Novel genetic loci associated with the plasma triglyceride response to an omega-3 fatty acid supplementation.

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ABSTRACT

Background: A recent genome-wide association study (GWAS) by our group identified 13 loci associated with the plasma triglyceride (TG) response to omega-3 (n-3) fatty acid (FA) supplementation. The present study aimed to test whether single nucleotide polymorphisms (SNPs) within the IQCJ, NXPH1, PHF17 and MYB genes are associated with the plasma TG response to an n-3 FA supplementation.

Methods: 208 subjects followed a 6-week n-3 FA supplementation of 5g/d of fish oil (1.9–2.2g of EPA and 1.1g of DHA). Measurements of plasma lipids were made before and after the supplementation. Tagged SNPs were selected to increase the density of markers near GWAS hits.

Results: In a repeated model, independent effects of the genotype and the gene by supplementation interaction were associated with plasma TG. Genotype effects were observed with two SNPs of NXPH1 and gene-diet interactions were observed with ten SNPs of IQCJ, four of NXPH1 and three of MYB. Positive and negative responders showed different genotype frequencies with nine SNPs of IQCJ, two of NXPH1 and two of MYB.

Conclusion: Fine mapping in GWAS-associated loci allowed the identification of SNPs explaining partly the large inter-individual variability observed in plasma TG levels in response to an n-3 FA supplementation.

Keywords: Gene-diet interactions, plasma lipid levels, omega-3 fatty acids, genome-wide association study, nutrigenetics.

BACKGROUND

It has been demonstrated that omega-3 (n-3) fatty acids (FA) from marine sources, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are beneficial in cardiovascular disease (CVD) prevention [1]. More specifically, the consumption of n-3 FA is associated with a decrease in plasma triglyceride (TG) levels, which are recognized to be a CVD risk factor [2]. However, there is an important and well-recognized inter-individual variability in the plasma TG response to an n-3 FA supplementation. Our research group has previously reported that 29 % of all participants of the *Fatty Acid Sensor* (*FAS*) *Study*, who received a 6-week n-3 FA supplementation of 5g of fish oil (1.9-2.2g EPA and 1.1g DHA) per day, did not reduce their plasma TG levels [3]. Likewise, in the *FINGEN* study, 31% of all volunteers did not show any reduction of their plasma TG levels after an 8-week supplementation of 1.8g of n-3 FA per day [4]. This heterogeneity in the plasma TG response may be partly due to genetic factors [5]. Numerous research groups around the world have been interested in the study of associations between polymorphisms (SNPs) in candidate genes such as the *apolipoprotein E* (*APOE*) or the *peroxisome proliferator-activated receptors* (*PPARs*) and their effect on plasma TG levels in response to n-3 FA [4;6-9]. However, these variations account for a very small proportion of the variance of the plasma TG levels in response to an n-3 FA supplementation. Other not yet identified genetic variations are thus likely to contribute to the heterogeneity of the response. Genome-wide association studies (GWAS)

provide a more complete and non-restrictive approach to test the impact of SNPs on a phenotype.

Recently, a GWAS performed by our research group on subjects of the *FAS Study* identified 13 SNPs associated with plasma TG levels [10]. A genetic risk score (GRS) constructed with these loci explained 21.53% of the variability in plasma TG levels in response to an n-3 FA supplementation. Most of these SNPs were located within or in the vicinity of *IQ motif containing J (IQCJ), Neurexophilin 1 (NXPH1), PHD finger protein 17 (PHF17) and V-MYB avian myeloblastosis viral oncogene homolog (MYB)* genes. *IQCJ* is mostly expressed in the brain and is part of an isoform of a transcriptional unit named *IQCJ-SCHIP1* that binds two distinct genes, *IQCJ* and *SCHIP1*, encoding different proteins. *IQCJ-SCHIP1* is implicated in the neuronal development, particularly of the nodes of Ranvier and the axon initial segment [11;12]. *NXPH1* encodes the Neurexophillin-1 protein, which binds to alpha-neurexins in the synapse and is implicated in neuronal regulation [13-15]. *PHF17* encodes the Jade-1 protein, which plays a role in cell growth, apoptosis and cancer [16-18]. Jade-1 is stabilized by the von Hippel-Lindau tumor suppressor [16]. It also interacts with HBO1 histone acetyltransferase (HAT) to promote acetylation of nucleosomal histones and to increase cell proliferation [18;19]. *MYB* encodes a transcriptional factor, whose role is mainly to regularize hematopoïesis, tumorigenesis and cell growth [20-22].

Thus, the objective of the present study was to increase the density of markers within the *IQCJ, NXPH1, PHF17* and *MYB* genes and to test their association

with plasma TG levels in response to an n-3 FA supplementation. The hypothesis is that genetic factors exert influence on plasma TG levels in response to an n-3 FA supplementation.

METHODS

Study population

A total of 254 subjects from the Quebec City metropolitan area were recruited between September 2009 and December 2011 to participate in the *FAS Study*. Recruitment was made via announcements in local newspapers and electronic messages sent to university students and employees. To be eligible, participants had to be aged between 18 and 50 years old and to have a body mass index (BMI) between 25 and 40 kg/m^2. They also had to be non-smokers, and be free of any thyroid or metabolic disorders requiring treatment such as diabetes, hypertension, dyslipidemia or coronary heart disease. Participants were excluded from the study if they had taken n-3 FA supplements for at least six months prior to the beginning of the study. In sum, 210 participants completed the intervention protocol. Two of them did not have plasma TG levels data available for further analyses, leaving 208 subjects included in the study sample. Statistical analyses were therefore conducted with 208 subjects. Subjects who completed the intervention protocol were divided into two subgroups: positive and negative responders to the n-3 FA supplementation. This classification was made according to the change of their plasma TG levels. Participants who had Δ TG $\geq$ 0 were considered as negative responders and those whose Δ TG was $<$ 0 were considered as positive responders.

Study design and diets

The whole study design and diets have been previously reported [10]. Briefly, subjects followed a run-in period of two weeks. During these two weeks, they

received individual dietary instructions by a trained registered dietitian to achieve the recommendations from *Canada's Food Guide to Healthy Eating*. Recommendations were given to ensure constant n-3 FA dietary intake and body weight stability throughout the protocol. After the run-in period, they received a bottle containing the needed n-3 FA capsules (Ocean Nutrition, Nova Scotia, Canada) that would be taken for the next six weeks. Each capsule contained 1 g of fish oil concentrate. They had to take five capsules a day, providing 3 g of n-3 FA, including 1.9 – 2.2 g of EPA and 1.1 g of DHA. Furthermore, they were asked to report any deviation from the protocol or experienced side effects, and to write down their alcohol intake as well as their fish consumption. They also received detailed oral and written instructions on their diet before each phase.

Laboratory methods

Plasma lipids

Methods to measure plasma lipids have previously been detailed [10]. Briefly, blood samples were collected after a 12h overnight fast and 48h alcohol abstinence. Blood samples were taken before the run-in period to verify whether individuals were presenting any metabolic disorders. The remaining participants who were eligible to the study had blood samples taken prior to and after the n-3 FA supplementation period. Enzymatic assays were used to measure plasma total cholesterol (TC) and TG concentrations [23;24].

SNP selection and genotyping

SNPs in *IQCJ, NXPH1, PHF17* and *MYB* were identified via the International HapMap Project SNP database, based on the National Center for Biotechnology information (NCBI) B36 assembly Data Rel 28, phase II + III, built 126. Gene Tagger procedure in the Haploview software v4.2 was used to determine tagged SNPs with a minor allele frequency (MAF) > 5% and pairwise tagging ($r^2 \geq 0.80$) located in gene regions and surrounding regions (2kb). The mean r^2 was 0.96 for *IQCJ*, 0.96 for *NXPH1*, 0.97 for *PHF17* and 0.95 for *MYB*. 16 tag SNPs in *IQCJ*, 34 in *NXPH1*, 8 in *PHF17* and 9 in *MYB* were selected, so that ≥ 85% of all common variations (MAF > 5%) in these genes were covered. The GenElute Gel Extraction Kit (Sigma-Aldrich Co., St. Louis, MO) was used to extract genomic DNA (gDNA) from the blood samples. Selected SNPs were genotyped using TaqMan technology. More specifically, 2.5 µL of each gDNA (40 ng/µL) and 2.5 µL of OpenArray Genotyper Master Mix (Life Technologies, Carlsbad, CA) were mixed with a validated primer in a 384-well plate and loaded onto the genotyping plates with the QuantStudio™ 12K Flex OpenArray® AccuFill™ System (Life Technologies). Genotyping was conducted on the QuantStudio™ 12K Flex Real-Time PCR System (Life Technologies). Thereafter, results were analysed in TaqMan Genotyper v1.3 (Life Technologies).

Statistical analyses

All statistical analyses were run in SAS Statistical Software v9.2 (SAS Institute, Cary, NC, USA), except for the ALLELE procedure, which was conducted using SAS Genetics v9.3. This procedure was used to test genotype distributions for any deviation from the Hardy-Weinberg equilibrium (HWE) and to calculate the

MAF. Normal distribution was evaluated with the box-plot, skewness and kurtosis ranges. Abnormally distributed variables were $\log_{10}$-transformed. Rare genotype homozygotes presenting a genotype frequency < 5% were merged with heterozygotes for statistical analyses (dominant model). Otherwise, all three genotypes were analysed separately as three groups, namely major allele homozygotes, heterozygotes and minor allele homozygotes (additive model). The MIXED procedure for repeated measures was used to test whether there was an effect of genotype, supplementation and genotype × supplementation (gene-diet) interaction on TG levels after the n-3 FA supplementation in a model adjusted for age, sex and BMI. The MIXED procedure for repeated measures was also used to test for significant differences of the study sample pre- vs post-supplementation in a model adjusted for age, sex and BMI. Finally, the FREQ procedure was used to verify differences in genotype frequency distribution between positive and negative responders to the n-3 FA supplementation. $P \leq 0.05$ was defined as the statistical significance threshold.

RESULTS

Table 1 shows allele frequencies of selected SNPs. All SNPs were in Hardy-Weinberg equilibrium and were therefore kept for further analyses. The percent coverage of each gene was 87% for *IQCJ*, 85% for *NXPH1*, 96% for *PHF17* and 100% for *MYB*. The vast majority of SNPs were located within introns. Three SNPs of *PHF17*, namely rs2217023, rs13143771 and rs13142964, were located in the upstream region of the gene and rs13148510 was located in the 3'UTR region. Finally, one SNP of *MYB* (rs210936) was located in the downstream region of the gene.

Baseline characteristics of the subjects are shown in **Table 2**. The average BMI pre- and post-supplementation of all participants were above 25 kg/m^2 as specified by the study design. The average plasma TG levels before the supplementation were above the cut-point value of 1.129 mmol/L according to the American Heart Association (AHA) for optimal plasma TG levels [2].

For each selected SNP, we tested the independent effect of genotype, supplementation (time) and genotype × supplementation (interaction) on plasma TG levels (**Table 3**). First, as expected, the supplementation alone had a significant effect on plasma TG levels ($p < 0.006$, for all). Second, two SNPs of *NXPH1* (rs2107779 and rs2107474) were associated with TG levels ($p < 0.05$, for both) during the supplementation. Two SNPs of *NXPH1* and another of *MYB* had marginal but not significant effects on TG levels ($p < 0.1$, for all). Third, significant gene-diet interaction effects were observed with ten SNPs of *IQCJ* (rs2044704, rs1962071, rs6800211, rs17782879, rs1868414, rs2595260, rs9827242,

rs1449009, rs2621309 and rs61332355), four SNPs of *NXPH1* (rs7806226, rs7805772, rs2349780 and rs6974252) and three SNPs of *MYB* (rs9321493, rs11154794 and rs210962) ($p < 0.05$, for all). Marginal effects of gene-diet interactions were observed with two additional SNPs, located in *IQCJ* and *NXPH1* respectively ($p < 0.1$, for both). Neither genotype effects nor gene-diet interaction effects were found with SNPs of the *PHF17* gene.

Significant differences were observed in the genotype distribution between positive and negative responders for several SNPs in *IQCJ*, *NXPH1* and *MYB* as shown in **Table 4.** Globally, higher frequencies of minor allele were observed in negative responders while for two SNPs, rs1868414 and rs9827242, there was a higher frequency of carriers of the minor allele among positive responders vs. negative responders. There was no significant difference in genotype distribution with SNPs from *PHF17*.

DISCUSSION

In the present study, we narrowed down regions previously identified in a GWAS performed on the *FAS Study* by increasing the density of markers in *IQCJ, NXPH1, PHF17* and *MYB*, and testing for associations with plasma TG levels during the supplementation. To do so, 67 SNPs were selected in order to cover ≥ 85% of the genetic variability near GWAS hits. This study is the first to report on follow-up of GWAS signals associated with plasma TG levels in a supplementation protocol with n-3 FAs.

In this study, it was observed that the n-3 FA supplementation alone induced a decrease of plasma TG levels, as previously reported [4]. Furthermore, two SNPs were independently associated with plasma TG levels and significant gene-diet interaction effects were found with 17 SNPs. 13 SNPs had different genotype frequency distribution between positive and negative responders. Results in **Tables 3** and **4** have some similarities. Indeed, nine out of the ten SNPs whose genotype frequency distribution was significantly different between positive and negative responders have either been found to exert significant influence on plasma TG levels or to show gene-diet interaction with the supplementation.

Most of these associations were observed with SNPs in the *IQCJ* and *NXPH1* genes. Both of these genes are mainly known to be expressed in the brain and to participate in the nervous system functions. The *IQCJ* gene alone has been barely studied. *IQCJ* is bound to *SCHIP1* to form a fusion gene called *IQCJ-SCHIP1* and up to now, the vast majority of researches have been done on the

whole *IQCJ-SCHIP1* segment. Nevertheless, it has been demonstrated that the IQCJ-SCHIP1 protein contains a calmodulin-binding IQ motif at the N-terminus that SCHIP1 does not have hereby suggesting that the latter has a different biological role [11]. *SCHIP1* has also been associated with neurofibromatosis type 2 (NF2), a tumor suppressor protein [11]. According to the literature, *IQCJ-SCHIP1* is mostly involved in the neuronal function and its protein has been observed in cytoplasm, actin-rich regions, differentiated PC12 cells and neurite extensions [11;12]. As to *NXPH1*, it is a member of the neurexophilin family, composed of four genes encoding for related glycoproteins that act as neuropeptides and interact with neurexins [13-15].

For two of these genes, *IQCJ* and *NXPH1*, very few associations with plasma lipid levels have been reported. It has recently been reported in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study, which aimed to investigate SNPs responsible of the heterogeneity observed in the response to a lipid-lowering therapy with fenofibrate, that these two genes may actually play a role in lipid metabolism [25]. Indeed, Kraja et al. conducted a GWAS and demonstrated that one SNP in *IQCJ-SCHIP1* (rs6805526) was associated with very low-density lipoprotein (VLDL) particle clearance and TG levels [25]. It is well known that a decrease of TG synthesis through fish oil rich in n-3 FAs is also associated with a decrease of VLDL secretion from the liver [26]. Moreover, fenofibrate and n-3 FA have similar mechanisms of action as they can both activate PPAR-alpha, a nuclear receptor involved in the regulation of intra- and extracellular lipid metabolism, and have beneficial effects on inflammation

[27;28]. Like n-3 FAs, fenofibrate therapy is used to lower plasma TG levels and its effectiveness to reduce the risk of CVD events for patients with hypertriglyceridemia has been demonstrated [29]. However, one can notice that rs6805526 is located in the *SCHIP1* section of *IQCJ-SCHIP1* and is thus not part of *IQCJ* alone. rs6805526 is not in LD with any SNP in *IQCJ* either. In the present study, we tested SNPs in *IQCJ* only and not *SCHIP1*. Nonetheless, because *IQCJ* and *SHIP1* are comprised within a single fusion transcript (*IQCJ-SCHIP1*), they are closely connected [11]. Even if the physiological roles of *IQCJ* and its impact on lipid metabolism remain unclear, these findings suggest that both *IQCJ* and *SCHIP1* are very likely to play a role in lipid metabolism.

In the same GWAS (Kraja et al.), several SNPs of *NXPH1* were associated with low-density lipoprotein LDL, high-density lipoprotein (HDL), TG levels, type 2 diabetes, C-reactive protein and blood pressure [25]. Another SNP in the vicinity of this gene, rs10952132, was strongly associated with LDL peak particle diameter and it is known that plasma TG concentrations influence the LDL particle size [30;31]. Comuzzie et al. found an association between *NXPH1* and some features of diabetes in a population of Hispanic children [32]. All these findings support the association between *NXPH1* and plasma lipids, but yet very little is known about the underlying mechanisms.

Furthermore, several significant results were observed with the *MYB* gene. It is recognized that *MYB* participates in leukemogenesis and hematopoiesis [33]. *MYB* is also known to promote cell proliferation, to suppress apoptosis and to block differentiation [33]. Gross et al. stated that this gene may be associated

with CVD development and intracellular lipid accumulation [34]. C-myb messenger RNA is expressed in vascular smooth cells and c-myb is induced in response to homocysteine [34]. High levels of homocysteine are an independent cardiovascular risk factor [35]. A recent study showed that one SNP in *MYB*, rs9494145, might be associated with some features of obesity such as BMI, even though no significant association was observed [36]. Besides that, some studies have reported that *MYB* may be involved in intestinal nutrient absorption and adipogenic differentiation in mesenchymal stem cells [37;38].

CONCLUSION

In sum, results from the present study suggest that the heterogeneity observed in plasma TG levels following an n-3 FA supplementation might be partly explained by SNPs within the *IQCJ*, *NXPH1* and *MYB* genes. Further investigations will be necessary to provide a better understanding of the underlying mechanisms through which these genes may exert their effects on plasma lipid levels.

COMPETING INTERESTS

The authors declare no competing interests

AUTHORS' CONTRIBUTIONS

BVM conducted genotyping and wrote the paper; BVM, HC and FG performed statistical analysis; IR, SL and MCV designed research; PC was responsible for the medical follow-up; BVM and MCV have primary responsibility for final content. All authors read and approved the final manuscript.

CONSENT

The 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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Bibliography

1. Marik PE, Varon J: Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. Clin Cardiol 2009;32:365-372.

2. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, Goldberg AC, Howard WJ, Jacobson MS, Kris-Etherton PM, Lennie TA, Levi M, Mazzone T, Pennathur S: Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 2011;123:2292-2333.

3. Cormier H, Rudkowska I, Paradis AM, Thifault E, Garneau V, Lemieux S, Couture P, Vohl MC: Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an n-3 PUFA supplementation. Nutrients 2012;4:1026-1041.

4. Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, Kimber AC, Grew JP, Farrell L, Stannard J, Napper FL, Sala-Vila A, West AL, Mathers JC, Packard C, Williams CM, Calder PC, Minihane AM: Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr 2008;88:618-629.

5. Masson LF, McNeill G, Avenell A: Genetic variation and the lipid response to dietary intervention: a systematic review. Am J Clin Nutr 2003;77:1098-1111.

6. Thifault E, Cormier H, Bouchard-Mercier A, Rudkowska I, Paradis AM, Garneau V, Ouellette C, Lemieux S, Couture P, Vohl MC: Effects of age, sex, body mass index and APOE genotype on cardiovascular biomarker response to an n-3 polyunsaturated fatty acid supplementation. J Nutrigenet Nutrigenomics 2013;6:73-82.

7. Minihane AM, Khan S, Leigh-Firbank EC, Talmud P, Wright JW, Murphy MC, Griffin BA, Williams CM: ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. Arterioscler Thromb Vasc Biol 2000;20:1990-1997.

8. Tai ES, Demissie S, Cupples LA, Corella D, Wilson PW, Schaefer EJ, Ordovas JM: Association between the PPARA L162V polymorphism and plasma lipid levels: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 2002;22:805-810.

9. Lindi V, Schwab U, Louheranta A, Laakso M, Vessby B, Hermansen K, Storlien L, Riccardi G, Rivellese A: Impact of the Pro12Ala polymorphism of the PPAR-gamma2 gene on serum triacylglycerol response to n-3 fatty acid supplementation. Mol Genet Metab 2003;79:52-60.

10. Rudkowska I, Guenard F, Julien P, Couture P, Lemieux S, Barbier O, Calder PC, Minihane AM, Vohl MC: Genome-wide association study of the plasma triglyceride response to an n-3 polyunsaturated fatty acid supplementation. J Lipid Res 2014;55:1245-1253.

11. Kwasnicka-Crawford DA, Carson AR, Scherer SW: IQCJ-SCHIP1, a novel fusion transcript encoding a calmodulin-binding IQ motif protein. Biochem Biophys Res Commun 2006;350:890-899.

12. Martin PM, Carnaud M, Garcia del CG, Irondelle M, Irinopoulou T, Girault JA, Dargent B, Goutebroze L: Schwannomin-interacting protein-1 isoform IQCJ-SCHIP-1 is a late component of nodes of Ranvier and axon initial segments. J Neurosci 2008;28:6111-6117.

13. Missler M, Hammer RE, Sudhof TC: Neurexophilin binding to alpha-neurexins. A single LNS domain functions as an independently folding ligand-binding unit. J Biol Chem 1998;273:34716-34723.

14. Missler M, Sudhof TC: Neurexophilins form a conserved family of neuropeptide-like glycoproteins. J Neurosci 1998;18:3630-3638.

15. Reissner C, Stahn J, Breuer D, Klose M, Pohlentz G, Mormann M, Missler M: Dystroglycan binding to alpha-neurexin competes with neurexophilin-1 and neuroligin in the brain. J Biol Chem 2014;289:27585-27603.

16. Zhou MI, Foy RL, Chitalia VC, Zhao J, Panchenko MV, Wang H, Cohen HT: Jade-1, a candidate renal tumor suppressor that promotes apoptosis. Proc Natl Acad Sci U S A 2005;102:11035-11040.

17. Doyon Y, Cayrou C, Ullah M, Landry AJ, Cote V, Selleck W, Lane WS, Tan S, Yang XJ, Cote J: ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. Mol Cell 2006;21:51-64.

18. Foy RL, Song IY, Chitalia VC, Cohen HT, Saksouk N, Cayrou C, Vaziri C, Cote J, Panchenko MV: Role of Jade-1 in the histone acetyltransferase (HAT) HBO1 complex. J Biol Chem 2008;283:28817-28826.

19. Siriwardana NS, Meyer R, Havasi A, Dominguez I, Panchenko MV: Cell cycle-dependent chromatin shuttling of HBO1-JADE1 histone acetyl transferase (HAT) complex. Cell Cycle 2014;13:1885-1901.

20. Vanhee S, De MK, Van CY, Verstichel G, Van RN, Menten B, Velghe I, Philippe J, De BD, Lambrecht BN, Taghon T, Leclercq G, Kerre T, Vandekerckhove B: In vitro human embryonic stem cell hematopoiesis mimics MYB-independent yolk sac hematopoiesis. Haematologica 2015;100:157-166.

21. Sarvaiya PJ, Schwartz JR, Hernandez CP, Rodriguez PC, Vedeckis WV: Role of c-Myb in the survival of pre B-cell acute lymphoblastic leukemia and leukemogenesis. Am J Hematol 2012;87:969-976.

22. Costa AF, Altemani A, Garcia-Inc, Fresno F, Suarez C, Llorente JL, Hermsen M: Analysis of MYB oncogene in transformed adenoid cystic carcinomas reveals distinct pathways of tumor progression. Lab Invest 2014;94:692-702.

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

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

25. Kraja AT, Borecki IB, Tsai MY, Ordovas JM, Hopkins PN, Lai CQ, Frazier-Wood AC, Straka RJ, Hixson JE, Province MA, Arnett DK: Genetic analysis of 16 NMR-lipoprotein fractions in humans, the GOLDN study. Lipids 2013;48:155-165.

26. Kiefer D, Pantuso T: Omega-3 fatty acids: An update emphasizing clinical use. Agro Food Ind Hi Tech 2012;23:10-13.

27. Delerive P, Fruchart JC, Staels B: Peroxisome proliferator-activated receptors in inflammation control. J Endocrinol 2001;169:453-459.

28. Bragt MC, Mensink RP: Comparison of the effects of n-3 long chain polyunsaturated fatty acids and fenofibrate on markers of inflammation and vascular function, and on the serum lipoprotein profile in overweight and obese subjects. Nutr Metab Cardiovasc Dis 2012;22:966-973.

29. Lee M, Saver JL, Towfighi A, Chow J, Ovbiagele B: Efficacy of fibrates for cardiovascular risk reduction in persons with atherogenic dyslipidemia: a meta-analysis. Atherosclerosis 2011;217:492-498.

30. Frazier-Wood AC, Aslibekyan S, Borecki IB, Hopkins PN, Lai CQ, Ordovas JM, Straka RJ, Tiwari HK, Arnett DK: Genome-wide association study indicates variants associated with insulin signaling and inflammation mediate lipoprotein responses to fenofibrate. Pharmacogenet Genomics 2012;22:750-757.

31. Kondo A, Muranaka Y, Ohta I, Notsu K, Manabe M, Kotani K, Saito K, Maekawa M, Kanno T: Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. Clin Chem 2001;47:893-900.

32. Comuzzie AG, Cole SA, Laston SL, Voruganti VS, Haack K, Gibbs RA, Butte NF: Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. PLoS One 2012;7:e51954.

33. Zhao L, Ye P, Gonda TJ: The MYB proto-oncogene suppresses monocytic differentiation of acute myeloid leukemia cells via transcriptional activation of its target gene GFI1. Oncogene 2014;33:4442-4449.

34. Gross CM, Kramer J, Pfeufer A, Dietz R, Gessner R, Praus M: The intron 6 G/T polymorphism of c-myb oncogene and the risk for coronary in-stent restenosis. Basic Res Cardiol 2004;99:309-314.

35. Lai WK, Kan MY: Homocysteine-Induced Endothelial Dysfunction. Ann Nutr Metab 2015;67:1-12.

36. Melen E, Himes BE, Brehm JM, Boutaoui N, Klanderman BJ, Sylvia JS, Lasky-Su J: Analyses of shared genetic factors between asthma and obesity in children. J Allergy Clin Immunol 2010;126:631-637.

37. Xu H, Inouye M, Hines ER, Collins JF, Ghishan FK: Transcriptional regulation of the human NaPi-IIb cotransporter by EGF in Caco-2 cells involves c-myb. Am J Physiol Cell Physiol 2003;284:C1262-C1271.

38. Chen Y, Liu J, Xu H, Luo Z, Zhang C: Overexpression of the c-Myb but not its leukemogenic mutant DNA-binding domain increased adipogenic differentiation in mesenchymal stem cells. Biochem Biophys Res Commun 2011;407:202-206.

Table 1. Selected polymorphisms in candidate genes from the *FAS Study* GWAS (n = 210)

Gene	dbSNP No.	Sequence	Function	Allele frequency	
IQCJ	rs12497650	TTT**[C/T]**ATTG	Intron	C (n = 286) 0.6810	T (n = 134) 0.3190
	rs4501157	ACA**[G/T]**TAA	Intron	G (n = 147) 0.3517	T (n = 271) 0.6483
	rs13091349	TCT**[C/T]**CTC	Intron	C (n = 350) 0.8333	T (n = 70) 0.1667
	rs2044704	TTT**[C/G]**TAG	Intron	C (n = 108) 0.2571	G (n = 312) 0.7429
	rs1962071	AGC**[A/C]**GCC	Intron	A (n = 306) 0.7286	C (n = 114) 0.2714
	rs7634829	TGT**[A/G]**TAA	Intron	A (n = 237) 0.5643	G (n = 183) 0.4357
	rs2621294	TGC**[A/G]**AAG	Intron	A (n = 260) 0.6220	G (n = 158) 0.3780
	rs6800211	AGG**[C/T]**GTC	Intron	C (n = 298) 0.7095	T (n = 122) 0.2905
	rs17782879	TCC**[A/G]**TAT	Intron	A (n = 128) 0.3048	G (n = 292) 0.6952
	rs1868414	CTG**[C/T]**GCC	Intron	C (n = 281) 0.6722	T (n = 137) 0.3278
	rs2595260	AGG**[C/T]**ATC	Intron	C (n = 316) 0.7524	T (n = 104) 0.2476
	rs6763890	ATG**[A/T]**CTT	Intron	A (n = 141) 0.3373	T (n = 277) 0.6627
	rs9827242	TCA**[C/T]**AGT	Intron	C (n = 78) 0.1857	T (n = 342) 0.8143
	rs1449009*	CAA**[C/T]**ATT	Intron	A (n = 295) 0.7091	G (n = 121) 0.2909
	rs2621309*	TTT**[C/G]**CTT	Intron	C (n = 300) 0.7211	G (n = 116) 0.2788
	rs61332355*	AGG**[A/C]**AAT	Intron	A (n = 75) 0.1803	C (n = 341) 0.8197
NXPH1	rs6956210	TTC**[C/T]**TTT	Intron	C (n = 99) 0.2357	T (n = 321) 0.7643
	rs2107779	ATG**[C/T]**TGA	Intron	C (n = 232) 0.5524	T (n = 188) 0.4476
	rs10273195	CTG**[A/T]**GGC	Intron	A (n = 336) 0.8000	T (n = 84) 0.2000
	rs12216689	TGA**[A/C]**TGA	Intron	A (n = 301) 0.7167	C (n = 119) 0.2833
	rs6963644	TGC**[A/G]**TTT	Intron	A (n = 32) 0.0762	G (n = 388) 0.9238
	rs17150341	AGG**[C/T]**ATT	Intron	C (n = 292) 0.6952	T (n = 128) 0.3048
	rs1013868	TTC**[A/G]**CTG	Intron	C (n = 281) 0.6690	T (n = 139) 0.3310
	rs12537067	CTA**[A/G]**CTC	Intron	A (n = 35) 0.0833	G (n = 385) 0.9167
	rs4318981	CAT**[C/T]**ATA	Intron	C (n = 150) 0.3571	T (n = 270) 0.6429
	rs17153997	GTG**[C/T]**GTA	Intron	C (n = 238) 0.5694	T (n = 180) 0.4306
	rs7801099	AAC**[A/G]**ACA	Intron	A (n = 233) 0.5548	G (n = 187) 0.4452
	rs4725120	ATA**[A/G]**AAG	Intron	A (n = 193) 0.4595	G (n = 227) 0.5405
	rs1859275	GTG**[A/G]**CTA	Intron	A (n = 147) 0.3517	G (n = 271) 0.6483
	rs10238726	CTC**[A/G]**TTC	Intron	A (n = 288) 0.6857	G (n = 132) 0.3143
	rs1012960	GTT**[A/T]**CTT	Intron	A (n = 210) 0.5000	T (n = 210) 0.5000
	rs11767429	ACA**[A/G]**GAG	Intron	A (n = 294) 0.7000	G (n = 126) 0.3000
	rs4333500	AAG**[G/T]**TGG	Intron	G (n = 253) 0.6024	T (n = 167) 0.3976
	rs7793115	GGA**[A/G]**GAG	Intron	A (n = 41) 0.0981	G (n = 377) 0.9019
	rs7799856	CAA**[A/C]**AAA	Intron	A (n = 240) 0.5714	C (n = 180) 0.4286
	rs7806226	ATA**[A/C]**TAT	Intron	A (n = 354) 0.8429	C (n = 66) 0.1571

	rs13221144	CCT**[C/T]**ACG	Intron	C (n = 97) 0.2310	T (n = 323) 0.7690
	rs17406479	TTG**[G/T]**TTT	Intron	G (n = 341) 0.8119	T (n = 79) 0.1881
	rs10486228	AAA**[C/T]**TGA	Intron	C (n = 76) 0.1810	T (n = 344) 0.8190
	rs17154569	TCT**[A/G]**AGA	Intron	A (n = 344) 0.8230	G (n = 74) 0.1770
	rs4141002	CAC**[C/T]**TGT	Intron	C (n = 368) 0.8762	T (n = 52) 0.1238
	rs7805772	GAG**[A/G]**TCC	Intron	A (n = 338) 0.8086	G (n = 80) 0.1914
	rs2349780	ACA**[A/G]**TGG	Intron	A (n = 260) 0.6190	G (n = 160) 0.3810
	rs2107474	CTT**[C/G]**AAC	Intron	C (n = 242) 0.5762	G (n = 178) 0.4238
	rs11769942	TTC**[C/T]**GAC	Intron	C (n = 263) 0.6262	T (n = 157) 0.3738
	rs6952383	CTA**[A/T]**TCT	Intron	A (n = 378) 0.9000	T (n = 42) 0.1000
	rs6974252	GTA**[A/G]**TTG	Intron	A (n = 59) 0.1405	G (n = 361) 0.8595
	rs10265408	CTG**[C/G]**ATG	Intron	C (n = 303) 0.7249	G (n = 115) 0.2751
	rs2189904	TAC**[C/T]**TTT	Intron	C (n = 281) 0.6690	T (n = 139) 0.3310
	rs2057862	AGA**[C/G]**TGT	Intron	C (n = 171) 0.4091	G (n = 247) 0.5909
PHF17	rs2217023	GCA**[C/G]**ATG	nearGene-5’	C (n = 81) 0.1938	G (n = 337) 0.8062
	rs4975270	TAC**[A/G]**CAT	intron	A (n = 240) 0.5714	G (n = 180) 0.4286
	rs11722830	CTT**[A/G]**TGG	Intron	A (n = 89) 0.2119	G (n = 331) 0.7881
	rs12505447	CCT**[C/T]**GGG	Intron	C (n = 341) 0.8119	T (n = 79) 0.1881
	rs6534704	GTA**[A/T]**CCT	Intron	A (n = 32) 0.0762	T (n = 388) 0.9238
	rs13148510	CAA**[C/G]**AGC	3'UTR	C (n = 403) 0.9595	G (n = 17) 0.0405
	rs13143771	ATA**[C/T]**AGT	nearGene-5’	C (n = 119) 0.2833	T (n = 301) 0.7167
	rs13142964	TAA**[C/G]**AAC	nearGene-5’	C (n = 389) 0.9262	G (n = 31) 0.0738
MYB	rs9321493	GTA**[C/T]**ACT	Intron	C (n = 233) 0.5548	T (n = 187) 0.4452
	rs11154794	AGC**[C/T]**TCC	Intron	C (n = 53) 0.1262	T (n = 367) 0.8738
	rs210798	GAA**[G/T]**TCC	Intron	G (n = 245) 0.5833	T (n = 175) 0.4167
	rs210936	CTT**[C/T]**TCC	nearGene-3’	A (n = 218) 0.5190	G (n = 202) 0.4810
	rs7757388	ATA**[A/G]**AAG	Intron	A (n = 351) 0.8397	G (n = 67) 0.1603
	rs210962	AGA**[C/T]**CCT	Intron	C (n = 318) 0.7608	T (n = 100) 0.2392
	rs17639758	GTA**[A/G]**CAT	Intron	A (n = 11) 0.0262	G (n = 409) 0.9738
	rs1013891	TAC**[A/G]**GCA	Intron	A (n = 146) 0.3476	G (n = 274) 0.6524
	rs2179308	GGT**[A/G]**TTG	Intron	A (n = 214) 0.5095	G (n = 206) 0.4905

FAS: Fatty Acid Sensor

Allelic frequencies were obtained using the ALLELE Procedure (SAS Genetics v9.3).

* GWAS hit identified in the *FAS study*

Table 2. Characteristics of the study sample pre- and post-supplementation (n = 208)

Characteristics	Pre	Post	*P*[a]
Study population, n	210	208	-
Age, years	30.8 ± 8.7	-	-
Weight, kg[b, c]	81.3 ± 13.9	81.6 ± 14.2	0.0009
BMI, kg/m^2 [b, c]	27.8 ± 3.7	27.9 ± 3.8	0.005
TG, mmol/L[b, d]	1.21 ± 0.63	1.02 ± 0.52	< 0.0001

Values are means ± SD. *P < 0.05

[b]P values are for $\log_{10}$-transformed values

[c]P values adjusted for age and sex

[d]P values adjusted for age, sex and BMI

Table 3. Plasma TG levels before and after a 6-week n-3 FA supplementation according to genotype for tagged SNPs with marginal or significant effect of genotype or gene-diet interaction (n = 208)

		Pre-n-3 FA supplementation[a]			Post-n-3 FA supplementation[a]			*P* values		
		11	12	22	11	12	22	Genotype	Supplementation	Interaction
IQCJ	rs12497650	1.175 ± 0.664	1.224 ± 0.554	1.293 ± 0.811	1.034 ± 0.563	1.010 ± 0.484	1.004 ± 0.555	0.96	<0.0001	0.09
	rs2044704	1.184 ± 0.538	1.266 ± 0.726	1.157 ± 0.791	0.957 ± 0.466	1.110 ± 0.633	1.103 ± 0.397	0.52	<0.0001	0.004
	rs1962071	1.262 ± 0.619	1.168 ± 0.677	1.051 ± 0.455	1.023 ± 0.509	1.041 ± 0.594	0.930 ± 0.304	0.74	<0.0001	0.03
	rs6800211	1.164 ± 0.590	1.250 ± 0.623	1.253 ± 0.881	1.038 ± 0.479	1.004 ± 0.569	1.003 ± 0.568	0.82	<0.0001	0.01
	rs17782879	1.251 ± 0.688	1.165 ± 0.543	1.185 ± 0.680	1.001 ± 0.532	1.016 ± 0.493	1.138 ± 0.624	0.43	<0.0001	0.01
	rs1868414	1.195 ± 0.707	1.208 ± 0.492	1.255 ± 0.714	1.055 ± 0.560	0.998 ± 0.503	0.966 ± 0.472	0.97	<0.0001	0.03
	rs2595260	1.202 ± 0.550	1.235 ± 0.708	1.157 ± 0.835	0.973 ± 0.485	1.108 ± 0.605	1.027 ± 0.454	0.67	<0.0001	0.01
	rs9827242[b]	1.212 ± 0.679	1.202 ± 0.527	-	1.055 ± 0.556	0.956 ± 0.458	-	0.85	<0.0001	0.02
	rs1449009*	1.201 ± 0.557	1.229 ± 0.683	1.173 ± 0.777	0.960 ± 0.483	1.082 ± 0.598	1.105 ± 0.419	0.83	<0.0001	0.002
	rs2621309*	1.194 ± 0.549	1.232 ± 0.699	1.201 ± 0.784	0.955 ± 0.476	1.090 ± 0.608	1.129 ± 0.414	0.67	<0.0001	0.002
	rs61332355[b]*	1.240 ± 0.592	1.145 ± 0.697	-	0.999 ± 0.485	1.064 ± 0.597	-	0.81	<0.0001	0.0002
NXPH1	rs2107779	1.347 ± 0.761	1.163 ± 0.516	1.094 ± 0.588	1.094 ± 0.508	0.995 ± 0.511	0.962 ± 0.572	0.05	<0.0001	0.55
	rs12216689	1.209 ± 0.675	1.194 ± 0.571	1.277 ± 0.622	1.044 ± 0.510	0.998 ± 0.487	0.979 ± 0.783	0.76	<0.0001	0.08
	rs1013868	1.268 ± 0.590	1.106 ± 0.568	1.400 ± 0.925	1.059 ± 0.543	0.948 ± 0.453	1.175 ± 0.688	0.08	<0.0001	0.52
	rs11767429	1.270 ± 0.724	1.085 ± 0.509	1.373 ± 0.431	1.096 ± 0.592	0.895 ± 0.382	1.123 ± 0.562	0.08	<0.0001	0.33
	rs7806226[b]	1.259 ± 0.671	1.072 ± 0.471	-	1.025 ± 0.529	1.007 ± 0.517	-	0.27	<0.0001	0.004
	rs7805772	1.266 ± 0.689	1.066 ± 0.408	1.165 ± 0.681	1.022 ± 0.529	0.944 ± 0.393	1.290 ± 0.849	0.28	0.006	0.002
	rs2349780	1.120 ± 0.621	1.254 ± 0.614	1.272 ± 0.703	1.006 ± 0.562	1.042 ± 0.509	0.973 ± 0.489	0.55	<0.0001	0.04
	rs2107474	1.156 ± 0.506	1.106 ± 0.567	1.383 ± 0.733	1.052 ± 0.464	0.913 ± 0.486	1.157 ± 0.578	0.01	<0.0001	0.26
	rs6974252[b]	1.241 ± 0.646	1.114 ± 0.571	-	1.021 ± 0.519	1.020 ± 0.546	-	0.23	<0.0001	0.03
MYB	rs9321493	1.191 ± 0.698	1.243 ± 0.651	1.158 ± 0.448	1.076 ± 0.589	1.032 ± 0.543	0.910 ± 0.340	0.52	<0.0001	0.04
	rs11154794[b]	1.229 ± 0.588	1.142 ± 0.748	-	1.024 ± 0.549	1.010 ± 0.442	-	0.61	<0.0001	0.02
	rs210962	1.168 ± 0.605	1.323 ± 0.688	1.027 ± 0.497	1.031 ± 0.533	1.060 ± 0.548	0.774 ± 0.270	0.08	<0.0001	0.05

The MIXED procedure (SAS v9.2) for repeated measures adjusted for age, sex and BMI was used to test for the interaction effects

P values are derived from $\log_{10}$-transformed TG levels

11: common genotype homozygotes, 12: heterozygotes, 22: rare genotype homozygotes

[a] Values are mean ± SD

[b] 12 were merged with 22 (dominant model)

* GWAS hit identified in the *FAS Study*

Table 4. Significant differences in genotype frequency distribution between positive and negative responders to the n-3 FA supplementation according to genotype for tagged SNPs (n=208)

		Positive responders[a]			Negative responders[a]			
		11	**12**	**22**	**11**	**12**	**22**	***P* value[b]**
IQCJ	rs2044704	64.9	29.7	5.4	41.7	40.0	18.3	0.001
	rs1962071	60.8	32.4	6.8	40.0	43.3	16.7	0.01
	rs17782879	52.7	41.2	6.1	36.7	45.0	18.3	0.01
	rs1868414	40.8	42.9	16.3	63.3	30.0	6.7	0.01
	rs2595260	66.2	27.0	6.8	43.3	43.3	13.3	0.01
	rs9827242[c]	58.8	41.2	-	80.0	20.0	-	0.004
	rs1449009*	59.5	34.5	6.1	35.0	43.3	21.7	0.0004
	rs2621309*	62.2	31.8	6.1	35.0	45.0	20.0	0.0003
	rs61332355[c]*	25.0	75.0	-	53.3	46.7	-	<0.0001
NXPH1	rs7806226[c]	79.1	21.0	-	58.3	41.7	-	0.002
	rs7805772	73.0	25.0	2.0	54.2	30.5	15.3	0.0005
MYB	rs11154794[c]	81.1	18.9	-	65.0	35.0	-	0.01
	rs210936	25.7	52.0	22.3	36.7	33.3	30.0	0.05

The FREQ procedure (SAS v9.2) was used to test the differences in allele frequency distribution

11: common genotype homozygotes, 12: heterozygotes, 22: rare genotype homozygotes

[a] Relative values in percent

[b] Chi-square test in SAS v9.2

[c] 12 were merged with 22 (dominant model)

* GWAS hit identified in the *FAS Study*