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Fine mapping of genome-wide association study signals to identify genetic markers of the plasma triglyceride response to an omega-3 fatty acid supplementation

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ABSTRACT

Background: Using a genome-wide association study (GWAS) approach, our group previously computed a genetic risk score (GRS) from single nucleotide polymorphisms (SNPs) of 10 loci that affect the plasma triglyceride (TG) response to an omega-3 (n-3) fatty acid (FA) supplementation.

Objectives: The objective was to compute a novel and more refined GRS using fine mapping to include a large number of genetic variants.

Methods: A total of 208 participants of the Fatty Acid Sensor (FAS) Study received 5 g fish oil/d, containing 1.9–2.2 g eicosapentaenoic acid and 1.1 g docosahexanoic acid, for 6 wk. Plasma TG concentrations were measured before and after supplementation. Dense genotyping and genotype imputation were used to refine mapping around GWAS hits. A GRS was computed by summing the number of at-risk alleles of tagging SNPs. Analyses were replicated in samples of the FINGEN study.

Results: A total of 31 tagging SNPs associated with the TG response were used for GRS calculation in the FAS study. In a general linear model adjusted for age, sex, and body mass index, the GRS explained 49.73% of TG response variance ($P < 0.0001$). Nonresponders to the n-3 FA supplementation had a higher GRS than did responders. In the FINGEN replication study, the GRS explained 3.67% of TG response variance ($P = 0.0006$).

Conclusions: Fine mapping proved to be effective to refine the previous GRS. Carrying increasing numbers of at-risk alleles of 31 SNPs confers a higher risk of being nonresponsive to n-3 FAs. The genetic profile therefore appears to be an important determinant of the plasma TG response to an n-3 FA supplementation and could be used to target those most likely to gain clinical benefit. This trial was registered at <http://www.clinicaltrials.gov> as NCT01343342. *Am J Clin Nutr* 2019;109:176–185.

Keywords: genetic risk score, plasma lipid levels, omega-3 fatty acids, genome-wide association study, nutrigenetics, gene–diet interactions

Introduction

The hypotriglyceridemic effect of marine omega-3 (n-3) fatty acids (FAs) has been consistently described (1). At a population level, a daily intake of 4 g n-3 FAs in the form of EPA and DHA can lower triglyceride (TG) concentrations by up to 30%

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Supplemental Figure 1 is available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: cAMP, cyclic AMP; FA, fatty acid; FAS study, Fatty Acid Sensor Study; GRS, genetic risk score; GWAS, genome-wide association study; *IQCF-SCHIP1*, IQ motif containing J-Schwannomin interacting protein 1 readthrough; *MYB*, V-MYB avian myeloblastosis viral oncogene homolog; *NELL1*, Neural EGFL like 1; *NXPH1*, Neurexophilin 1; *PHF17*, PHD finger protein 17; *SLIT2*, Slit guidance ligand 2; SNP, single nucleotide polymorphism; TG, triglyceride.

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(1). However, it remains unclear whether n-3 FA consumption actually translates into a decrease in cardiovascular events and mortality risk because discrepancies have been reported in the literature (2–5). A possible explanation for these inconsistent results is that not all individuals benefit equally from n-3 FA consumption. Accordingly, a large interindividual heterogeneity in the plasma TG response to an n-3 FA supplementation has been observed by many research groups. In the FINGEN study, 40% of participants who underwent an 8-wk n-3 FA supplementation did not show any decrease in TG concentrations (6). Likewise, 29% of participants of the Fatty Acid Sensor (FAS) Study, conducted by our research group, did not have decreased TG concentrations after a 6-wk supplementation of 3 g n-3 FAs/d, comprising 1.9–2.2 g EPA and 1.1 g DHA (7).

The etiology of the variability in the hypotriglyceridemic response is likely to be multifactorial, with genetic factors partly accounting for the interindividual variability of the TG response to an n-3 FA supplementation (8). Our group previously conducted a genome-wide association study (GWAS) in participants of the FAS study to identify potential variants associated with the plasma TG response to n-3 FA supplementation and identified 13 loci located in 6 genes: IQ motif containing J-Schwannomin interacting protein 1 readthrough (*IQCJ-SCHIP1*), Neurexophilin 1 (*NXP1*), PHD finger protein 17 (*PHF17*), V-MYB avian myeloblastosis viral oncogene homolog (*MYB*), Neural EGFL like 1 (*NELL1*), and Slit guidance ligand 2 (*SLIT2*) (9). A genetic risk score (GRS) was computed from 10 GWAS hits, and this explained 21.53% of the TG response (9). We recently reported several gene–diet interactions modulating TG concentrations following the n-3 FA supplementation after increasing the density of markers around GWAS hits by dense genotyping (10). These results demonstrated the importance of pursuing mapping refinement around GWAS-associated loci to identify actual causative single nucleotide polymorphisms (SNPs). It is therefore very likely that the current GRS on FAS participants could be improved by using fine mapping to add variants, which would bring more power and accuracy. The objective of the present study was to generate a more refined and improved GRS using fine mapping of GWAS hit regions to add SNPs in order to predict more accurately the individual TG response to an n-3 FA supplementation.

Methods

Study population

A total of 254 healthy Canadian Caucasian subjects living in the Quebec City metropolitan area were recruited into the FAS study from September 2009 to December 2011 using announcements in local newspapers, as well as electronic messages sent to students and employees of Université Laval (Quebec). Inclusion criteria were as follows: aged between 18 and 50 y, a BMI (kg/m^2) between 25 and 40, nonsmokers, and free from thyroid or metabolic disorders requiring a treatment such as diabetes, hypertension, dyslipidemia, or cardiovascular disease. Subjects were not eligible if they had taken n-3 FA supplements for a minimum of 6 mo before the intervention. A total of 210 participants completed the intervention. However, 2 participants did not have plasma TG concentration data available for further analyses and were therefore excluded, yielding a final sample of 208 participants. Subjects were subsequently separated into 2 subgroups: 1) responders to the n-3 FA supplementation and 2) nonresponders. Responders were defined as participants

whose plasma TG concentrations decreased after the n-3 FA supplementation, whereas nonresponders were participants whose TG concentrations remained stable or increased through the n-3 FA supplementation.

Study design and diets

The study design and diets have been previously reported (9, 11). A flow chart of participants and intervention is presented as online supporting material (**Supplemental Figure 1**). First, participants followed a run-in period of 2 wk, where they were given dietary instructions by a trained registered dietitian to achieve the recommendations from *Canada's Food Guide* (12). The purpose of these recommendations was to ensure that participants had a constant n-3 FA dietary intake and maintained a stable body weight throughout the study period. More specifically, they were asked not to consume >150 g fish or seafood/wk, to avoid food enriched with n-3 FAs, to limit alcohol consumption to a maximum of 2 regular drinks/wk, and to avoid any dietary supplement (n-3 FA supplements, vitamins, or natural products) during the intervention. Thereafter, participants were asked to consume the n-3 FA capsules (Ocean Nutrition) daily for 6 wk. The 5 capsules/d provided a total of 3 g n-3 FAs/d, including 1.9–2.2 g EPA and 1.1 g DHA. Participants were asked to report any deviation from the protocol. They were also asked to record any experienced side effects, alcohol intake, and fish consumption.

Laboratory methods

Plasma lipids.

Methods used to measure blood lipids have previously been published (7). Briefly, blood samples were collected after a 12-h overnight fast and 48-h alcohol abstinence at the beginning and end of the intervention. Plasma TG concentrations were assessed by enzymatic assays (13, 14).

Gene mapping.

Two methods were used for fine mapping: dense genotyping and genotype imputation. For genotyping, the whole procedure was previously described (10). Briefly, SNPs were identified using the International HapMap Project SNP database, based on the National Center for Biotechnology Information B36 assembly Data Release 28, phase II + III, build 126. Tagging SNPs were selected using the Tagger procedure in Haploview v4.2 according to their minor allele frequency (>5%) and pairwise tagging ($r^2 \geq 0.80$). A total of 16 SNPs in *IQCJ*, 34 in *NXP1*, 8 in *PHF17*, and 9 in *MYB* were chosen to cover all common variations at these chromosomal regions. To the 16 SNPs in *IQCJ*, 23 genotyped SNPs in *SCHIP1* were added in order to cover the full *IQCJ-SCHIP1* gene. All SNPs were in Hardy–Weinberg equilibrium. The GenElute Gel Extraction Kit (Sigma-Aldrich Co.) was used to extract genomic DNA from the blood samples. Genotyping was conducted by polymerase chain reaction using TaqMan technology (Life Technologies) in 210 subjects.

For genotype imputation, the 1000 Genomes project data (release 1000G Phase I v3, updated 26 August 2012) was used as a reference set for the imputation of genotypes (genotyped from Illumina BeadChip) of previously identified GWAS loci (9). A

total of 1684 markers in *IQCJ-SCHIP1*, 1684 in *NXP1*, 885 in *PHF17*, and 777 in *MYB*, which were originally used to conduct the GWAS in 141 participants, were used to infer other genotypes using algorithms implemented in IMPUTE2 (15). The imputation cutoff was 0.90, with a 99.0% success rate. A total of 52,770 informative SNPs in *IQCJ-SCHIP1*, 50,218 in *NXP1*, 30,140 in *PHF17*, 29,725 in *MYB*, 61,560 in *NELL1*, and 61,736 in *SLIT2* were obtained from genotype imputation, including initial markers.

Imputed SNPs were then submitted to quality-control tests, where only polymorphic SNPs with a genotype call rate >95% and minor allele frequency $\geq 1\%$ were conserved. Quality-control tests left 5205 SNPs in *IQCJ-SCHIP1*, 6040 in *NXP1*, 3028 in *PHF17*, 2616 in *MYB*, 7846 in *NELL1*, and 7124 in *SLIT2* available for SNP analysis.

SNP analysis and selection.

Allele frequency between responders and nonresponders was calculated and compared using PLINK (16). The OR reporting the ratio between the proportion of nonresponders carrying the minor allele of an SNP and the proportion of responders carrying the minor allele of the same SNP was calculated. OR *P* values were calculated using a chi-square test.

Before statistical analyses, tagging SNPs were selected using PLINK from genotyping and imputation data. For inclusion criteria, r^2 was set at ≥ 0.80 , and the *P* value was 0.05. Final samples of 88 independent tagging SNPs in *IQCJ-SCHIP1*, 88 in *NXP1*, 56 in *PHF17*, 97 in *MYB*, 58 in *NELL1*, and 118 in *SLIT2* were kept for statistical analyses.

Replication study.

Analyses were replicated in the European FINGEN study, a trial conducted at the Universities of Glasgow, Newcastle, Reading, and Southampton in the United Kingdom from 2003 to 2005. Over 95% of participants were British Caucasians. The study design has been previously published (6, 17). Briefly, it was a double-blind, placebo-controlled, dose-response crossover study in which participants received a placebo, 0.7 g EPA and DHA/d, or 1.8 g EPA and DHA/d for 8 wk with 12-wk washouts in between. Responsiveness to n-3 FA supplementation was defined in the same way as outlined above for the FAS participants, with only the response to the 1.8-g/d dose used in the current analysis. TG data were available for 310 of the 312 subjects. Genotyping was conducted by LCG genomics (18).

Ethics

The FAS study was approved by the Université Laval and CHU de Québec ethics committees and was performed in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Statistical analyses

Statistical analyses were conducted using SAS software version 9.4. Normal distribution was evaluated with the box-plot, skewness, and kurtosis ranges. Abnormally distributed variables were $\log_{10}$ -transformed. Statistical significance was set at $P < 0.05$. An unpaired *t* test was used to assess differences between responders and nonresponders before the supplementation.

A GRS was calculated for each participant from the sum of risk alleles of tagging SNPs in an additive way. To select the most relevant SNPs to include in the GRS calculation, stepwise bidirectional regressions adjusted for age, sex, and BMI were conducted in each gene separately (REG procedure in SAS) to assess the contribution of SNPs to the TG variation (Δ TG). Significant SNPs ($P < 0.05$) were kept for the GRS calculation.

A *t* test was performed to compare mean GRSs between responders and nonresponders to the n-3 FA supplementation. A general linear model (GLM procedure in SAS) adjusted for age, sex, and BMI was used to assess the effect of the GRS on the plasma TG response to the n-3 FA supplementation. The sensitivity and specificity of the GRS were calculated by measuring the area under the receiver operating characteristic curve using the logistic procedure in SAS with adjustments for age, sex, BMI, and baseline TG concentrations.

Results

Characteristics of participants and FA profiles have been previously reported (9). Table 1 presents a summary of baseline and postsupplementation characteristics of responders and nonresponders to the n-3 FA supplementation. Participants were overweight, with mean $\pm$ SD baseline BMIs of 28.9 ± 3.6 and 27.8 ± 3.9 in the responder and nonresponder groups, respectively. Responders had higher TG concentrations at baseline than nonresponders ($P < 0.0001$), and their TG concentrations decreased significantly by, on average, 0.50 mmol/L through supplementation ($P < 0.0001$), whereas nonresponders had a mean 0.17-mmol/L increase ($P < 0.0001$).

TABLE 1 Characteristics of subjects pre- and postsupplementation¹

Characteristics	Responders (<i>n</i> = 81)			Nonresponders (<i>n</i> = 60)		
	Presupplementation	Postsupplementation	<i>P</i>	Presupplementation	Postsupplementation	<i>P</i>
Sex (men/women), <i>n/n</i>	38/43	—		30/30	—	
Age, y	31.9 $\pm$ 8.8	—		31.1 $\pm$ 9.0	—	
BMI, kg/m ²	28.9 $\pm$ 3.6	28.9 $\pm$ 3.7	0.7	27.8 $\pm$ 3.9	28.0 $\pm$ 4.0	0.0007
Triglycerides, mmol/L	1.53 $\pm$ 0.74	1.03 $\pm$ 0.56	<0.0001	1.03 $\pm$ 0.48	1.20 $\pm$ 0.54	<0.0001

¹Values are means $\pm$ SDs; *n* = 141. The TTEST procedure (SAS version 9.4) was used to assess differences between pre- and postsupplementation in responders and nonresponders.

FIGURE 1 Manhattan plot showing P values for differences in allele frequency between responders and nonresponders to n-3 fatty acid supplementation in each gene region identified by the initial genome-wide association study (9). Single nucleotide polymorphisms obtained from genotype imputation are shown in red in the panels below. Differences in allele frequency were assessed using a chi-square test in PLINK. Genes: *IQCJ-SCHIP1*, IQ motif containing J-Schwannomin interacting protein 1 readthrough; *MYB*, V-MYB avian myeloblastosis viral oncogene homolog; *NELL1*, Neural EGFL like 1; *NXPH1*, Neurexophilin 1; *PHF17*, PHD finger protein 17; and *SLIT2*, Slit guidance ligand 2.

Figure 1 shows the P values for differences in allele frequency between responders and nonresponders in GWAS-associated genes after mapping refinement by genotype imputation (A: *IQCJ-SCHIP1*, B: *SLIT2*, C: *PHF17*, D: *MYB*, E: *NXPH1*, F:

NELL1). A total of 62 markers passed the significance threshold used in the GWAS ($P = 10^{-5}$): 12 in *IQCJ-SCHIP1*, 1 in *NXPH1*, 22 in *PHF17*, 6 in *MYB*, 1 in *NELL1*, and 20 in *SLIT2*.

TABLE 2 Characteristics of the 31 SNPs used to compute the genetic risk score¹

Gene and SNP (rs number)	Position, base pairs	Location	Alleles ²	Minor allele frequency		<i>P</i> ³	OR (95% CI) ⁴
				Responders	Nonresponders		
<i>IQCF-SCHIP1</i>							
rs7639707	159,148,087	Intron	<u>A</u> /G	0.019	0.075	0.020	4.297 (1.138, 16.231)
rs62270407	159,597,626	Intron	C/ <u>T</u>	0.352	0.192	0.003	0.437 (0.25, 0.763)
<i>NXPH1</i>							
rs61569932	8,299,207	Upstream <i>NXPH1</i> , intron of <i>ICA1</i>	<u>G</u> /T	0	0.025	0.045	0
rs1990554	8,344,530	Upstream <i>NXPH1</i> , intron of <i>ICA1</i>	<u>A</u> /C	0	0.025	0.043	0
rs6463808	8,476,787	Intron	<u>A</u> /G	0.086	0.300	<0.0001	4.531 (2.312, 8.881)
rs6966968	8,840,378	Downstream <i>NXPH1</i> , intergenic	A/ <u>G</u>	0.111	0.220	0.013	2.261 (1.174, 4.354)
rs28473103	8,842,073	Downstream <i>NXPH1</i> , intergenic	A/ <u>G</u>	0.420	0.283	0.018	0.546 (0.33, 0.906)
rs28673635	8,855,531	Downstream <i>NXPH1</i> , intergenic	<u>A</u> /G	0.111	0.208	0.025	2.105 (1.089, 4.068)
rs12702829	9,049,555	Downstream <i>NXPH1</i> , intergenic	C/ <u>T</u>	0.365	0.509	0.018	1.797 (1.105, 2.922)
rs78943417	9,062,499	Downstream <i>NXPH1</i> , intergenic	A/ <u>AT</u>	0.144	0.034	0.003	0.213 (0.071, 0.633)
rs293180	9,159,909	Downstream <i>NXPH1</i> , intergenic	G/ <u>T</u>	0.063	0.158	0.010	2.784 (1.243, 6.234)
rs1837523	9,201,284	Downstream <i>NXPH1</i> , intergenic	C/ <u>T</u>	0.317	0.188	0.018	0.499 (0.279, 0.891)
<i>PHF17</i>							
rs1216346	129,555,929	Upstream <i>PHF17</i> , intergenic	C/ <u>T</u>	0.231	0.526	<0.0001	3.694 (2.196, 6.211)
rs114348423	130,112,033	Downstream <i>PHF17</i> , intergenic	<u>A</u> /G	0.006	0.042	0.041	6.998 (0.807, 60.716)
rs75007521	130,286,406	Downstream <i>PHF17</i> , intergenic	<u>G</u> /T	0.049	0	0.014	—
<i>MYB</i>							
rs72560788	135,200,886	Upstream <i>MYB</i> , intergenic	C/ <u>T</u>	0.117	0.050	0.049	0.396 (0.153, 1.024)
rs72974149	135,395,122	Upstream <i>MYB</i> , intergenic	A/ <u>G</u>	0.130	0.042	0.012	0.292 (0.107, 0.798)
rs210962	135,503,785	Intron	C/ <u>T</u>	0.303	0.183	0.023	0.518 (0.292, 0.917)
rs6933462	135,584,967	Downstream <i>MYB</i> , intergenic	C/ <u>G</u>	0.106	0.200	0.028	2.103 (1.073, 4.122)
<i>NELLI</i>							
rs79624996	20,211,262	Upstream <i>NELLI</i> , intergenic	<u>A</u> /G	0.092	0.181	0.032	2.179 (1.055, 4.498)
rs1850875	20,731,343	Intron	C/ <u>T</u>	0.340	0.533	0.001	2.223 (1.37, 3.608)
rs78786240	20,735,026	Intron	C/ <u>T</u>	0.045	0	0.021	—
rs117114492	21,008,313	Intron	<u>G</u> /T	0	0.042	0.009	0
<i>SLIT2</i>							
rs184945470	19,334,808	Upstream <i>SLIT2</i> , intergenic	C/ <u>T</u>	0.069	0.158	0.016	2.548 (1.163, 5.583)
rs143662727	19,634,162	Upstream <i>SLIT2</i> , intergenic	A/ <u>G</u>	0.037	0	0.033	—
rs10009109	19,655,475	Upstream <i>SLIT2</i> , intergenic	C/ <u>T</u>	0.388	0.534	0.015	1.811 (1.119, 2.931)
rs10009535	19,747,014	Upstream <i>SLIT2</i> , intergenic	A/ <u>G</u>	0.407	0.558	0.012	1.839 (1.141, 2.964)
rs61790364	19,921,757	Upstream <i>SLIT2</i> , intergenic	<u>A</u> /G	0.117	0.258	0.002	2.621 (1.397, 4.919)
rs73241936	20,008,049	Upstream <i>SLIT2</i> , intergenic	C/ <u>T</u>	0.111	0.200	0.038	2.000 (1.03, 3.883)
rs16869663	20,485,683	Intron	A/ <u>G</u>	0.043	0.110	0.032	2.741 (1.059, 7.102)
rs76015249	20,735,742	Downstream <i>SLIT2</i> , intron of <i>KCNIP4</i>	<u>A</u> /G	0	0.025	0.043	0

¹GRS, genetic risk score; SNP, single nucleotide polymorphism. Genes: *IQCF-SCHIP1*, IQ motif containing J-Schwannomin interacting protein 1 readthrough; *MYB*, V-MYB avian myeloblastosis viral oncogene homolog; *NELLI*, Neural EGFL like 1; *NXPH1*, Neurexophilin 1; *PHF17*, PHD finger protein 17; *SLIT2*, Slit guidance ligand 2.

²Minor alleles are underlined.

³*P* values for differences in allele frequency between responders and nonresponders were assessed using a chi-square test in PLINK.

⁴ORs reporting the ratio of the proportion of nonresponders carrying the minor allele over the proportion of responders.

Six stepwise bidirectional models (1 for each gene) adjusted for age, sex, and BMI were conducted to select SNPs to include in the GRS calculation. According to the stepwise models, 31 SNPs—namely 2 in *IQCF-SCHIP1*, 10 in *NXPH1*, 3 in *PHF17*, 4 in *MYB*, 4 in *NELLI*, and 8 in *SLIT2*—were associated with the TG response (Table 2) and used in the GRS calculation.

Figure 2 presents the GRS distribution in the study population. The higher the GRS score is, the more a subject carries at-risk alleles. Responders had a lower GRS score (1.26 ± 2.34) than nonresponders (6.32 ± 2.21) ($P < 0.0001$). In a general linear model adjusted for age, sex, and BMI, the GRS was significantly associated with the TG response ($P < 0.0001$). The

FIGURE 2 GRS distribution in the study population ($n = 141$). If a GRS is positive, the subject carries more at-risk alleles. If a GRS is negative, the subject carries more beneficial alleles. GRS, genetic risk score.

GRS accounted for 49.73% of TG change following the n-3 FA supplementation ($P < 0.0001$).

The sensitivity and specificity of the genetic risk model were assessed with the area under the receiver operating characteristic curve. The AUC was 0.9366 (95% CI: 0.8976, 0.9756) for the GRS solely and 0.7537 (95% CI: 0.6721, 0.8353) for the sum of other determinants (general model), including BMI, sex, age, and baseline TG concentrations. The addition of the GRS to the general model significantly increased the predictive power ($P < 0.0001$) for an AUC of 0.9455 (95% CI: 0.9084, 0.9826) (Figure 3). After cross-validation, the AUCs were 0.9187 for the GRS alone and 0.9280 for the fully adjusted model (data not shown).

With regard to the FINGEN replication study, there were 122 nonresponders (39.7%) and 188 responders (60.3%), according to the same definition as mentioned above. Genotyping in FINGEN participants was conducted on the 31 SNPs of the GRS. Among these 31 SNPs, 8 were monomorphic in the FINGEN cohort (rs61569932, rs1216346, rs79624996, rs10009535, and rs76015249) or failed genotyping (rs6966968, rs78943417, and rs184945470), leaving 23 SNPs for GRS calculation. In the general linear model adjusted for age, sex, and BMI, this GRS was also significantly associated with the TG response ($P = 0.0006$) and accounted for 3.67% of the TG change following the n-3 FA supplementation ($P = 0.0006$). Regarding sensitivity and specificity, the AUCs were 0.6417 (95% CI: 0.5795, 0.7039) for the GRS solely, 0.7109 (95% CI: 0.6530, 0.7688) for the general model, and 0.7553 (95% CI: 0.7010, 0.8095) for the general model + GRS (Figure 4).

Discussion

The present study follows up a GWAS of the plasma TG response to an n-3 FA supplementation in which a GRS was

constructed (9). We used dense genotyping, as well as genotype imputation, to further increase the density of markers identified in the GWAS. Association studies were performed, and a new GRS was computed from imputed and genotyped tagging SNPs to better predict the plasma TG response to an n-3 FA supplementation. The previous GRS was computed using a total of 10 GWAS hits (9). Using genotype imputation and dense genotyping data, we were able to construct a more refined GRS using 31 SNPs. As shown in Figure 2, we now have a clear disparity between responders and nonresponders regarding their number of carried at-risk alleles. Nonresponders have much higher GRS levels than responders. The 31-SNPs GRS explains a much larger proportion of the TG variance (49.73%) than the 10-SNPs GRS (21.53%), and its predictive capacity for classifying individuals into responders and nonresponders categories is also highly accurate. However, the GRS poorly explained TG variance in the FINGEN population (3.67%) compared with FAS. This is consistent with previous results of the 10-SNPs GRS, where only 2% of the TG variation was explained by the GRS in the FINGEN population (nonsignificant) compared with 21.53% in the FAS population ($P = 0.0002$) (9). This important divergence could be explained by the 8 missing SNPs in the GRS in the replication study. Also, differences in allele frequency between the 2 populations might be a contributing factor. Accordingly, several SNPs (rs62270407, rs10009109, rs76015249, rs61569932, and rs293180) showed significant differences in allele frequency between the FAS and FINGEN populations. These differences can be explained by their ancestry background. FAS study participants are French-Canadians of European descent, a more homogeneous population with a founder effect, whereas >95% of FINGEN study participants were British Caucasians (19). Moreover, the proportion of nonresponders among FAS participants was 29%, compared with almost 40% among FINGEN participants.

FIGURE 3 Receiver operating characteristic curve analysis for the GRS in the Fatty Acid Sensor Study population. Sensitivity and specificity were assessed using the logistic procedure in SAS version 9.4. GRS, genetic risk score.

Despite the clear influence of SNPs on the responsiveness of TG concentrations to n-3 FA supplementation, the exact mechanisms by which the 6 genes in GWAS-associated loci contribute to TG variation still remain unclear. We previously demonstrated that SNPs in GWAS-associated loci may exert their effect on TG concentrations by influencing gene expression via modulation of DNA methylation (20). However, most of these genes are poorly connected to lipid metabolism as detailed in our previous article (10). This is especially the case for *IQCJ-SCHIP1*. Nevertheless, even though *IQCJ-SCHIP1* has not been explicitly linked to lipid metabolism, we hypothesize that its action on the TG response could be mediated through calcitonin action.

Accordingly, Martin et al. (21) demonstrated that, in the absence of calcium, *IQCJ* binds calmodulin via its motif IQ, a sequence motif not shared with the *SCHIP1* segment (22).

Calmodulin is a messenger protein that normally binds to calcium to modulate its action (23). However, other proteins, such as IQ motif-containing proteins, also interact with calmodulin in a calcium-independent way, more specifically when calcium levels are low (23). Calmodulin is implicated in lipid metabolism via its effect on calcitonin (24), a thyroid hormone that regulates calcium and phosphate in the blood (25). In a previous study, it has been reported that the administration of calcitonin decreased LDL-cholesterol and TG concentrations in rats, whereas the administration of a calmodulin inhibitor prevented this effect, thus suggesting that calmodulin suppressed the action of calcitonin (24). Werner and Low (26) also observed that calcitonin inhibited lipolysis during basal and stimulated lipolysis with parathyroid hormone, noradrenaline, or dibutyryl cyclic AMP (cAMP; bucladesine) in rat adipose tissue. One of the main mechanisms of action of calcitonin lies in the capacity of its

FIGURE 4 Receiver operating characteristic curve analysis for the GRS in the FINGEN study population. Sensitivity and specificity were assessed using the logistic procedure in SAS version 9.4. GRS, genetic risk score.

receptor, a member of G-protein-coupled receptors, to couple with the cAMP signal transduction (27). cAMP is a ubiquitous second messenger implicated in lipid metabolism by activating cAMP-dependent protein kinase, an enzyme that enhances TG hydrolysis in adipocytes (28, 29). cAMP-dependent mechanisms also inhibit lipolysis during refeeding (29). Moreover, dietary compounds, including calcium, caffeine, and ethanol, can affect adipocyte lipolysis through modulation of cAMP levels, with increased cAMP levels resulting in stimulated lipolysis (29).

Another possible explanation is the presence of linkage disequilibrium between tagging SNPs and other SNPs located in transcriptional units other than GWAS-associated loci. As shown in Table 2, the majority of the 31 tagging SNPs are not located within GWAS-associated loci but are rather intergenic, and some GWAS-associated loci are located close to genes of interest. For instance, *NXPH1* is located next to the Islet cell autoantigen 1 (*ICAI*), a gene involved in the pathogenesis of type 1 diabetes

(30). An SNP of *ICAI*, along with several SNPs of *NXPH1*, was identified in a GWAS of childhood obesity in a Hispanic population (31). These results not only highlight the importance of refining GWAS signals to properly identify the most causative SNPs but also reinforce the hypothesis that genetic profile is a significant determinant of the metabolic response to an n-3 FA supplementation, and these observations could probably extend to other dietary interventions. It is therefore crucial to stop overlooking genetic factors for the assessment of responsiveness to such interventions. For instance, a recently published meta-analysis of randomized clinical trials on the association between n-3 FA supplement use and cardiovascular disease concluded that n-3 FA consumption was not associated with a reduction in coronary artery disease or major vascular events (32). Another recent meta-analysis of randomized, double-blind, placebo-controlled trials on the efficacy of n-3 FA consumption for the treatment of hypertriglyceridemia concluded that n-3 FAs

were ineffective (33). Although n-3 FA consumption overall significantly reduced TG concentrations, the authors asserted that evidence regarding the effectiveness of n-3 FAs in the management of dyslipidemia, especially hypertriglyceridemia, is inconclusive, in part because of the heterogeneity in studies, low methodological quality of studies, and small sample sizes (33). As raised by Calder (34) in a commentary on the meta-analysis, their findings are very consistent with the literature and actually clearly confirm that n-3 FAs in supplemental form can lower TG concentrations. Moreover, this inconclusiveness regarding the beneficial effects of n-3 FA intake can probably be attributable to the lack of consideration of contributors to the interindividual variability in the metabolic response to n-3 FA, including genetic factors. Based on our results, it appears that not all individuals can benefit from the TG-lowering effects of n-3 FA supplements. Future research should focus on addressing the effects of n-3 FAs on responders and nonresponders separately by stratifying subjects according to their TG response and taking determinants including genetic factors into account.

In conclusion, this study further demonstrates the relevance of refining GWAS hits, here providing a novel, refined GRS highly predictive of the responsiveness to n-3 FAs. The genetic profile appears to be a major determinant of the TG response to n-3 FA supplementation. Future studies on n-3 FA and other nutrients should pay more attention to the importance of genetic factors on the interindividual variability in lipid responsiveness.

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