.07(.05)	.22	
rs1800154	55875926	T/C	.31		.99	-.12(.04)	.003	.01
rs1800159	55880161	A/G	.34		.79	-.12(.04)	.01	.02
rs1800168	55878824	C/T	.31		.98	-.12(.04)	.003	.015
rs1800175	55847646	T/G	.31		.99	-.12(.04)	.004	.01
rs1800176	55847718	T/C	.31	.84		-.12(.04)	.003	.01
rs1800181	55864555	T/C	.31		<.99	-.12(.04)	.003	.01
rs4074308	55866681	T/C	.32	.69		-.12(.04)	.004	.01
rs4759044	55816937	C/T	.43		.89	.03(.04)	.54	
rs4759275	55812023	A/G	.44		.93	.06(.04)	.12	
rs4759277	55819957	A/C	.37		<.99	-.03(.04)	.45	
rs6581128	55869939	A/C	.29	.56	.91	-.12(.04)	.006	.02
rs715948	55819249	T/C	.30			-.05(.05)	.32	
rs7398375	55827115	G/C	.23			-.05(.07)	.50	
rs7956957	55889082	C/G	.35			-.11(.04)	.008	.02
rs7968719 ⁴	55827018	C/G	.46			-.08(.04)	.052	
rs7975818	55844772	C/G	.31	<.99		-.12(.04)	.005	.02
rs7978567	55882251	T/C	.34			-.12(.04)	.003	.01

¹No HWE value indicates an imputed SNP²Transformed phenotype mean=-.29, SD=.71

³FDR corrected P

⁴SNP not in LD >.8 with other SNPs tested