Fine mapping of GWAS signals to identify genetic markers of the plasma triglyceride response to an omega-3 fatty acid supplementation

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Short running head: Genetic markers of the triglyceride response

Abbreviations:

BMI: Body mass index; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FA: fatty acid; *FAS* study: *Fatty Acid Sensor* study; GRS: genetic risk score; GWAS: genome-wide association study; n-3: omega-3; SNPs: single nucleotide polymorphisms; TG: triglyceride.

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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 ten loci which affect the plasma triglyceride (TG) response to an omega-3 (n-3) fatty acid (FA) supplementation.

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

Design: A total of 208 participants of the *Fatty Acid Sensor (FAS)* study received 5g of fish oil per day, containing 1.9–2.2g of eicosapentanoic acid and 1.1g of docosahexanoic acid, for six weeks. Plasma TG levels were measured before and after supplementation. Dense genotyping and genotype imputation were employed 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 ($p < 0.0001$). Non-responders to the n-3 FA supplementation had a higher GRS than responders. In the *FINGEN* replication study, the GRS explained 3.67% of TG response variance ($p = 0.0006$).

Conclusion: 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 non-responsive to n-3 FA. 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.

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

BACKGROUND

The hypotriglyceridemic effect of marine omega-3 (n-3) fatty acids (FA) has been consistently described (1). At a population level, a daily intake of 4g of n-3 FA in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can lower triglyceride (TG) levels by up to 30% (1). However, it remains unclear whether n-3 FA consumption actually translates into a decrease of cardiovascular events and mortality risk, for discrepancies have been reported in the literature (2-5). A possible explanation for these inconsistent results is that not all individuals equally benefit from n-3 FA consumption. Accordingly, a large inter-individual 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-week n-3 FA supplementation did not show a decrease in TG levels (6). Likewise, 29% of participants of the *Fatty Acid Sensor* (*FAS*) study, conducted by our research group, did not have decreased TG levels after a 6-week supplementation of 3g of n-3 FA a day, comprising 1.9-2.2 g of EPA and 1.1 g DHA (7).

The etiology of the variability in the hypotriglyceridaemic response is likely to be multi-factorial, with genetic factors partly accounting for the inter-individual variability of the TG response to an n-3 FA supplementation (8). Our group previously conducted a genome-wide association study (GWAS) on 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 six genes, namely *IQCJ-SCHIP1*, *NXPH1*, *PHF17*, *MYB*, *NELL1* and *SLIT2* (9). A genetic risk score (GRS) was computed from ten GWAS hits and this explained 21.53% of the TG response (9). We recently reported several gene-diet interactions modulating TG levels 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 hits regions to add SNPs in order to more accurately predict the individual TG response to an n-3 FA supplementation.

MATERIALS AND 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 years; a body mass index (BMI) between 25 and 40 kg/m^2; non-smokers; 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 six months prior to the intervention. A total of 210 participants completed the intervention. However, two participants did not have plasma TG levels data available for further analyses and were therefore excluded, yielding a final sample of 208 participants. Subjects were subsequently separated into two subgroups: 1- responders to the n-3 FA supplementation; and 2- non-responders. Responders were defined as participants whose plasma TG levels decreased after the n-3 FA supplementation, whereas non-responders were participants whose TG levels 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). Flow chart of participants and intervention is presented as online supporting material (**Supplemental Figure 1**). First, participants followed a run-in period of two weeks, where they were given dietary instructions by a trained registered dietitian to achieve the recommendations from the *Canada's Food Guide*. 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 more than 150 g of fish or seafood per week, to avoid food enriched with n-3 FA, to limit alcohol consumption to a maximum of two regular drinks per week 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 daily for six weeks. (Ocean Nutrition, Nova Scotia, Canada). The five capsules per day provided a total of 3 g of n-3 FA a day, including 1.9 – 2.2 g of EPA and 1.1 g of 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 already been published (7). Briefly, blood samples were collected after a 12h overnight fast and 48h alcohol abstinence at the beginning and end of the intervention. Plasma TG concentrations were assessed by enzymatic assays (12, 13).

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 (NCBI) B36 assembly Data Release 28, phase II + III, built 126. Tagging SNPs were selected using the Tagger procedure in Haploview v4.2

according to their minor allele frequency (MAF >5%) and pairwise tagging (r^2 ≥0.80). A total of 16 SNPs in *IQCJ*, 34 in *NXPH1*, 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., St. Louis, MO) was used to extract genomic DNA (gDNA) from the blood samples. Genotyping was conducted by polymerase chain reaction (PCR) using TaqMan technology (Life Technologies, Carlsbad, CA) in 210 subjects.

For genotype imputation, the 1000 Genomes project data (release 1000G Phase I v3, updated 26 Aug 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 *NXPH1*, 885 in *PHF17* and 777 in *MYB*, that were originally used to conduct the GWAS in 141 participants, were used to infer other genotypes using algorithms implemented in IMPUTE2 (14). The imputation cut-off was 0.90, with 99.0% of success rate. A total of 52770 informative SNPs in *IQCJ-SCHIP1*, 50218 in *NXPH1*, 30140 in *PHF17*, 29725 in *MYB*, 61560 in *NELL1* and 61736 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 MAF ≥1% were conserved. Quality control tests left 5205 SNPs in *IQCJ-SCHIP1*, 6040 in *NXPH1*, 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 non-responders was calculated and compared using PLINK. Odds ratio reporting the ratio between the proportion of non-responders carrying the minor allele of a SNP and the proportion of responders carrying the minor allele of the same SNP was calculated. Odds ratio *P* values were calculated using a Chi-square test.

Prior to statistical analyses, tagging SNPs were selected using PLINK from genotyping and imputation data. For inclusion criteria, the r^2 was set at ≥0.80 and the *P* value was 0.05. A final sample of 88 independent tagging SNPs in *IQCJ-SCHIP1*, 88 in *NXPH1*, 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, 15). Briefly, it was a double-blind, placebo-controlled, dose-response crossover study in which participants received either a placebo, 0.7 g of EPA and DHA a day or 1.8 g of EPA and DHA a day for eight weeks with 12-week 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 1.8g/day dose used in the current analysis. TG data was available for 310 of the 312 subjects. Genotyping was conducted by LCG genomics (16).

Statistical analyses

Statistical analyses were conducted using SAS software v9.4. 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 non-responders prior to 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 (ΔTG). Significant SNPs ($p<0.05$) were kept for the GRS calculation.

A *t*-test was performed to compare mean GRS scores between responders and non-responders 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. Sensitivity and specificity of the GRS were calculated by measuring the area under the receiver operating characteristic (ROC) curve using the logistic procedure in SAS with adjustments for age, sex, BMI and baseline TG levels.

RESULTS

Characteristics of participants and FA profiles were previously reported (9). **Table 1** presents a summary of baseline and post-supplementation characteristics of responders and non-responders to the n-3 FA supplementation. Participants were overweight, with mean (SD) baseline BMIs of 28.9 (3.6) kg/m^2 and 27.8 (3.9) kg/m^2 in the responder and non-responder groups, respectively. Responders had higher TG levels at baseline compared to non-responders ($p < 0.0001$), and their TG levels significantly decreased by on average 0.50 mmol/l through supplementation ($p < 0.0001$), whereas non-responders had a mean 0.17 mmol/l increase ($p < 0.0001$).

Figure 1 shows *p* value for differences in allele frequency between responders and non-responders 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 ($p = 10^{-5}$), counting 12 in *IQCJ-SCHIP1*, one in *NXPH1*, 22 in *PHF17*, six in *MYB*, one in *NELL1* and 20 in *SLIT2*.

Six stepwise bidirectional models (one 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 two in *IQCJ-SCHIP1*, 10 in *NXPH1*, three in *PHF17*, four in *MYB*, four in *NELL1* and eight in *SLIT2*, were associated with the TG response (**Table 2)** which were 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 lower GRS score (1.26 ± 2.34) in comparison to non-responders (6.32 ± 2.21) ($p < 0.0001$). In a general linear model adjusted for age, sex and BMI, the GRS was

189 significantly associated with the TG response (p < 0.0001). The GRS accounted for
190 49.73% of TG change following the n-3 FA supplementation (p < 0.0001).

191 Sensitivity and specificity of the genetic risk model were assessed with the area under the
192 ROC curve. The area under the curve was 0.9366 [95% confidence interval (CI): 0.8976,
193 0.9756] for the GRS solely, and 0.7537 [95% CI: 0.6721, 0.8353] for the sum of other
194 determinants (general model), including BMI, sex, age and baseline TG levels. The
195 addition of the GRS to the general model significantly increased the predictive power
196 (p<0.0001), for an area under the curve of 0.9455 [95% CI: 0.9084, 0.9826] (**Figure 3**).
197 After cross-validation, the area under the curve was 0.9187 for the GRS alone and 0.9280
198 for the full adjusted model (data not shown).

199 As to the *FINGEN* replication study, there were 122 non-responders (39.7%) and 188
200 responders (60.3%), according to the same definition as mentioned above. Genotyping in
201 *FINGEN* participants was conducted on the 31 SNPs of the GRS. Among these 31 SNPs,
202 eight were either monomorphic in the *FINGEN* cohort (rs61569932, rs1216346,
203 rs79624996, rs10009535 and rs76015249) or failed genotyping (rs6966968, rs78943417
204 and rs184945470), leaving 23 SNPs for GRS calculation. In the general linear model
205 adjusted for age, sex and BMI, this GRS was also significantly associated with the TG
206 response (p = 0.0006) and accounted for 3.67% of the TG change following the n-3 FA
207 supplementation (p = 0.0006). Regarding sensitivity and specificity, the areas under the
208 curve were 0.6417 [95% CI: 0.5795, 0.7039] for the GRS solely, 0.7109 [95% CI): 0.6530,
209 0.7688] for the general model and 0.7553 [95% CI: 0.7010, 0.8095] for the general model
210 + GRS (**Figure 4**).

211

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 ten GWAS hits (9). Using genotype imputation and dense genotyping data, we were able to construct a more refined GRS using 31 SNPs. As illustrated in **Figure 2**, we now have a clear disparity between responders and non-responders regarding their number of carried at-risk alleles. Non-responders 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 non-responders categories is also highly accurate. However, the GRS poorly explained TG variance in the *FINGEN* population (3.67%) compared to *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 to 21.53% in the *FAS* population (p = 0.0002) (9). This important divergence could be explained by the eight missing SNPs in the GRS in the replication study. Also, differences in allele frequency between the two 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 while over 95% of *FINGEN* study participants were British Caucasians (17). Moreover, the proportion of non-responders among *FAS* participants is 29% vs almost 40% among *FINGEN* participants.

Despite the clear influence of SNPs on the responsiveness of TG levels to n-3 FA supplementation, the exact mechanisms by which the six 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 levels by influencing gene expression via modulation of DNA methylation (18). However, most of these genes are poorly connected to lipid metabolism as detailed in our previous paper (10). This is especially the case for *IQCJ-SCHIP1*. Nevertheless, even though *IQCJ-SCHIP1* has not been explicitdly linked to lipid metabolism, we hypothesize that its action on the TG response could be mediated through calcitonin action.

Accordingly, Martin PM *et al*. demonstrated that, in absence of calcium, *IQCJ* binds calmodulin via its motif IQ, a sequence motif not shared with the *SCHIP1* segment (19, 20). Calmodulin is a messenger protein that normally binds to calcium to modulate its action (21). However, other proteins, like IQ motif-containing proteins, also interact with calmodulin in a calcium-independent way, more specifically when calcium levels are low (21). Calmodulin is implicated in lipid metabolism via its effect on calcitonin (22), a thyroid hormone that regulates calcium and phosphate in the blood (23). In a previous study, it has been reported that the administration of calcitonin decreased LDL-cholesterol and TG levels in rats, while the administration of a calmodulin inhibitor prevented this effect, thus suggesting that calmodulin suppressed the action of calcitonin (22). Werner and Low also

observed that calcitonin inhibited lipolysis during basal and stimulated lipolysis with parathyroid hormone, noradrenaline or dibutyryl cAMP (bucladesine) in rat adipose tissue (24). One of the main mechanism of action of calcitonin lies in the capacity of its receptor, a member of G-protein coupled receptors, to couple with the cyclic adenosine monophosphate (cAMP) signal transduction (25). cAMP is an ubiquitous second messenger implicated in lipid metabolism by activating cAMP-dependent protein kinase, an enzyme that enhances TG hydrolysis in adipocytes (26, 27). cAMP-dependent mechanisms also inhibit lipolysis during refeeding (27). 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 (27).

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 3**, 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 *ICA1*, a gene involved in the pathogenesis of type 1 diabetes (28). A SNP of *ICA1*, along with several SNPs of *NXPH1*, were identified in a GWAS of childhood obesity in an hispanic population (29). 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 of coronary heart disease or major vascular events (30). 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 FA were ineffective (31). Although n-3 FA consumption overall significantly reduced TG levels, authors asserted that evidence regarding the effectiveness of n-3 FA in the management of dislipidemia, especially hypertriglyceridemia, is inconclusive, in part because of the heterogeneity in studies, low methodological quality of studies and small sample sizes (31). As raised by Calder P. in a commentary on the meta-analysis, their findings are very consistent with literature and actually clearly confirm that n-3 FA in supplemental form can lower TG levels (32). Moreover, this inconclusiveness regarding the beneficial effects of n-3 FA intake can probably be attributable to the lack of consideration of contributors of 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 FA on responders and non-responders 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 FA. 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 inter-individual variability in lipid responsiveness.

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Authors' contributions

BVM conducted genotyping, SNP analysis and wrote the paper; BVM and FG conducted genotype imputation and statistical analysis; IR, SL and MCV designed research; PC was responsible for the medical follow-up; PCC and AMM contributed data from the *FINGEN* study; BVM and MCV have primary responsibility for final content. All authors read and approved the final manuscript. Authors declare no conflict of interest.

Consent

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.

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TABLES

Table 1. Characteristics of subjects pre- and post-supplementation (n=141)

	Responders (n=81)			Non-responders (n=60)		
Characteristics	Pre-supplementation	Post-supplementation	*P* [1]	Pre-supplementation	Post-supplementation	*P* [1]
Sex (men/women)	38/43	-	-	30/30	-	-
Age (years) [2]	31.9 ± 8.8	-	-	31.1 ± 9.0	-	-
Body mass index (kg/m^2) [2]	28.9 ± 3.6	28.9 ± 3.7	0.7	27.8 ± 3.9	28.0 ± 4.0	0.0007
Triglycerides (mmol/l) [2]	1.53 ± 0.74	1.03 ± 0.56	< 0.0001	1.03 ± 0.48	1.20 ± 0.54	< 0.0001

[1] TTEST procedure (SAS v9.4) was used to assess differences pre- vs post-supplementation in responders and non-responders

[2] Mean ± standard deviation

Table 2. Characteristics of the 31 SNPs used to compute the genetic risk score (GRS)

Gene	SNP, rs number	Position, base pairs	Location	Alleles [1]	Minor allele frequency		*P* [2]	22 OR [95%CI] [3]
					Responders	Non-responders		
IQCJ-SCHIP1	rs7639707	159148087	Intron	**A**/G	0.019	0.075	0.020	4.297 [1.138, 16.231]
	rs62270407	159597626	Intron	C/**T**	0.352	0.192	0.003	0.437 [0.25, 0.763]
NXPH1	rs61569932	8299207	Upstream *NXPH1*, intron of *ICA1*	**G**/T	0	0.025	0.045	0
	rs1990554	8344530	Upstream *NXPH1*, intron of *ICA1*	**A**/C	0	0.025	0.043	0
	rs6463808	8476787	Intron	**A**/G	0.086	0.300	<0.0001	4.531 [2.312, 8.881]
	rs6966968	8840378	Downstream *NXPH1*, intergenic	A/**G**	0.111	0.220	0.013	2.261 [1.174, 4.354]
	rs28473103	8842073	Downstream *NXPH1*, intergenic	A/**G**	0.420	0.283	0.018	0.546 [0.33, 0.906]
	rs28673635	8855531	Downstream *NXPH1*, intergenic	**A**/G	0.111	0.208	0.025	2.105 [1.089, 4.068]
	rs12702829	9049555	Downstream *NXPH1*, intergenic	**C**/T	0.365	0.509	0.018	1.797 [1.105, 2.922]
	rs78943417	9062499	Downstream *NXPH1*, intergenic	A/**AT**	0.144	0.034	0.003	0.213 [0.071, 0.633]
	rs293180	9159909	Downstream *NXPH1*, intergenic	G/**T**	0.063	0.158	0.010	2.784 [1.243, 6.234]
	rs1837523	9201284	Downstream *NXPH1*, intergenic	**C**/T	0.317	0.188	0.018	0.499 [0.279, 0.891]
PHF17	rs1216346	129555929	Upstream *PHF17*, intergenic	**C**/T	0.231	0.526	<0.0001	3.694 [2.196, 6.211]

	rs114348423	130112033	Downstream *PHF17*, intergenic	**A**/G	0.006	0.042	0.041	6.998 [0.807, 60.716]
	rs75007521	130286406	Downstream *PHF17*, intergenic	**G**/T	0.049	0	0.014	-
MYB	rs72560788	135200886	Upstream *MYB*, intergenic	C/**T**	0.117	0.050	0.049	0.396 [0.153, 1.024]
	rs72974149	135395122	Upstream *MYB*, intergenic	A/**G**	0.130	0.042	0.012	0.292 [0.107, 0.798]
	rs210962	135503785	Intron	C/**T**	0.303	0.183	0.023	0.518 [0.292, 0.917]
	rs6933462	135584967	Downstream *MYB*, intergenic	**C**/G	0.106	0.200	0.028	2.103 [1.073, 4.122]
NELL1	rs79624996	20211262	Upstream *NELL1*, intergenic	**A**/G	0.092	0.181	0.032	2.179 [1.055, 4.498]
	rs1850875	20731343	Intron	**C**/T	0.340	0.533	0.001	2.223 [1.37, 3.608]
	rs78786240	20735026	Intron	C/**T**	0.045	0	0.021	-
	rs117114492	21008313	Intron	**G**/T	0	0.042	0.009	0
SLIT2	rs184945470	19334808	Upstream *SLIT2*, intergenic	C/**T**	0.069	0.158	0.016	2.548 [1.163, 5.583]
	rs143662727	19634162	Upstream *SLIT2*, intergenic	A/**G**	0.037	0	0.033	-
	rs10009109	19655475	Upstream *SLIT2*, intergenic	**C**/T	0.388	0.534	0.015	1.811 [1.119, 2.931]
	rs10009535	19747014	Upstream *SLIT2*, intergenic	A/**G**	0.407	0.558	0.012	1.839 [1.141, 2.964]
	rs61790364	19921757	Upstream *SLIT2*, intergenic	**A**/G	0.117	0.258	0.002	2.621 [1.397, 4.919]
	rs73241936	20008049	Upstream *SLIT2*, intergenic	**C**/T	0.111	0.200	0.038	2.000 [1.03, 3.883]

	rs16869663	20485683	Intron	A/**G**	0.043	0.110	0.032	2.741 [1.059, 7.102]
	rs76015249	20735742	Downstream *SLIT2*, intron of *KCNIP4*	**A**/G	0	0.025	0.043	0

[1] Minor allele in bold

[2] P values for differences in allele frequency between responders and non-responders were assessed using a Chi square test in PLINK.

[3] Odds ratio reporting the ratio of the proportion of non-responders carrying the minor allele over the proportion of responders

FIGURE TITLES/LEGENDS

Figure 1. Manhattan plot showing *p* values for differences in allele frequency between responders and non-responders to omega-3 fatty acid supplementation in each gene region identified by the initial genome-wide association study (GWAS) (9). SNPs obtained from genotype imputation are shown in red in panels below. Differences in allele frequency were assessed using a Chi square test in PLINK.

Figure 2. Genetic risk score (GRS) distribution in 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.

Figure 3. Receiver Operating Characteristic (ROC) Curve Analysis for the genetic risk score (GRS) in the *FAS* study population. Sensitivity and specificity were assessed using the logistic procedure in SAS v9.4.

Figure 4. Receiver Operating Characteristic (ROC) Curve Analysis for the genetic risk score (GRS) in the *FINGEN* study population. Sensitivity and specificity were assessed using the logistic procedure in SAS v9.4.