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.

Clinical Trial Registry number and website (FAS study): http://www.clinicaltrials.gov (NCT01343342)

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

23 determinant of the plasma TG response to an n-3 FA supplementation and could be

24 used to target those most likely to gain clinical benefit.

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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

52   increasing the density of markers around GWAS hits by dense genotyping (10). These

53   results demonstrated the importance of pursuing mapping refinement around GWAS-

54   associated loci to identify actual causative single nucleotide polymorphisms (SNPs). It is

55   therefore very likely that the current GRS on *FAS* participants could be improved by

56   using fine mapping to add variants, which would bring more power and accuracy.

57   The objective of the present study was to generate a more refined and improved GRS

58   using fine mapping of GWAS hits regions to add SNPs in order to more accurately

59   predict the individual TG response to an n-3 FA supplementation.

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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

86 recommendations from the *Canada's Food Guide.* The purpose of these

87 recommendations was to ensure that participants had a constant n-3 FA dietary intake

88 and maintained a stable body weight throughout the study period. More specifically, they

89 were asked not to consume more than 150 g of fish or seafood per week, to avoid food

90 enriched with n-3 FA, to limit alcohol consumption to a maximum of two regular drinks

91 per week and to avoid any dietary supplement (n-3 FA supplements, vitamins or natural

92 products) during the intervention. Thereafter, participants were asked to consume the n-

93 3 FA capsules daily for six weeks. (Ocean Nutrition, Nova Scotia, Canada). The five

94 capsules per day provided a total of 3 g of n-3 FA a day, including 1.9 – 2.2 g of EPA

95 and 1.1 g of DHA. Participants were asked to report any deviation from the protocol.

96 They were also asked to record any experienced side effects, alcohol intake and fish

97 consumption.

98 *Laboratory methods*

99 *Plasma lipids*

100 Methods used to measure blood lipids have already been published (7). Briefly, blood

101 samples were collected after a 12h overnight fast and 48h alcohol abstinence at the

102 beginning and end of the intervention. Plasma TG concentrations were assessed by

103 enzymatic assays (12, 13).

104 *Gene mapping*

105 Two methods were used for fine mapping: dense genotyping and genotype imputation.

106 For genotyping, the whole procedure was previously described (10). Briefly, SNPs were

107 identified using the International HapMap Project SNP database, based on the National

108 Center for Biotechnology information (NCBI) B36 assembly Data Release 28, phase II +

109 III, built 126. Tagging SNPs were selected using the Tagger procedure in Haploview

110 v4.2 according to their minor allele frequency (MAF >5%) and pairwise tagging (r^2

111 ≥0.80). A total of 16 SNPs in *IQCJ*, 34 in *NXPH1*, 8 in *PHF17* and 9 in *MYB* were

112 chosen to cover all common variations at these chromosomal regions. To the 16 SNPs

113 in *IQCJ*, 23 genotyped SNPs in *SCHIP1* were added in order to cover the full *IQCJ-*

114 *SCHIP1* gene. All SNPs were in Hardy-Weinberg Equilibrium. The GenElute Gel

115 Extraction Kit (Sigma-Aldrich Co., St. Louis, MO) was used to extract genomic DNA

116 (gDNA) from the blood samples. Genotyping was conducted by polymerase chain

117 reaction (PCR) using TaqMan technology (Life Technologies, Carlsbad, CA) in 210

118 subjects.

119 For genotype imputation, the 1000 Genomes project data (release 1000G Phase I v3,

120 updated 26 Aug 2012) was used as a reference set for the imputation of genotypes

121 (genotyped from Illumina BeadChip) of previously identified GWAS loci (9). A total of

122 1684 markers in *IQCJ-SCHIP1*, 1684 in *NXPH1*, 885 in *PHF17* and 777 in *MYB*, that

123 were originally used to conduct the GWAS in 141 participants, were used to infer other

124 genotypes using algorithms implemented in IMPUTE2 (14). The imputation cut-off was

125 0.90, with 99.0% of success rate. A total of 52770 informative SNPs in *IQCJ-SCHIP1*,

126 50218 in *NXPH1*, 30140 in *PHF17*, 29725 in *MYB*, 61560 in *NELL1* and 61736 in *SLIT2*

127 were obtained from genotype imputation, including initial markers.

128 Imputed SNPs were then submitted to quality control tests, where only polymorphic

129 SNPs with a genotype call rate >95% and MAF ≥1% were conserved. Quality control

130   tests left 5205 SNPs in *IQCJ-SCHIP1*, 6040 in *NXPH1*, 3028 in *PHF17*, 2616 in *MYB*,

131   7846 in *NELL1* and 7124 in *SLIT2* available for SNP analysis.

132   *SNP analysis and selection*

133   Allele frequency between responders and non-responders was calculated and compared

134   using PLINK. Odds ratio reporting the ratio between the proportion of non-responders

135   carrying the minor allele of a SNP and the proportion of responders carrying the minor

136   allele of the same SNP was calculated. Odds ratio *P* values were calculated using a Chi-

137   square test.

138   Prior to statistical analyses, tagging SNPs were selected using PLINK from genotyping

139   and imputation data. For inclusion criteria, the r^2 was set at ≥0.80 and the *P* value was

140   0.05. A final sample of 88 independent tagging SNPs in *IQCJ-SCHIP1*, 88 in *NXPH1*, 56

141   in *PHF17*, 97 in *MYB*, 58 in *NELL1* and 118 in *SLIT2* were kept for statistical analyses.

142   *Replication study*

143   Analyses were replicated in the European *FINGEN* study, a trial conducted at the

144   Universities of Glasgow, Newcastle, Reading and Southampton in the United Kingdom

145   from 2003 to 2005. Over 95% of participants were British Caucasians. The study design

146   has been previously published (6, 15). Briefly, it was a double-blind, placebo-controlled,

147   dose-response crossover study in which participants received either a placebo, 0.7 g of

148   EPA and DHA a day or 1.8 g of EPA and DHA a day for eight weeks with 12-week

149   washouts in between. Responsiveness to n-3 FA supplementation was defined in the

150   same way as outlined above for the *FAS* participants, with only the response to the

151   1.8g/day dose used in the current analysis. TG data was available for 310 of the 312

152   subjects. Genotyping was conducted by LCG genomics (16).

153   *Statistical analyses*

154   Statistical analyses were conducted using SAS software v9.4. Normal distribution was

155   evaluated with the box-plot, skewness and kurtosis ranges. Abnormally distributed

156   variables were log_{10}-transformed. Statistical significance was set at $P < 0.05$. An

157   unpaired t-test was used to assess differences between responders and non-responders

158   prior to the supplementation.

159   A GRS was calculated for each participant from the sum of risk alleles of tagging SNPs

160   in an additive way. To select the most relevant SNPs to include in the GRS calculation,

161   stepwise bidirectional regressions adjusted for age, sex and BMI were conducted in

162   each gene separately (REG procedure in SAS) to assess the contribution of SNPs to the

163   TG variation (ΔTG). Significant SNPs (p<0.05) were kept for the GRS calculation.

164   A *t*-test was performed to compare mean GRS scores between responders and non-

165   responders to the n-3 FA supplementation. A general linear model (GLM procedure in

166   SAS) adjusted for age, sex and BMI was used to assess the effect of the GRS on the

167   plasma TG response to the n-3 FA supplementation. Sensitivity and specificity of the

168   GRS were calculated by measuring the area under the receiver operating characteristic

169   (ROC) curve using the logistic procedure in SAS with adjustments for age, sex, BMI and

170   baseline TG levels.

171 **RESULTS**

172 Characteristics of participants and FA profiles were previously reported (9). **Table 1**

173 presents a summary of baseline and post-supplementation characteristics of responders

174 and non-responders to the n-3 FA supplementation. Participants were overweight, with

175 mean (SD) baseline BMIs of 28.9 (3.6) kg/m^2 and 27.8 (3.9) kg/m^2 in the responder and

176 non-responder groups, respectively. Responders had higher TG levels at baseline

177 compared to non-responders ($p < 0.0001$), and their TG levels significantly decreased

178 by on average 0.50 mmol/l through supplementation ($p < 0.0001$), whereas non-

179 responders had a mean 0.17 mmol/l increase ($p < 0.0001$).

180 **Figure 1** shows p value for differences in allele frequency between responders and non-

181 responders in GWAS-associated genes after mapping refinement by genotype

182 imputation (A: *IQCJ-SCHIP1*, B: *SLIT2*, C: *PHF17*, D: *MYB*, E: *NXPH1*, F: *NELL1*). A

183 total of 62 markers passed the significance threshold used in the GWAS ($p = 10^{-5}$),

184 counting 12 in *IQCJ-SCHIP1*, one in *NXPH1*, 22 in *PHF17*, six in *MYB*, one in *NELL1*

185 and 20 in *SLIT2*.

186 Six stepwise bidirectional models (one for each gene) adjusted for age, sex and BMI

187 were conducted to select SNPs to include in the GRS calculation. According to the

188 stepwise models, 31 SNPs, namely two in *IQCJ-SCHIP1*, 10 in *NXPH1*, three in *PHF17*,

189 four in *MYB*, four in *NELL1* and eight in *SLIT2*, were associated with the TG response

190 (**Table 2)** which were used in the GRS calculation. **Figure 2** presents the GRS

191 distribution in the study population. The higher the GRS score is, the more a subject

192 carries at-risk alleles. Responders had lower GRS score (1.26 ± 2.34) in comparison to

193 non-responders (6.32 ± 2.21) ($p < 0.0001$). In a general linear model adjusted for age,

194 sex and BMI, the GRS was significantly associated with the TG response ($p < 0.0001$).

195 The GRS accounted for 49.73% of TG change following the n-3 FA supplementation (p

196 < 0.0001).

197 Sensitivity and specificity of the genetic risk model were assessed with the area under

198 the ROC curve. The area under the curve was 0.9366 [95% confidence interval (CI):

199 0.8976, 0.9756] for the GRS solely, and 0.7537 [95% CI: 0.6721, 0.8353] for the sum of

200 other determinants (general model), including BMI, sex, age and baseline TG levels.

201 The addition of the GRS to the general model significantly increased the predictive

202 power (p<0.0001), for an area under the curve of 0.9455 [95% CI: 0.9084, 0.9826]

203 (**Figure 3**). After cross-validation, the area under the curve was 0.9187 for the GRS

204 alone and 0.9280 for the full adjusted model (data not shown).

205 As to the *FINGEN* replication study, there were 122 non-responders (39.7%) and 188

206 responders (60.3%), according to the same definition as mentioned above. Genotyping

207 in *FINGEN* participants was conducted on the 31 SNPs of the GRS. Among these 31

208 SNPs, eight were either monomorphic in the *FINGEN* cohort (rs61569932, rs1216346,

209 rs79624996, rs10009535 and rs76015249) or failed genotyping (rs6966968, rs78943417

210 and rs184945470), leaving 23 SNPs for GRS calculation. In the general linear model

211 adjusted for age, sex and BMI, this GRS was also significantly associated with the TG

212 response ($p = 0.0006$) and accounted for 3.67% of the TG change following the n-3 FA

213 supplementation ($p = 0.0006$). Regarding sensitivity and specificity, the areas under the

214 curve were 0.6417 [95% CI: 0.5795, 0.7039] for the GRS solely, 0.7109 [95% CI):

215 0.6530, 0.7688] for the general model and 0.7553 [95% CI: 0.7010, 0.8095] for the

216 general model + GRS (**Figure 4**).

217

DISCUSSION

219 The present study follows up a GWAS of the plasma TG response to an n-3 FA

220 supplementation in which a GRS was constructed (9). We used dense genotyping, as

221 well as genotype imputation to further increase the density of markers identified in the

222 GWAS. Association studies were performed and a new GRS was computed from

223 imputed and genotyped tagging SNPs to better predict the plasma TG response to an n-

224 3 FA supplementation. The previous GRS was computed using a total of ten GWAS hits

225 (9). Using genotype imputation and dense genotyping data, we were able to construct a

226 more refined GRS using 31 SNPs. As illustrated in **Figure 2**, we now have a clear

227 disparity between responders and non-responders regarding their number of carried at-

228 risk alleles. Non-responders have much higher GRS levels than responders. The 31-

229 SNPs GRS explains a much larger proportion of the TG variance (49.73%) than the 10-

230 SNPs GRS (21.53%) and its predictive capacity for classifying individuals into

231 responders and non-responders categories is also highly accurate. However, the GRS

232 poorly explained TG variance in the *FINGEN* population (3.67%) compared to *FAS*. This

233 is consistent with previous results of the 10-SNPs GRS, where only 2% of the TG

234 variation was explained by the GRS in the *FINGEN* population (nonsignificant)

235 compared to 21.53% in the *FAS* population (p = 0.0002) (9). This important divergence

236 could be explained by the eight missing SNPs in the GRS in the replication study. Also,

237 differences in allele frequency between the two populations might be a contributing

238 factor. Accordingly, several SNPs (rs62270407, rs10009109, rs76015249, rs61569932

239 and rs293180) showed significant differences in allele frequency between the *FAS* and

240 *FINGEN* populations. These differences can be explained by their ancestry background.

241 *FAS* study participants are French-Canadians of european descent, a more

242 homogeneous population with a founder effect while over 95% of *FINGEN* study

243 participants were British Caucasians (17). Moreover, the proportion of non-responders

244 among *FAS* participants is 29% vs almost 40% among *FINGEN* participants.

245 Despite the clear influence of SNPs on the responsiveness of TG levels to n-3 FA

246 supplementation, the exact mechanisms by which the six genes in GWAS-associated

247 loci contribute to TG variation still remain unclear. We previously demonstrated that

248 SNPs in GWAS-associated loci may exert their effect on TG levels by influencing gene

249 expression via modulation of DNA methylation (18). However, most of these genes are

250 poorly connected to lipid metabolism as detailed in our previous paper (10). This is

251 especially the case for *IQCJ-SCHIP1*. Nevertheless, even though *IQCJ-SCHIP1* has not

252 been explicitdly linked to lipid metabolism, we hypothesize that its action on the TG

253 response could be mediated through calcitonin action.

254 Accordingly, Martin PM *et al.* demonstrated that, in absence of calcium, *IQCJ* binds

255 calmodulin via its motif IQ, a sequence motif not shared with the *SCHIP1* segment (19,

256 20). Calmodulin is a messenger protein that normally binds to calcium to modulate its

257 action (21). However, other proteins, like IQ motif-containing proteins, also interact with

258 calmodulin in a calcium-independent way, more specifically when calcium levels are low

259 (21). Calmodulin is implicated in lipid metabolism via its effect on calcitonin (22), a

260 thyroid hormone that regulates calcium and phosphate in the blood (23). In a previous

261 study, it has been reported that the administration of calcitonin decreased LDL-

262 cholesterol and TG levels in rats, while the administration of a calmodulin inhibitor

263 prevented this effect, thus suggesting that calmodulin suppressed the action of calcitonin

264   (22). Werner and Low also observed that calcitonin inhibited lipolysis during basal and

265   stimulated lipolysis with parathyroid hormone, noradrenaline or dibutyryl cAMP

266   (bucladesine) in rat adipose tissue (24). One of the main mechanism of action of

267   calcitonin lies in the capacity of its receptor, a member of G-protein coupled receptors, to

268   couple with the cyclic adenosine monophosphate (cAMP) signal transduction (25).

269   cAMP is an ubiquitous second messenger implicated in lipid metabolism by activating

270   cAMP-dependent protein kinase, an enzyme that enhances TG hydrolysis in adipocytes

271   (26, 27). cAMP-dependent mechanisms also inhibit lipolysis during refeeding (27).

272   Moreover, dietary compounds, including calcium, caffeine and ethanol, can affect

273   adipocyte lipolysis through modulation of cAMP levels, with increased cAMP levels

274   resulting in stimulated lipolysis (27).

275   Another possible explanation is the presence of linkage disequilibrium between tagging

276   SNPs and other SNPs located in transcriptional units other than GWAS-associated loci.

277   As shown in **Table 3**, the majority of the 31 tagging SNPs are not located within GWAS-

278   associated loci, but are rather intergenic, and some GWAS-associated loci are located

279   close to genes of interest. For instance, *NXPH1* is located next to *ICA1*, a gene involved

280   in the pathogenesis of type 1 diabetes (28). A SNP of *ICA1*, along with several SNPs of

281   *NXPH1*, were identified in a GWAS of childhood obesity in an hispanic population (29).

282   These results not only highlight the importance of refining GWAS signals to properly

283   identify the most causative SNPs, but also reinforce the hypothesis that genetic profile is

284   a significant determinant of the metabolic response to an n-3 FA supplementation, and

285   these observations could probably extend to other dietary interventions. It is therefore

286   crucial to stop overlooking genetic factors for the assessment of responsiveness to such

287   interventions. For instance, a recently published meta-analysis of randomized clinical

288   trials on the association between n-3 FA supplement use and cardiovascular disease

289   concluded that n-3 FA consumption was not associated with a reduction of coronary

290   heart disease or major vascular events (30). Another recent meta-analysis of

291   randomized, double-blind, placebo-controlled trials on the efficacy of n-3 FA

292   consumption for the treatment of hypertriglyceridemia concluded that n-3 FA were

293   ineffective (31). Although n-3 FA consumption overall significantly reduced TG levels,

294   authors asserted that evidence regarding the effectiveness of n-3 FA in the management

295   of dislipidemia, especially hypertriglyceridemia, is inconclusive, in part because of the

296   heterogeneity in studies, low methodological quality of studies and small sample sizes

297   (31). As raised by Calder P. in a commentary on the meta-analysis, their findings are

298   very consistent with literature and actually clearly confirm that n-3 FA in supplemental

299   form can lower TG levels (32). Moreover, this inconclusiveness regarding the beneficial

300   effects of n-3 FA intake can probably be attributable to the lack of consideration of

301   contributors of the interindividual variability in the metabolic response to n-3 FA including

302   genetic factors. Based on our results, it appears that not all individuals can benefit from

303   the TG lowering effects of n-3 FA supplements. Future research should focus on

304   addressing the effects of n-3 FA on responders and non-responders separately by

305   stratifying subjects according to their TG response and taking determinants including

306   genetic factors into account.

307   In conclusion, this study further demonstrates the relevance of refining GWAS hits, here

308   providing a novel, refined GRS highly predictive of the responsiveness to n-3 FA.

309   Genetic profile appears to be a major determinant of the TG response to n-3 FA

310 supplementation. Future studies on n-3 FA and other nutrients should pay more

311 attention to the importance of genetic factors on the inter-individual variability in lipid

312 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.

329   References

330

331   1.   Talayero BG, Sacks FM. The role of triglycerides in atherosclerosis. Curr Cardiol Rep 2011;13:544-
332   52.

333   2.   Amiano P, Machon M, Dorronsoro M, Chirlaque MD, Barricarte A, Sanchez MJ, Navarro C, Huerta
334   JM, Molina-Montes E, Sanchez-Cantalejo E, et al. Intake of total omega-3 fatty acids,
335   eicosapentaenoic acid and docosahexaenoic acid and risk of coronary heart disease in the
336   Spanish EPIC cohort study. Nutr Metab Cardiovasc Dis 2014;24:321-7.

337   3.   Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid
338   supplementation and risk of major cardiovascular disease events: a systematic review and meta-
339   analysis. JAMA 2012;308:1024-33.

340   4.   Kotwal S, Jun M, Sullivan D, Perkovic V, Neal B. Omega 3 Fatty acids and cardiovascular
341   outcomes: systematic review and meta-analysis. Circ Cardiovasc Qual Outcomes 2012;5:808-18.

342   5.   Group ASC. Effects of n-3 Fatty Acid Supplements in Diabetes Mellitus. N Engl J Med 2018.

343   6.   Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, Kimber AC, Grew JP, Farrell L,
344   Stannard J, et al. Effect of sex and genotype on cardiovascular biomarker response to fish oils:
345   the FINGEN Study. Am J Clin Nutr 2008;88:618-29.

346   7.   Rudkowska I, Paradis AM, Thifault E, Julien P, Barbier O, Couture P, Lemieux S, Vohl MC.
347   Differences in metabolomic and transcriptomic profiles between responders and non-responders
348   to an n-3 polyunsaturated fatty acids (PUFAs) supplementation. Genes Nutr 2013;8:411-23.

349   8.   Masson LF, McNeill G, Avenell A. Genetic variation and the lipid response to dietary intervention:
350   a systematic review. Am J Clin Nutr 2003;77:1098-111.

351   9.   Rudkowska I, Guenard F, Julien P, Couture P, Lemieux S, Barbier O, Calder PC, Minihane AM, Vohl
352   MC. Genome-wide association study of the plasma triglyceride response to an n-3
353   polyunsaturated fatty acid supplementation. J Lipid Res 2014;55:1245-53.

354 10.   Vallee Marcotte B, Cormier H, Guenard F, Rudkowska I, Lemieux S, Couture P, Vohl MC. Novel
355   Genetic Loci Associated with the Plasma Triglyceride Response to an Omega-3 Fatty Acid
356   Supplementation. J Nutrigenet Nutrigenomics 2016;9:1-11.

357 11.   Cormier H, Rudkowska I, Paradis AM, Thifault E, Garneau V, Lemieux S, Couture P, Vohl MC.
358   Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma
359   triacylglycerol response to an n-3 PUFA supplementation. Nutrients 2012;4:1026-41.

360 12.   BURSTEIN M, SAMAILLE J. [On a rapid determination of the cholesterol bound to the serum
361   alpha- and beta-lipoproteins]. Clin Chim Acta 1960;5:609.

362 13.   McNamara JR, Schaefer EJ. Automated enzymatic standardized lipid analyses for plasma and
363   lipoprotein fractions. Clin Chim Acta 1987;166:1-8.

364 14.   Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the
365   next generation of genome-wide association studies. PLoS Genet 2009;5:e1000529.

366 15.   Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H, Mathers JC, Minihane AM. The
367   impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD)
368   risk to increased fish oil fatty acids intakes. Annu Rev Nutr 2011;31:203-34.

369 16.   LGC Limited. Version current 2017. Internet: https://www.lgcgroup.com/.

370 17.   Heyer E, Tremblay M. Variability of the genetic contribution of Quebec population founders
371   associated to some deleterious genes. Am J Hum Genet 1995;56:970-8.

372 18.   Vallee Marcotte B, Guenard F, Cormier H, Lemieux S, Couture P, Rudkowska I, Vohl MC. Plasma
373   Triglyceride Levels May Be Modulated by Gene Expression of IQCJ, NXPH1, PHF17 and MYB in
374   Humans. Int J Mol Sci 2017;18.

375 19.   Kwasnicka-Crawford DA, Carson AR, Scherer SW. IQCJ-SCHIP1, a novel fusion transcript encoding
376   a calmodulin-binding IQ motif protein. Biochem Biophys Res Commun 2006;350:890-9.

377 20. Martin PM, Carnaud M, Garcia del CG, Irondelle M, Irinopoulou T, Girault JA, Dargent B,
378   Goutebroze L. Schwannomin-interacting protein-1 isoform IQCJ-SCHIP-1 is a late component of
379   nodes of Ranvier and axon initial segments. J Neurosci 2008;28:6111-7.
380 21. Bahler M, Rhoads A. Calmodulin signaling via the IQ motif. FEBS Lett 2002;513:107-13.
381 22. Nishizawa Y, Okui Y, Inaba M, Okuno S, Yukioka K, Miki T, Watanabe Y, Morii H.
382   Calcium/calmodulin-mediated action of calcitonin on lipid metabolism in rats. J Clin Invest
383   1988;82:1165-72.
384 23. Felsenfeld AJ, Levine BS. Calcitonin, the forgotten hormone: does it deserve to be forgotten? Clin
385   Kidney J 2015;8:180-7.
386 24. Werner S, Low H. Inhibitory effects of calcitonin on lipolysis and 47 calcium accumulation in rat
387   adipose tissue in vivo. Horm Metab Res 1974;6:30-6.
388 25. Masi L, Brandi ML. Calcitonin and calcitonin receptors. Clin Cases Miner Bone Metab 2007;4:117-
389   22.
390 26. Halls ML, Cooper DM. Regulation by Ca2+-signaling pathways of adenylyl cyclases. Cold Spring
391   Harb Perspect Biol 2011;3:a004143.
392 27. Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes.
393   Annu Rev Nutr 2007;27:79-101.
394 28. Friday RP, Pietropaolo SL, Profozich J, Trucco M, Pietropaolo M. Alternative core promoters
395   regulate tissue-specific transcription from the autoimmune diabetes-related ICA1 (ICA69) gene
396   locus. J Biol Chem 2003;278:853-63.
397 29. Comuzzie AG, Cole SA, Laston SL, Voruganti VS, Haack K, Gibbs RA, Butte NF. Novel genetic loci
398   identified for the pathophysiology of childhood obesity in the Hispanic population. PLoS One
399   2012;7:e51954.
400 30. Aung T, Halsey J, Kromhout D, Gerstein HC, Marchioli R, Tavazzi L, Geleijnse JM, Rauch B, Ness A,
401   Galan P, et al. Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease
402   Risks: Meta-analysis of 10 Trials Involving 77917 Individuals. JAMA Cardiol 2018.
403 31. Nam GE MS, and Choi Y-J. Use of Omega-3 Fatty Acid Supplements Has Insufficient Clinical
404   Evidence for Treatment of Hypertriglyceridemia: A Meta-Analysis of Randomized, Double-Blind,
405   Placebo-Controlled Trials. Eur J Lipid Sci Technol, 2017;1-8.
406 32. C. CP. Commentary: New Meta-Analysis Confirms the Long Established Triacylglycerol-Lowering
407   Effect of Omega-3 Fatty Acids Given in Supplemental Form. European Journal of Lipid Science
408   and Technology 2017;119:1700239.

TABLES

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

Characteristics	Responders (n=81)			Non-responders (n=60)		
	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

Gene	SNP, rs number	Position, base pairs	Location	Alleles [1]	Minor allele frequency		P [2]	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.