Abstract Studies have shown a large interindividual variability in plasma TG response to long-chain n-3 PUFA supplementation, which may likely be attributable to genetic variability within the populations studied. The objective is to compare the frequency of SNPs in a genome-wide association study between responders (reduction in plasma TG levels >0.01 mM) and nonresponders (increase in plasma TG of ≥0 mM) to supplementation. Genomic DNA from 141 subjects who completed a 2-week run-in period followed by 6-week supplementation with 5 g of fish oil daily (1.9–2.2 g EPA and 1.1 g DHA daily) were genotyped on Illumina HumanOmni-5-QuadBeadChip. Thirteen loci had frequency differences between responders and nonresponders (P < 1 × 10−5), including SNPs in or near IQCSCHIPI, MYB, NELL1, NXP1, PHF17, and SLIT2 genes. A genetic risk score (GRS) was constructed by summing the number of risk alleles. This GRS explained 21.53% of the variation in TG response to n-3 PUFA supplementation when adjusted for age, sex, and BMI (P = 0.0002). Using Fish Oil Intervention and Genotype as a replication cohort, the GRS was able to explain 2% of variation in TG response when adjusted. In conclusion, subjects who decrease their plasma TG levels following n-3 PUFA supplementation may have a different genetic profile than individuals who do not respond. —Rudkowska, I., F. Guénard, P. Julien, P. Couture, S. Lemieux, O. Barbier, P. C. Calder, A. M. Minihane, and M-C. Vohl. Genome-wide association study of the plasma triglyceride response to an n-3 polyunsaturated fatty acid supplementation. J. Lipid Res. 2014. 55: 1245–1253.

Numerous meta-analyses have demonstrated that the long-chain n-3 PUFAs (LC n-3 PUFAs; EPA and DHA) significantly reduce plasma TG levels in a dose-dependent manner, with the TG lowering being proportional to baseline levels (1, 2). In trials of subjects with high TG levels, n-3 PUFAs in dosages of 3.4–4.0 g/day decreased TG levels by 16% to 45% (1). However, there is well-recognized heterogeneity in the plasma TG response to LC n-3 PUFA supplementation (3); for example, 31% of all volunteers from the Fish Oil Intervention and Genotype (FINGEN) Study showed no reduction in TG after 1.8 g EPA and DHA per day for 8 weeks (4). Similarly, our research group has reported that 29% of all subjects showed no reduction

Abbreviations: CI, confidence interval; CRP, C-reactive protein; FINGEN, Fish Oil Intervention and Genotype; GRS, genetic risk score; GWAS, genome-wide association study; HDL-C, HDL cholesterol; IPA, Ingenuity Pathway Analysis; IQCSCHIPI, IQ motif containing J-schwannomin interacting protein 1 fusion protein; LC n-3 PUFA, long-chain n-3 PUFA; LD, linkage disequilibrium; MYB, V-myb myeloblastosis viral oncogene homolog; NELL1, NEL-like 1 (chicken); NXP1, neuroexophilin-1; PHF17, PHD finger protein 17; PL, plasma phospholipid; SLIT2, slit homolog 2; TC, total cholesterol; Vohl, Marie-Claude.

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This work was supported by an operating grant from Canadian Institutes of Health Research (CIHR) (MOP229488). F. Guénard received a research fellowship from the Heart and Stroke Foundation of Canada. O. Barbier received a scholarship from the CHIR (New Investigator Award, MSHP5330). P. Couture received a scholarship from the Fonds de recherche du Québec - Santé (FRQS). M-C. Vohl holds a Tier 1 Canada Research Chair in Genomics Applied to Nutrition and Health. The Fish Oil Intervention and Genotype Study was supported by the Biotechnology and Biological Sciences Research Council. The authors do not declare any conflicts of interest.

Manuscript received 20 November 2013 and in revised form 16 April 2014.

Published, JLR Papers in Press, May 19, 2014
DOI 10.1194/jlr.M045898

Supplementary Material can be found at: http://www.jlr.org/content/suppl/2014/05/19/jlr.M045898.DC1

http://www.jlr.org/content/suppl/2014/05/19/jlr.M045898.DC1.html

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The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one data file.
in plasma TG after a 6-week supplementation with 5 g of fish oil daily (1.9–2.2 g EPA and 1.1 g DHA daily) (5, 6).

This large heterogeneity in the TG response is likely to be partly attributable to genetic variability within the study populations. Studies have examined whether the APOL1 genotype contributes to the lipid response to n-3 PUFAs; these studies have yielded inconsistent results (4, 7–9). Further, research has demonstrated that the effect of the PPARα L162V polymorphism (rs1800206) on plasma TG and apoC-III concentrations depends on dietary PUFAs, with a high intake triggering lower TG in carriers of the V162 allele (10). Likewise, Lindi et al. (11), investigated the influence of the Pro12Ala (rs1801282) polymorphism of the PPARγ gene: carriers of the Ala12 allele presented a greater decrease in plasma TG concentrations in response to LC n-3 PUFA supplementation than did homozygotes for Pro12 when the total dietary fat or saturated fat intake was low (11). Recently, studies have also supported the notion that the fatty acid desaturation 1 and 2 (FADS1 and 2) gene cluster is a major determinant of plasma TG levels (5). Thus, these genetic variations may influence the response to LC n-3 PUFA supplementation; however, other unknown genetic variations may also exist.

Genome-wide association studies (GWASs) provide a more comprehensive approach unconstrained by existing knowledge to test common genetic variants across the genome. Therefore, a GWAS approach is ideal to identify SNPs that have not been discovered previously. The objective of this study was to compare the frequency of alleles in individuals with and without a decrease in plasma TG levels after LC n-3 PUFA supplementation, in order to determine whether an association exists between the responsiveness to LC n-3 PUFA supplementation and specific SNPs. In addition, a genetic risk model was developed with the specific SNPs identified to be able to predict the response to LC n-3 PUFA supplementation. To validate the results, we genotyped the DNA of participants in the FINGEN Study (3, 4) to demonstrate whether the genetic risk score (GRS) may explain, at least partly, the variability of the TG-lowering effect of LC n-3 PUFA supplementation.

SUBJECTS AND METHODS

Study population

A total of 254 subjects from the greater Quebec City metropolitan area were recruited to participate in the study. In total, 210 subjects completed the intervention protocol. Further, subjects who completed the study were separated into subgroups: responders and nonresponders. Responders are defined as having a reduction in plasma TG (change in plasma TG levels ≥0.01 mM; TG postsupplementation with n-3 PUFAs minus TG presupplementation with n-3 PUFAs), and nonresponders are defined by no reduction in plasma TG concentrations after the LC n-3 PUFA supplementation (change in plasma TG of ≥0.01 mM; TG postsupplementation with n-3 PUFAs minus TG presupplementation with n-3 PUFAs). From this classification, a total of 141 subjects with the most extreme response to LC n-3 PUFA supplementation were selected including 81 responders and 60 nonresponders.

Participants had a BMI between 25 and 40 kg/m² and were not currently taking any lipid-lowering medications. Subjects were excluded from the study if they had taken LC n-3 PUFA supplements for at least 6 months prior, used oral hypolipidemic therapy, or had been diagnosed with diabetes, hypertension, hypothyroidism, or other known metabolic disorders such as severe dyslipidemia or coronary heart disease. The experimental protocol was approved by the ethics committees of Centre Hospitalier Universitaire de Quebec Research Center and Laval University. This trial was registered at ClinicalTrials.gov as NCT01343342.

Study design and diets

First, subjects followed a run-in period of 2 weeks. Individual dietary instructions were given by a trained dietician to achieve the recommendation from Canada’s Food Guide (http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php). Subjects were asked to follow these dietary recommendations and maintain a stable body weight throughout the study period. Some specifications were given regarding LC n-3 PUFA dietary intake: not to exceed two fish or seafood servings per week (maximum 150 g), preferably white flesh fish instead of fatty fish (examples were given), and to avoid enriched LC n-3 PUFA dietary products such as some milks, juices, breads, and eggs. Subjects were also asked to limit their alcohol consumption during the protocol: two regular drinks per week (≤28 g of alcohol per week) were allowed. In addition, subjects were not allowed to take n-3 PUFA supplements (such as flaxseed or fish oils), vitamins, or natural health products during the study period.

Second, after the run-in period, each participant received a bottle containing the fish oil capsules to be consumed in the following 6 weeks. They were asked to take five (each 1 g of fish oil concentrate) capsules per day (Ocean Nutrition, Nova Scotia, Canada), providing a total of 3 g of LC n-3 PUFAs (including 1.9–2.2 g EPA and 1.1 g DHA) per day. To aid digestion, we recommended taking the fish oil capsules while eating. Compliance was assessed from the counting of returned capsules. Subjects were asked to report any deviation from the protocol, to write down their alcohol and fish consumption, and to record any side effects. Before each phase, subjects received detailed written and oral instructions on their diet.

Laboratory methods

Plasma lipids. Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after a 12 h overnight fast and 48 h alcohol abstinence. Blood samples were taken to identify and exclude individuals with any metabolic disorders. Afterward, selected participants had blood samples taken prior to and after the LC n-3 PUFA supplementation period. Plasma was separated by centrifugation (2,500 g for 10 min at 4°C), and samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (TC) and TG concentrations were measured using enzymatic assays (12). The HDL cholesterol (HDL-C) fraction was obtained after precipitation of VLDL and LDL particles in the infranatant with heparin manganese chloride (13). HDL cholesterol was calculated with the Friedewald formula (14). Fasting insulin concentrations were measured by radioimmunoassay with polyethylene glycol separation (15). Fasting glucose concentrations were enzymatically measured (16). ApoB100 concentrations were measured in plasma by the rocket immunoelectrophoretic method of Laurell (17), as previously described. Plasma C-reactive protein (CRP) concentration was measured by nephelometry (Prospec equipment, Behring) using a sensitive assay, as described previously (18).

FA composition of plasma phospholipids. Plasma lipids were extracted with chloroform-methanol (2:1, by volume) according
to a modified Folch method (19). Total plasma phospholipids (PLs) were separated by thin layer chromatography using a combination of isopropyl ether and acetic acid, and FAs of isolated PLs were then methylated. Capillary gas chromatography was then used to obtain FA profiles. The technique used for plasma analyses has been previously validated (20). Values of FA concentrations are expressed as percent of total FA in plasma PL.

DNA extraction and genotyping. The GenElute Gel Extraction Kit (Sigma-Aldrich Co., St. Louis, MO) was used to extract genomic DNA (gDNA) from whole blood. After spectrophotometric quantification and verification of DNA quality via an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), samples (n = 141) were sent for genotyping. This was performed at the McGill University and Genome Quebec Innovation Centre (Montreal, Canada) using Illumina HumanOmni-5-Quad BeadChip (Illumina, San Diego, CA), according to the manufacturer’s instructions. Each HumanOmni-5-Quad BeadChip contained 4,301,331 markers.

Sample quality was assessed by signal intensity using R and overall call rate using PLINK (version 1.07; http://pngu.mgh.harvard.edu/purcell/plink/). From the 141 samples analyzed, no subjects were excluded due to low signal intensity exhibited across all chromosomes. Furthermore, no samples were excluded due to overall call rate lower than 90% (default value in PLINK), with subjects having mean call rates of 99.75%. Therefore, all 141 subjects met eligibility criteria for further analysis.

SNP analysis. Calculations of allele frequencies and tests of SNP data for Hardy-Weinberg equilibrium (HWE) were performed using PLINK software. The quality control inclusion criteria for the SNPs were the following: minor allele frequency >1%, SNP call rate >95%, and HWE P > 1.87 × 10⁻⁸. A total of 1,632,526 SNPs were excluded, leaving 2,668,805 SNPs for statistical analyses.

For each of these SNPs, we investigated whether the allele frequency was significantly different between nonresponders and responders. The odds ratio reports the ratio between two proportions, which in the context of GWAS is the proportion of individuals in the nonresponders group having a specific allele and the proportion of individuals in the responders group having the same allele. Additionally, the P value for significance of the odds ratio was calculated using a Chi-square test. After quality control and exclusion of SNPs, the calculated threshold for statistical significance was P < 1.87 × 10⁻⁸ (as calculated with 0.05/2,668,805 SNPs). One critical, but often overlooked, assumption is that all such tests are independent. However, SNPs in close proximity are not independent, and therefore the traditional method to adjust significance thresholds for multiple testing over-correction when used in GWASs (21). Thus, a statistically suggestive P value was defined as P ≤ 10⁻⁵ (22) and was used in order to avoid discounting true positive associations. ANOVA was used to test for a dose-response crossover study consisting of three intervention arms each of 8 weeks duration and separated by 12 week washout intervals. During the intervention periods, participants consumed either A) the control, B) 0.7 g EPA+DHA/day, or C) 1.8 g EPA+DHA/day in random order. The study subjects and recruitment are described in full detail elsewhere (3, 4). In the context of the current study, we used the TG results from 310 subjects who completed the 1.8 g EPA+DHA/day intervention phase to classify individuals as responders and nonresponders. In the same matter as the original study, responders are defined as demonstrating a reduction in plasma TG (change in plasma TG levels ≥0.01 mM; TG postsupplementation with n-3 PUFAs minus TG presupplementation with n-3 PUFAs), and nonresponders are defined by no reduction in plasma TG concentrations after the LC n-3 PUFAs supplementation (change in plasma TG of ≥ 0.01 mM; TG postsupplementation with n-3 PUFAs minus TG presupplementation with n-3 PUFAs).

Genotyping in the replication study. The 10 SNPs revealed by the GWAS were run on the QuantStudio™12K Flex Real-Time PCR System (Life Technologies, Carlsbad, CA). A total of 310 DNA samples were normalized to 20 ng/μL. Two microliters of TaqMan OpenArray Genotyper Master Mix (Life Technologies) and 2 μl of each gDNA sample were premixed in a 384-well plate and loaded onto the genotyping plates using the QuantStudio™ 12K Flex OpenArray® AccuFill™ System. The results were analyzed in TaqMan Genotyper v1.3 (Life Technologies).

Statistical analyses. Results are presented as mean ± SD. Variables not normally distributed were transformed by natural logarithm to normalize their distribution. Data were analyzed using a paired t-test to determine significant changes between pre- and post-supplementation periods for each of the subgroups: responders and nonresponders. An unpaired t-test was used to establish differences between predata between the subgroups.

A genetic risk model was derived for each subject from the sum of risk alleles. GRS was computed with SNPs that are uncorrelated [linkage disequilibrium (LD) < 80%] and in turn contributed to the effect of LC n-3 PUFAs on plasma TG concentration in an additive way. Based on this genetic risk model, subjects were dichotomized with the median GRS. Sensitivity and specificity of the genetic risk model to classify individuals into responder and nonresponder categories were calculated. The general linear model was used to examine the effect of the GRS on TG response to LC n-3 PUFAs taking into account the confounding effects of age, sex, and BMI. Statistical analyses were performed with SAS statistical software, version 9.2 (SAS Institute Inc, Cary, NC) and statistical significance defined as P ≤ 0.05.

RESULTS

Subjects’ characteristics

Subjects with extreme responses were used to determine whether SNPs were of different frequency between the 81 responders [including 38 men (47%) and 43 women (53%)] and 60 nonresponders [including 30 men (50%) and 30 women (50%)]. Table 1 describes the characteristics of the responders and nonresponders presupplementation. The average population age was 31 years, and the subjects were slightly overweight with a mean BMI of 28.4 kg/m². The characteristics of responder and nonresponder subgroups are comparable to those previously

GWAS between responders and nonresponders
reported (6). The presupplementation characteristics of nonresponders were similar to those of responders, except that higher plasma TG (P < 0.0001), glucose (P = 0.0144), insulin (P = 0.0199), and TC/HDL-C ratio (P = 0.0018) together with lower HDL-C levels (P = 0.0142) were observed in responders. Table 2 demonstrates the response of each group to LC n-3 PUFA supplementation. Clearly, the plasma TG decreased in responders (P < 0.0001) and increased in nonresponders (P < 0.0001). Further, responders showed decreased plasma TC (P = 0.0294) and increased plasma HDL-C levels (P < 0.0001), as well as decreased TC/HDL-C ratio (P < 0.0001) after LC n-3 PUFA supplementation. In contrast, nonresponders showed increased TC/HDL-C ratio (P = 0.0054) and apoB levels (P = 0.0015). Fasting glucose levels tended to increase in both the responders (P = 0.0629) and nonresponders (P = 0.0576). Plasma insulin levels decreased in responders (P = 0.0177) and increased in nonresponders (P = 0.0250).

There were no differences in FA composition of plasma PL between the responders and the nonresponders presupplementation with n-3 PUFAs. As expected, the 6 weeks of LC n-3 PUFA supplementation increased the concentration of total n-3 PUFAs and decreased total n-6 PUFAs in PLs in both subgroups (Table 3). In brief, the LC n-3 PUFA supplementation was associated with a similar increase in total n-3 PUFAs in both subgroups, from 5.95% to 10.48% of total FA for responders and from 5.86% to 10.65% of total FA for nonresponders. Similar increases were also observed for percentages of EPA and DHA and the ratio of total n-3/n-6 for the responders and nonresponders.

GWAS
Figure 1 demonstrates the GWAS Manhattan plot showing the association of the TG response to LC n-3 PUFA supplementation. Thirteen loci showed P values less than the suggestive threshold (P < 1 × 10−5) as described in detail in Table 4. Suggestive SNPs included rs2952724/rs2629715 intergenic SNPs upstream of the slit homolog 2 (Drosophila) (SLIT2) gene, rs1216352/rs1216365/rs931681 intergenic upstream of the PHD finger protein 17 (PHF17) gene, rs2621308/rs1449009/rs61332355/rs2621309 intron variants of IQ motif containing J-schwannomin interacting protein 1 fusion protein (IQGJ-SCHIP1) gene, rs6463808 intron variant of neurexinophilin-1 (NXPH1) gene, rs752088 intron variant of NEL-like 1 (chicken) (NELLI1) gene, and rs6920829 intron variant of V-myb myeloblastosis viral oncogene homolog (MYB) gene. Additional analysis showed

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**Table 1.** Presupplementation subject characteristics

| Characteristics | Responders (n = 81) | Nonresponders (n = 60) | P |
|-----------------|---------------------|------------------------|---|
| Sex             | 38 men (47%) and 43 women (53%) | 30 men (50%) and 30 women (50%) |   |
| Age (years)     | 31.9 ± 8.78         | 31.1 ± 9.85            |   |
| BMI (kg/m²)     | 28.9 ± 5.64         | 27.8 ± 3.91            | 0.0612 |
| TGs (mM)        | 1.53 ± 0.74         | 1.03 ± 0.48            | <0.0001 |
| Cholesterol (mM) | Total               | 5.00 ± 0.93            | 4.77 ± 1.01 | 0.2619 |
|                 | LDL                 | 2.94 ± 0.85            | 2.79 ± 0.91 | 0.5598 |
|                 | HDL                 | 1.50 ± 0.37            | 3.33 ± 0.95 | 0.0018 |
|                 | Ratio TC/HDL-C      | 3.91 ± 1.15            | 3.33 ± 0.95 | 0.0018 |
|                 | ApoB (g/l)          | 0.90 ± 0.26            | 0.83 ± 0.26 | 0.1687 |
|                 | Ratio LDL/apoB      | 3.28 ± 0.46            | 3.36 ± 0.43 | 0.3479 |
|                 | Glucose (mM)        | 5.05 ± 0.50            | 4.94 ± 0.39 | 0.0144 |
|                 | Insulin (pM)        | 102 ± 110              | 80.2 ± 36.8 | 0.0199 |
|                 | CRP (mg/l)          | 2.89 ± 3.71            | 2.87 ± 5.20 | 0.3798 |

Data are shown as mean ± SD.

**Table 2.** Characteristics of responders and nonresponders pre- and post supplementation with n-3 PUFAs

| Characteristics | Responders (n = 81) | Nonresponders (n = 60) | P |
|-----------------|---------------------|------------------------|---|
| BMI (kg/m²)     | 28.9 ± 3.64         | 28.9 ± 3.79            | 0.7906 |
| TGs (mM)        | 1.53 ± 0.74         | 1.03 ± 0.56            | <0.0001 |
| Cholesterol (mM) | Total               | 5.00 ± 0.93            | 4.87 ± 1.07 | 0.10 ± 0.53 | 0.1196 |
|                 | LDL                 | 2.94 ± 0.85            | 2.84 ± 0.97 | 0.05 ± 0.45 | 0.4044 |
|                 | HDL                 | 1.53 ± 0.37            | 1.48 ± 0.41 | -0.02 ± 0.17 | 0.3467 |
|                 | Ratio TC/HDL-C      | 3.91 ± 1.15            | 3.33 ± 0.95 | 0.15 ± 0.39 | 0.0054 |
|                 | ApoB (g/l)          | 0.90 ± 0.26            | 0.83 ± 0.26 | 0.05 ± 0.12 | 0.0015 |
|                 | Glucose (mM)        | 5.05 ± 0.50            | 4.94 ± 0.39 | 0.10 ± 0.41 | 0.0576 |
|                 | Insulin (pM)        | 102 ± 110              | 80.2 ± 36.8 | 0.0177 |
|                 | CRP (mg/l)          | 2.89 ± 3.71            | 2.87 ± 5.20 | 0.16 ± 5.45 | 0.5972 |

Data are shown as mean ± SD.

"P value derived from transformed data."
that individually most of the SNPs were associated with the change in TG levels as well as with other metabolic variables when adjusted for age, sex, and BMI (see supplementary data).

A GRS was computed using 10 out of the 13 SNPs showing suggestive associations because 3 SNPs were in LD ≥80% (Table 4). Based on this genetic risk model, subjects were categorized into two genetic risk groups using the median score: lower genetic risk (scores from −1 to 3) and higher genetic risk (scores from 4 to 8) (Fig. 2). The sensitivity and specificity of the GRS to correctly identify nonresponders and responders were calculated as 83.95% [95% confidence interval (CI): 74.12% to 91.16%] and 68.33% (95% CI: 55.04% to 79.74%), respectively (Table 5). In the general linear model, the GRS was significantly associated with TG response when adjusted for age, sex, and BMI (P = 0.0002, respectively). The GRS explained 21.53% of variation in TG during the LC n-3 PUFA supplementation protocol when adjusted for age, sex, and BMI (P = 0.0002).

Pathway analysis results

Using IPA, the top three pathways associated with the suggestive SNPs were PPAR signaling, tight junction signaling pathway, and ceramide signaling (data not shown).

FINGEN replication cohort

Briefly, subjects were identified as 188 responders [including 95 men (51%) and 93 women (49%)] and 122 nonresponders [including 53 men (43%) and 69 women (57%)]. Table 6 describes the presupplementation characteristics of the responders and nonresponders. Clearly, the plasma TG decreased in responders from 1.45 to 1.05 mM (change of −0.40 mM; P < 0.0001) and increased in nonresponders from 1.02 to 1.26 mM (change of 0.24 mM; P < 0.0001) after the daily intake of 1.8 g EPA+DHA.

Risk allele frequency and GRS were determined in the FINGEN population using the 10 newly discovered SNPs (Fig. 3). No differences in allele frequencies were observed between the two populations (data not shown). Further, the subjects were categorized into two genetic risk groups using the median score of the genetic risk model developed: lower genetic risk (scores from −1 to 3) and higher genetic risk (scores from 4 to 9). The sensitivity and specificity of the GRS to correctly identify nonresponders and responders were calculated as 69.68% (95% CI: 62.57% to 76.16%) and 24.59% (95% CI: 17.25% to 33.21%), respectively (Table 7). Although not significant, the GRS explained 2% of variation in TG response to LC n-3 PUFA supplementation in the replication cohort from the regression analyses when adjusted for age, sex, and BMI (P = not significant).

DISCUSSION

This study compares the frequency of alleles of SNPs in individuals with and without a decrease in plasma TG levels following LC n-3 PUFA supplementation. TG levels were significantly different between responders and nonresponders postsupplementation with n-3 PUFAs, representing the interindividual variability in response to LC n-3 PUFA supplementation. However, no significant locus (P < 1.87 × 10−8) was identified by the GWAS, most likely because of the small sample size of the cohort. However, a suggestive association threshold (<10−5) should be used to identify SNPs for consideration in follow-up studies. Results suggested that 13 SNPs had different frequencies be-

TABLE 3. PL FA profiles of responders and nonresponders pre- and postsupplementation with n-3 PUFAs

| (% of FA) | Responders (n = 81) | Nonresponders (n = 60) | P | P between Responders and Nonresponders |
| --- | --- | --- | --- | --- |
| EPA (20:5n-3) | 1.15 ± 0.46 | 3.91 ± 1.21 | <0.0001 | 0.6442 |
| DHA (22:6n-3) | 5.50 ± 0.78 | 5.02 ± 0.83 | <0.0001 | 0.9362 |
| Total n-3 PUFAs | 34.80 ± 1.86 | 30.68 ± 2.56 | <0.0001 | 0.1145 |
| Total n-6 PUFAs | 5.95 ± 1.06 | 10.48 ± 1.83 | <0.0001 | 0.4364 |
| Ratio total n-3/total n-6 | 0.17 ± 0.04 | 0.35 ± 0.09 | <0.0001 | 0.3156 |

Data are shown as mean ± SD; supplementation is the effect of n-3 PUFA supplementation on parameters.
compared with responders. The \textit{NELL1} gene encodes a heterotrimeric protein that is involved in cell growth regulation and differentiation. Specifically, \textit{NELL1} plays a role in the promotion of osteogenic differentiation and bone formation. A recent study has suggested that \textit{NELL1} is also a potent antiadipogenic agent (23). Previously, a GWAS examined the use of hydrochlorothiazide, an antihypertensive medication that is known to be between responders and nonresponders to LC n-3 PUFA supplementation, including SNPs in or near \textit{IQCJ-SCHIP1}, \textit{MYB}, \textit{NELL1}, \textit{NXPH1}, \textit{PHF17}, and \textit{SLIT2}. Further, a GRS based on these identified SNPs together with traditional risk factors can potentially differentiate subjects who may or may not respond to LC n-3 PUFAs for TG lowering.

In the current study, the rs752088 in the intron of the \textit{NELL1} gene was more frequent in nonresponders compared with responders. The \textit{NELL1} gene encodes a heterotrimeric protein that is involved in cell growth regulation and differentiation. Specifically, \textit{NELL1} plays a role in the promotion of osteogenic differentiation and bone formation. A recent study has suggested that \textit{NELL1} is also a potent antiadipogenic agent (23). Previously, a GWAS examined the use of hydrochlorothiazide, an antihypertensive medication that is known to be

\begin{table}
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\begin{tabular}{lcccccc}
\hline
SNP & CHR & Base pairs & Location & Allele & Responders & Nonresponders & \textit{P} between Responders and Nonresponders & Odds Ratio between Responders and Nonresponders \\
\hline
rs2621308$^a$ & 3 & 158,980,014 & Intron variant \textit{IQCJ-SCHIP1} & A & 0.15 & 0.40 & $3.21 \times 10^{-6}$ & 3.65 \\
rs1440909$^a$ & 3 & 158,977,883 & Intron variant \textit{IQCJ-SCHIP1} & G & 0.19 & 0.43 & $5.71 \times 10^{-6}$ & 3.36 \\
rs61392355 & 3 & 158,921,902 & Intron variant \textit{IQCJ-SCHIP1} & A & 0.09 & 0.29 & $6.88 \times 10^{-6}$ & 4.35 \\
rs2621309 & 3 & 158,980,745 & Intron variant \textit{IQCJ-SCHIP1} & G & 0.18 & 0.43 & $7.90 \times 10^{-6}$ & 3.34 \\
rs2952724$^a$ & 4 & 20,024,979 & Intergenic (300 kb upstream of \textit{SLIT2}) & A & 0.48 & 0.18 & $3.80 \times 10^{-7}$ & 0.25 \\
rs2629715$^a$ & 4 & 20,010,995 & Intergenic (300 kb upstream of \textit{SLIT2}) & C & 0.48 & 0.18 & $3.80 \times 10^{-7}$ & 0.25 \\
rs1216352$^a$ & 4 & 129,559,799 & Intergenic (300 kb upstream of \textit{PHF17}) & A & 0.23 & 0.51 & $1.06 \times 10^{-6}$ & 3.49 \\
rs1216365 & 4 & 129,569,808 & Intergenic (500 kb upstream of \textit{PHF17}) & C & 0.27 & 0.53 & $5.67 \times 10^{-6}$ & 3.16 \\
rst31681$^a$ & 4 & 129,578,480 & Intergenic (300 kb upstream of \textit{PHF17}) & G & 0.27 & 0.53 & $7.81 \times 10^{-6}$ & 3.06 \\
rs6920829 & 6 & 135,519,500 & Intron variant \textit{MYB} & G & 0.05 & 0.23 & $9.76 \times 10^{-6}$ & 5.59 \\
rs6463808 & 7 & 8,476,787 & Intron variant \textit{NXPH1} & A & 0.09 & 0.30 & $3.43 \times 10^{-6}$ & 4.53 \\
rst752088 & 11 & 20,781,004 & Intron variant \textit{NELL1} & G & 0.27 & 0.54 & $3.73 \times 10^{-6}$ & 3.23 \\
\hline
\end{tabular}
\caption{Significant differences in frequency of alleles of SNPs in individuals with and without a decrease in TG levels following LC n-3 PUFA supplementation.}
\end{table}

$^a$The same subscript letter within the same gene determines an LD between these SNPs (100%).

Fig. 2. GRS distribution in study population (n = 141). If a GRS is positive, then the subject carries more at-risk alleles from the SNPs identified in the GWAS. If a GRS is negative, then the subject carries more beneficial alleles from the SNPs identified in the GWAS.
TABLE 5. Sensitivity and specificity of GRS in study population

| Characteristics | Responders | Nonresponders | Total |
|-----------------|------------|---------------|-------|
| Low GRS         | 68         | 19            | 87    |
| High GRS        | 13         | 41            | 54    |
| Total           | 81         | 60            |       |
| Sensitivity     | 83.95% (95% CI: 74.12% to 91.16%) |
| Specificity     | 68.33% (95% CI: 55.04% to 79.74%) |

TABLE 6. Presupplementation subject characteristics in FINGEN replication population

| Characteristics | Responders (n = 188) | Nonresponders (n = 122) | P     |
|-----------------|----------------------|-------------------------|-------|
| Sex (years)     | 95 men (51%) and 93 women (49%) | 55 men (43%) and 69 women (57%) |       |
| BMI (kg/m²)     | 46.2 ± 12.3          | 43.9 ± 13.9              | 0.0672 |
| TGs (mM)        | 25.3 ± 3.27          | 25.0 ± 3.55              | 0.0669 |
| Cholesterol (mM)| 1.21 ± 0.57          | 1.15 ± 0.50              | 0.0052 |
| Total           | 5.16 ± 0.96          | 5.15 ± 1.05              |       |
| HDL             | 1.40 ± 0.37          | 1.50 ± 0.37              |       |
| Ratio TC/HDL-C  | 3.96 ± 1.27          | 3.65 ± 1.14              |       |
| Glucose (mM)    | 4.98 ± 0.64          | 4.90 ± 0.67              |       |

associated with hyperglycemia and hypertriglyceridemia. Results indicate that two SNPs (rs12279250 and rs4319515) located at the NELL1 gene locus achieved genome-wide significance for association with change in fasting plasma TG levels in African Americans, whereby each variant allele was associated with a 0.32 mM increase in the change in TG (23). The authors postulated that hydrochlorothiazide could modulate adipocyte differentiation through NELL1, leading to accumulation of plasma TG in susceptible subjects (23). Additionally, a recent study, namely the Genetics of Lipid Lowering Drugs and Diet Network study cohort, investigated which genetic variants may explain the observed heterogeneity in response to lipid-lowering therapy with fenofibrate (24). The results demonstrate that seven proxy genes showed pleiotropic effects on nuclear magnetic resonance spectroscopy lipoprotein measurements including the NELL1 gene on HDL and VLDL size (24). In the same study, SNPs near the IQCJ-SCHIP1 gene were also associated with VLDL particle clearance (24). In the current study, SNPs in the intron of IQCJ-SCHIP1 were more frequent in the nonresponders compared with the responders. It is well known in the literature that hypertriglyceridemia is characterized by elevated TG content in both VLDL particles and chylomicrons. Furthermore, recent studies suggested that the TG-lowering effect of LC n-3 PUFAs is associated with the decreased hepatic VLDL-TG secretion rate (25, 26). Thus, the functional impact of these genetic variations on plasma lipid metabolism and LC n-3 PUFA response should be further investigated.

Results demonstrate that SNPs in two genes, MYB and SLIT2, were of different frequency between responders and nonresponders. These two genes have been previously related to features of obesity. First, rs6920829 in the intron of the MYB gene had higher frequency of G allele in nonresponders than in responders. This gene encodes a transcription factor that plays an essential role in the regulation of hematopoiesis and tumorigenesis. Recently, a study showed that rs9494145 in MYB was associated with both asthma and BMI, although associations with BMI were modest and not significant after correction for multiple comparisons (27). Further, the rs2952724/rs2629715 upstream of the SLIT2 gene, which is known to suppress tumor growth, had a lower frequency in nonresponders compared with responders. Previously, researchers have shown that SLIT2 was overexpressed in abdominal subcutaneous adipocytes from obese compared with nonobese Pima Indians (28). It is recognized that obese subjects frequently have atherogenic dyslipidemia, including elevated TG, VLDL, and apoB, as well as decreased HDL-C levels. The role of these specific genes and their SNPs in obesity and the plasma lipid response to LC n-3 PUFA supplementation still needs to be determined.

Further, two genes identified here have been previously associated with cancer: PHF17 and NXPH1. However, very few studies have been published relating to the functions of the proteins encoded by these genes. Overall, further confirmation of these findings is needed to determine whether the signals from these SNPs are indirectly associated due to LD or are directly related to a functional impact of these genes.

Pathway analysis reveals that these suggestive SNPs are related to the following pathways: PPAR signaling, tight junction signaling, and ceramide production. PPARs act as ligand activated transcriptional regulators. Their ligands include LC n-3 PUFAs, which makes the PPARs closely linked to intracellular lipid levels (29). Tight junctions are the most apical cell-cell junctions of epithelial cells, and evidence indicates that they participate in the suppression of cell proliferation and stimulation of differentiation. Ceramides are a class of sphingolipids that contribute to membrane structure and functions as an intracellular signaling molecule (30). Plasma ceramides have been shown to be elevated in obese subjects with type 2 diabetes and correlated with the severity of insulin resistance (31).
also have an additive or interactive effect with this GRS to modify TG variation after LC n-3 PUFA supplementation. In sum, the addition of a GRS to traditional risk factors could be an essential part of therapeutic stratification for preventive LC n-3 PUFA treatment.

To our knowledge, this is the first GWAS to examine TG response to LC n-3 PUFA supplementation. In the current study, genes of interest were identified that explain inter-individual variability in lipid metabolism. Functional studies are needed to explore these novel biological pathways that may explain variability in the plasma lipid response to LC n-3 PUFA supplementation. Further studies need to confirm this GRS classification scheme together with other known SNPs or risk factors in other demographic and ethnic groups. Overall, this study shows that subjects who decrease their plasma TG levels following LC n-3 PUFA supplementation have a different genetic profile than individuals who do not respond to the LC n-3 PUFA supplementation.

Overall, these pathways are closely linked to the suggestive SNPs relating to lipid and cellular metabolism in disease. Based on 10 SNPs from GWAS results, we propose a GRS that can effectively differentiate subjects with high or low genetic risk who may or may not respond to LC n-3 PUFA supplementation. The replication study suggests that subjects with a low GRS are more likely to be responders to LC n-3 PUFAs. Previous studies have found that a GRS from 32 BMI-associated variants explained 1.5–1.8% of variation in BMI (32, 33). Thus, in the same way, our GRS from 10 SNPs related to LC n-3 PUFA supplementation explained 2% of TG response in the replication study, but this effect did not reach statistical significance. Differences may be attributable to, among other factors, differences in the study design including the length of the supplementation period, the dose of LC n-3 PUFAs, habitual diet and lifestyle of the participants, or inclusion/exclusion criteria. The addition of other genetic variations and risk factors could strengthen the predictive power of this model. Other risk factors may also have an additive or interactive effect with this GRS to modify TG variation after LC n-3 PUFA supplementation. In sum, the addition of a GRS to traditional risk factors could be an essential part of therapeutic stratification for preventive LC n-3 PUFA treatment.

To our knowledge, this is the first GWAS to examine TG response to LC n-3 PUFA supplementation. In the current study, genes of interest were identified that explain inter-individual variability in lipid metabolism. Functional studies are needed to explore these novel biological pathways that may explain variability in the plasma lipid response to LC n-3 PUFA supplementation. Further studies need to confirm this GRS classification scheme together with other known SNPs or risk factors in other demographic and ethnic groups. Overall, this study shows that subjects who decrease their plasma TG levels following LC n-3 PUFA supplementation have a different genetic profile than individuals who do not respond to the LC n-3 PUFA supplementation.

![Fig. 3. GRS distribution in FINGEN replication population (n = 310). If a GRS is positive, then the subject carries more at-risk alleles from the SNPs identified in the GWAS. If a GRS is negative, then the subject carries more beneficial alleles from the SNPs identified in the GWAS.](http://www.jlr.org/content/suppl/2014/05/19/jlr.M045898.DC1.html)

| TABLE 7. Sensitivity and specificity of GRS in FINGEN replication population |
|---------------------------------|-----------------|-----------------|
|                                 | Responders      | Nonresponders   | Total            |
| Low GRS                        | 131             | 92              | 223              |
| High GRS                       | 57              | 30              | 87               |
| Total                          | 188             | 122             |                  |

Sensitivity 69.68% (95% CI: 62.57% to 76.16%)  
Specificity 24.59% (95% CI: 17.23% to 33.21%)
The authors thank the subjects for their excellent collaboration. We thank Catherine Raymond for contributing to the laboratory work. We would also like to thank Line Berthiaume who performed gas chromatographic analyses to determine FA profiles in PLs.

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