Plasma Triglyceride Levels May Be Modulated by Gene Expression of IQCJ, NXPH1, PHF17 and MYB in Humans

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Academic Editors: Lynnette Ferguson and Virginia R. Parslow
Received: 27 October 2016; Accepted: 17 January 2017; Published: date

Abstract: A genome-wide association study (GWAS) by our group identified loci associated with the plasma triglyceride (TG) response to ω-3 fatty acid (FA) supplementation in IQCJ, NXPH1, PHF17 and MYB. Our aim is to investigate potential mechanisms underlying the associations between single nucleotide polymorphisms (SNPs) in the four genes and TG levels following ω-3 FA supplementation. 208 subjects received 3 g/day of ω-3 FA (1.9–2.2 g of EPA and 1.1 g of docosahexaenoic acid (DHA)) for six weeks. Plasma TG were measured before and after the intervention. 67 SNPs were selected to increase the density of markers near GWAS hits. Genome-wide expression and methylation analyses were conducted on respectively 30 and 35 participants’ blood sample together with in silico analyses. Two SNPs of IQCJ showed different affinities to splice sites depending on alleles. Expression levels were influenced by genotype for one SNP in NXPH1 and one in MYB. Associations between 12 tagged SNPs of IQCJ, 26 of NXPH1, seven of PHF17 and four of MYB and gene-specific CpG site methylation levels were found. The response of plasma TG to ω-3 FA supplementation may be modulated by the effect of DNA methylation on expression levels of genes revealed by GWAS.

Keywords: gene-diet interactions; plasma lipid levels; ω-3 fatty acids; genome-wide association study; nutrigenetics; epigenetics

1. Introduction

Cardiovascular diseases (CVD) are known to have several causes, including environmental and genetic predispositions. Nutrition is an important environmental factor affecting the risk of developing CVD, and several dietary recommendations have been issued for prevention. However, these recommendations currently do not take into account the fact that individuals respond differently to dietary interventions although this phenomenon is well documented in the literature [1]. For instance, it is recognized that ω-3 fatty acids (FA) from marine sources, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exert beneficial effects on cardiovascular health owing, among others, to their hypotriglyceridemic properties [2]. However, a large inter-individual variability of the metabolic response to an ω-3 FA supplementation has been observed. In the European FINGEN study, 31% of participants who received a supplement of 1.8 g/day of ω-3 FA for eight weeks did not reduce their plasma triglyceride (TG) levels [3]. Similarly, our research group reported that 29% of all participants of the Fatty Acid Sensor (FAS) Study, who received ω-3 FA supplementation providing 5 g of fish oil per day (1.9–2.2 g of EPA and 1.1 g DHA)
over a period of six weeks did not have their plasma TG levels decreased [4,5]. This heterogeneity of the plasma TG response is partly due to genetic factors, including gene-diet interactions [1,6].

Research groups around the world have been studying genes associated with lipid metabolism such as apolipoprotein E and peroxisome proliferator-activated receptor α, and discovered several single nucleotide polymorphisms (SNPs) implicated in this phenomenon [3,7-10]. However, these variations explain a very small proportion of the variance of the plasma TG levels in response to ω-3 FA supplementation. Since many other associated variations may remain unknown, our team recently conducted a genome-wide association study (GWAS) on subjects of the FAS Study and identified 13 SNPs associated with the plasma TG response [6]. A genetic risk score derived with these SNPs explained 21.5% of the variance of the plasma TG response [6].

Due to linkage disequilibrium (LD) between SNPs in neighbouring genes, it is difficult to discern actual causative SNPs solely with GWAS results. We therefore recently increased the density of markers at TG response-associated loci for the IQ motif-containing J (IQ CJ), neurexophilin-1 (NXPH1), PHD finger protein 17 (PHF17) and V-Myb avian myeloblastosis viral oncogene homolog (MYB) genes, and found numerous gene-diet interactions and effects of SNPs in the IQ CJ, NXPH1 and MYB genes on plasma TG levels [11]. However, the underlying mechanisms relating these genes and lipid metabolism remain unclear. IQ CJ and NXPH1 are known for their functions in the central and peripheral nervous systems [12-15]. PHF17 is involved in mitosis, meiosis and tumor suppression [16,17]. MYB regulates various cell functions and plays an important role in hematopoiesis and cancer development [18,19]. Yet, very little associations between these genes and lipid metabolism have been established. Thus, the purpose of this study is to investigate the mechanisms underlying associations between SNPs of candidate genes and TG levels following ω-3 FA supplementation by analyzing gene expression, DNA methylation levels and conducting in silico analyses.

2. Results

Table 1 shows the baseline characteristics of the participants. In accordance with the inclusion criteria, the average body mass index (BMI) pre- and post-supplementation of all subjects was >25 kg/m². The average pre-supplementation plasma TG level was above the cut-point value of 1.129 mmol/l based on the last recommendations of the American Heart Association [2].

| Characteristics | Before Supplementation | After Supplementation | p Value * |
|-----------------|-----------------------|----------------------|-----------|
| Study population, n | 210 | 208 | - |
| Age, years | 30.8 ± 8.7 | - | - |
| Weight, kg b | 81.3 ± 13.9 | 81.6 ± 14.2 | 0.0009 |
| BMI, kg/m² b | 27.8 ± 3.7 | 27.9 ± 3.8 | 0.005 |
| TG, mmol/L b | 1.21 ± 0.63 | 1.02 ± 0.52 | <0.0001 |

Values are means ± standard deviation (SD) unless otherwise indicated; p Values were obtained using the Student’s t-test (TTEST) procedure (SAS Genetics v9.2); BMI = body mass index; TG = triglycerides; * p < 0.05 was considered significant; b p Values are for log10-transformed values.

Table 2 shows allele frequencies of tagged SNPs. All SNPs were in Hardy-Weinberg equilibrium (HWE). 87% of the genetic variability of IQ CJ, as well as 85% of NXPH1, 96% of PHF17 and 100% of MYB were covered. Most of the SNPs were located in introns, except for three SNPs of PHF17 that were located in its upstream region, another of PHF17 that was in the three prime untranslated region (3' UTR) and one of MYB that was in its downstream region.
Table 2. Selected polymorphisms in candidate genes from the genome-wide association study (GWAS) of the Fatty Acid Sensor (FAS) study (n = 210 individuals).

| Gene  | dbSNP No. | Sequence | Location | Genotype Frequency |
|-------|-----------|----------|----------|--------------------|
| IQC   | rs12497650 | TTT[T/C]ATTG | Intron   | C/C (n = 95) 0.4524 C/T (n = 96) 0.4571 T/T (n = 19) 0.0905 |
|       | rs4501157  | ACA[G/T]TAA | Intron   | G/G (n = 29) 0.1388 G/T (n = 89) 0.4258 T/T (n = 91) 0.4354 |
|       | rs13091349 | TCT[C/T]TCT | Intron   | C/C (n = 147) 0.7000 C/T (n = 56) 0.2667 T/T (n = 7) 0.0333 |
|       | rs2044704  | TTT[C/G]TAG | Intron   | C/C (n = 20) 0.0952 C/G (n = 68) 0.3238 G/G (n = 122) 0.5810 |
|       | rs1962071  | AGC[A/C]GCC | Intron   | A/A (n = 116) 0.5524 A/C (n = 74) 0.3524 C/C (n = 20) 0.0952 |
|       | rs7634829  | TGT[A/G]TAA | Intron   | A/A (n = 68) 0.3238 A/G (n = 101) 0.4810 G/G (n = 41) 0.1952 |
|       | rs2621294  | TGC[A/G]AGG | Intron   | A/A (n = 85) 0.4067 A/G (n = 90) 0.4306 G/G (n = 34) 0.1627 |
|       | rs6800211  | AGG[C/T]GTC | Intron   | C/C (n = 104) 0.4952 C/T (n = 90) 0.4286 T/T (n = 16) 0.0762 |
|       | rs17782879 | TCG[A/G]TAT | Intron   | A/A (n = 20) 0.0952 A/G (n = 88) 0.4190 G/G (n = 102) 0.4857 |
|       | rs1868414  | CTG[C/T]GCC | Intron   | C/C (n = 100) 0.4785 C/T (n = 81) 0.3876 T/T (n = 28) 0.1340 |
|       | rs2595260  | AGG[C/T]TAC | Intron   | C/C (n = 125) 0.5952 C/T (n = 66) 0.3143 T/T (n = 19) 0.0905 |
|       | rs6763890  | ATG[A/T]CTT | Intron   | A/A (n = 28) 0.1340 A/T (n = 85) 0.4067 T/T (n = 96) 0.4593 |
|       | rs9827242  | TCA[C/T]AGT | Intron   | C/C (n = 5) 0.0238 C/T (n = 68) 0.3238 T/T (n = 137) 0.6524 |
|       | rs1449009  | CA[A/T]ATT | Intron   | A/A (n = 110) 0.5238 A/G (n = 77) 0.3667 G/G (n = 23) 0.1095 |
|       | rs2621309  | TTT[C/G]TTC | Intron   | C/C (n = 114) 0.5429 C/T (n = 72) 0.3429 G/G (n = 21) 0.1000 |
|       | rs61332355 | AGG[A/C]AAT | Intron   | A/A (n = 6) 0.0286 A/C (n = 64) 0.3048 C/C (n = 140) 0.6667 |
| NXP1  | rs69566210 | TCT[C/T]TTT | Intron   | C/C (n = 14) 0.0667 C/T (n = 71) 0.3381 T/T (n = 125) 0.5952 |
|       | rs2107779  | ATG[C/T]TGA | Intron   | C/C (n = 69) 0.3286 C/T (n = 94) 0.4476 T/T (n = 47) 0.2238 |
|       | rs10271895 | CTG[A/T]GTC | Intron   | A/A (n = 134) 0.6381 A/T (n = 68) 0.3238 T/T (n = 8) 0.0381 |
|       | rs12216689 | TGA[A/C]TGA | Intron   | A/A (n = 108) 0.5143 A/C (n = 85) 0.0717 T/T (n = 17) 0.0810 |
|       | rs6963644  | TGC[A/G]TTT | Intron   | A/A (n = 0) 0 A/G (n = 32) 0.1524 G/G (n = 178) 0.8476 |
|       | rs17150341 | AGG[C/T]ATT | Intron   | C/C (n = 102) 0.4857 C/T (n = 88) 0.4190 T/T (n = 20) 0.0952 |
|       | rs1013868  | TTC[A/G]CTG | Intron   | C/C (n = 93) 0.4429 C/T (n = 95) 0.4524 T/T (n = 22) 0.1048 |
|       | rs12530767 | CAT[A/G]CTC | Intron   | A/A (n = 1) 0.0048 A/G (n = 33) 0.1571 G/G (n = 176) 0.8381 |
|       | rs4318981  | CAT[C/T]ATA | Intron   | C/C (n = 31) 0.1476 C/T (n = 88) 0.4190 T/T (n = 91) 0.4333 |
|       | rs17153997 | GTC[G/T]GTA | Intron   | C/C (n = 73) 0.3493 C/T (n = 92) 0.4402 T/T (n = 44) 0.2105 |
|       | rs7801099  | AAC[A/G]ACA | Intron   | A/A (n = 64) 0.5127 A/G (n = 105) 0.5000 G/G (n = 41) 0.1952 |
|       | rs4725120  | ATA[A/G]AAG | Intron   | A/A (n = 47) 0.2238 A/G (n = 99) 0.4714 G/G (n = 64) 0.3048 |
|       | rs1859275  | GTG[A/G]CTA | Intron   | A/A (n = 30) 0.1435 A/G (n = 87) 0.4163 G/G (n = 92) 0.4402 |
|       | rs10238726 | CTG[A/G]TTC | Intron   | A/A (n = 102) 0.4857 A/G (n = 84) 0.4000 G/G (n = 24) 0.1143 |
| SNP         | Gene       | Position | Minor Allele | HWE | A/T | G/G | C/C | T/T |
|-------------|------------|----------|--------------|-----|-----|-----|-----|-----|
| rs1012960   | PHF17      | Intron   | A/A (n=46)   | 0.2190 | A/T (n=118) | 0.5619 | T/T (n=46) | 0.2190 |
| rs11767429  | MYB        | Intron   | A/A (n=106)  | 0.5048 | A/G (n=82)  | 0.3905 | G/G (n=22) | 0.4/A18 |
| rs4333500   |            | Intron   | G/G (n=74)   | 0.3524 | G/T (n=105) | 0.5000 | T/T (n=31) | 0.1476 |
| rs7793115   |            | Intron   | A/A (n=2)    | 0.0096 | A/G (n=37)  | 0.1770 | G/G (n=170) | 0.8134 |
| rs7799856   |            | Intron   | A/A (n=76)   | 0.3619 | A/C (n=88)  | 0.4190 | C/C (n=46) | 0.2190 |
| rs7806222   |            | Intron   | A/A (n=154)  | 0.7333 | A/G (n=46)  | 0.2190 | C/C (n=10) | 0.0476 |
| rs13221144  |            | Intron   | C/C (n=11)   | 0.0524 | C/T (n=75)  | 0.3571 | T/T (n=124) | 0.5905 |
| rs17406479  |            | Intron   | G/G (n=136)  | 0.6476 | G/T (n=69)  | 0.3286 | T/T (n=5)  | 0.0238 |
| rs10486228  |            | Intron   | C/C (n=6)    | 0.0286 | C/T (n=64)  | 0.3048 | T/T (n=140) | 0.6667 |
| rs17154569  |            | Intron   | A/A (n=140)  | 0.6699 | A/G (n=64)  | 0.3062 | G/G (n=5)  | 0.0239 |
| rs4141002   |            | Intron   | C/C (n=161)  | 0.7667 | C/T (n=46)  | 0.2190 | T/T (n=3)  | 0.0143 |
| rs7805772   |            | Intron   | A/A (n=141)  | 0.6746 | A/G (n=56)  | 0.2679 | G/G (n=12) | 0.0574 |
| rs2349780   |            | Intron   | A/A (n=76)   | 0.3619 | A/G (n=108) | 0.5143 | G/G (n=26) | 0.1238 |
| rs2107474   |            | Intron   | C/C (n=71)   | 0.3381 | C/G (n=100) | 0.4762 | G/G (n=39) | 0.1857 |
| rs11769942  |            | Intron   | C/C (n=84)   | 0.4000 | C/T (n=95)  | 0.4524 | T/T (n=31) | 0.1476 |
| rs6952383   |            | Intron   | A/A (n=171)  | 0.8143 | A/T (n=36)  | 0.1714 | T/T (n=3)  | 0.0143 |
| rs6974252   |            | Intron   | A/A (n=4)    | 0.0190 | A/G (n=51)  | 0.2429 | G/G (n=155) | 0.7381 |
| rs10265408  |            | Intron   | C/C (n=110)  | 0.5263 | C/G (n=83)  | 0.3971 | G/G (n=16) | 0.0766 |
| rs2189904   |            | Intron   | C/C (n=93)   | 0.4429 | C/T (n=95)  | 0.4524 | T/T (n=22) | 0.1048 |
| rs2057862   |            | Intron   | C/C (n=38)   | 0.1818 | C/G (n=95)  | 0.4545 | G/G (n=76) | 0.3636 |

**PHF17**

| SNP         | Gene       | Position | Minor Allele | HWE | A/T | G/G | C/C | T/T |
|-------------|------------|----------|--------------|-----|-----|-----|-----|-----|
| rs2217023   | PHF17      | Intron   | C/C (n=10)   | 0.0478 | C/G (n=61) | 0.2919 | G/G (n=138) | 0.6603 |
| rs4975270   |            | Intron   | A/A (n=76)   | 0.3619 | A/G (n=88)  | 0.4190 | G/G (n=46) | 0.2190 |
| rs11722830  |            | Intron   | A/A (n=8)    | 0.0381 | A/G (n=73)  | 0.3476 | G/G (n=129) | 0.6143 |
| rs12505447  |            | Intron   | C/C (n=138)  | 0.6571 | C/T (n=65)  | 0.3095 | T/T (n=7)  | 0.0333 |
| rs6534704   |            | Intron   | A/A (n=3)    | 0.0143 | A/T (n=26)  | 0.1238 | T/T (n=181) | 0.8619 |
| rs13148510  |            | 3' UTR   | C/C (n=193)  | 0.9190 | C/G (n=17)  | 0.0810 | G/G (n=0)  | 0 |
| rs13143771  |            | Intron   | C/C (n=19)   | 0.0905 | C/T (n=81)  | 0.3857 | T/T (n=110) | 0.5238 |
| rs13142964  |            | Intron   | C/C (n=179)  | 0.8524 | C/G (n=31)  | 0.1476 | G/G (n=0)  | 0 |

**MYB**

| SNP         | Gene       | Position | Minor Allele | HWE | A/T | G/G | C/C | T/T |
|-------------|------------|----------|--------------|-----|-----|-----|-----|-----|
| rs9321493   | MYB        | Intron   | C/C (n=68)   | 0.3238 | C/T (n=97) | 0.4619 | T/T (n=45) | 0.2143 |
| rs11154794  |            | Intron   | C/C (n=3)    | 0.0143 | C/T (n=47)  | 0.2238 | T/T (n=160) | 0.7619 |
| rs210798    |            | Intron   | G/G (n=75)   | 0.3571 | G/T (n=95)  | 0.4524 | T/T (n=40) | 0.1905 |
| rs210936    |            | Intron   | A/A (n=60)   | 0.2857 | A/G (n=98)  | 0.4667 | G/G (n=52) | 0.2476 |
| rs      | SNP            | Location | Genotype | Frequency |
|---------|----------------|----------|----------|-----------|
| rs7757388 | ATA[A/G]AAG    | Intron  | A/A (n = 148) 0.7081 | A/G (n = 55) 0.2632 | G/G (n = 6) 0.0287 |
| rs210962  | AGA[C/T]CCT    | Intron  | C/C (n = 125) 0.5981 | C/T (n = 68) 0.3254 | T/T (n = 16) 0.0766 |
| rs17639758 | GTA[A/G]CAT   | Intron  | A/A (n = 0) 0 | A/G (n = 11) 0.0524 | G/G (n = 199) 0.9476 |
| rs1013891 | TAC[A/G]GCA    | Intron  | A/A (n = 26) 0.1238 | A/G (n = 94) 0.4476 | G/G (n = 90) 0.4286 |
| rs2179308  | GGT[A/G]TTG    | Intron  | A/A (n = 54) 0.2571 | A/G (n = 106) 0.5048 | G/G (n = 50) 0.2381 |

Genotype frequency was obtained using the ALLELE procedure (SAS Genetics v9.3); dbSNP = Single-Nucleotide Polymorphism (SNP) Database; *GWAS hit identified in the FAS study.
We searched for possible connections between IQCJ, NXPH1, PHF17 and MYB and lipid metabolism by conducting in silico analyses. First, RNA splicing analyses revealed two SNPs of IQCJ, rs2595260 and rs9827242, showing different affinities to splice sites depending on alleles. Besides, LD analyses indicated no LD between tagged SNPs and other SNPs located in coding or promoter regions. Transcription factor affinity predictions demonstrated the poor affinity of tagged SNPs with transcription factors. We also investigated expression levels of IQCJ, NXPH1, PHF17 and MYB in specific tissues using the Tissue-specific Gene Expression and Regulation (TiGER) database and the Expression Atlas. IQCJ was especially expressed in the brain. Similarly, NXPH1 was expressed in the brain, but also in the peripheral nervous system. PHF17 was more ubiquitous. It was mostly expressed in the small intestine, prostate, mammary gland and kidney. MYB was expressed in bone marrow, thymus, testis and blood. Analyses of the expression microarrays revealed several effects of genotype on expression levels (Table 3). Two SNPs, namely rs10486228 (NXPH1) and rs17639758 (MYB), were significantly associated with gene expression levels. Three other SNPs, rs17782879 in IQCJ, rs11769942 in NXPH1 and rs11154794 in MYB, had marginal but non-significant effects on gene expression. No associations were observed between SNPs in PHF17 and corresponding gene expression levels.

Table 3. Effects of genotype on the expression of transcripts associated with the IQCJ, NXPH1, PHF17 and MYB genes (n = 30 individuals).

| Gene | SNP       | Transcript        | p Value |
|------|-----------|--------------------|---------|
| IQCJ | rs17782879| NM_001042706.1    | 0.06    |
|      | rs10486228| NM_152745.2       | 0.01    |
|      | rs11769942| NM_152745.2       | 0.07    |
|      | rs11154794*| NM_005375.2      | 0.09    |
|      | rs17639758| NM_005375.2       | 0.02    |

The General Linear Model (GLM) procedure (SAS v9.2) adjusted for age, sex and body mass index was used to test for the effects of genotype on the expression of transcripts; SNP = Single-Nucleotide Polymorphism; * Heterozygotes merged with rare homozygotes (dominant model).

Significant associations of tagged SNPs from GWAS-associated genes with methylation levels are presented in Table 4. A total of 17 significant associations were found with CpG sites and IQCJ SNPs. There were also 71 significant associations with NXPH1 SNPs, 15 with PHF17 SNPs and seven with MYB SNPs. Because of the large number of results and the number of statistical tests computed, we accounted for multiple testing with a false discovery rate. 6 SNPs; rs2044704, rs1962071, rs2595260, rs1449009, rs2621309 and rs61332355, all in IQCJ, remained significant after false discovery rate correction.

Table 4. Significant associations of tagged single-nucleotide polymorphisms (SNPs) from genome-wide association study (GWAS)-associated genes with pre-supplementation methylation levels (n = 35 individuals).

| Gene | SNP      | CpG Site | Position | p Value |
|------|----------|----------|----------|---------|
| IQCJ | rs4501157| cg09784347| CHR 3, 158786963 | 0.0457  |
|      | rs2044704| cg17255703| CHR 3, 158787141 | 0.0077  |
|      | rs1962071| cg16975599| CHR 3, 158962761 | 0.0001  *|
|      | rs7634829| cg17255703| CHR 3, 158787141 | 0.0025  |
|      | rs2621294| cg26659666| CHR 3, 158786242 | 0.0238  |
|      | rs17782879| cg16975599| CHR 3, 158962761 | 0.0001  *|
|      | rs1868414| cg16975599| CHR 3, 158962761 | 0.0362  |

The General Linear Model (GLM) procedure (SAS v9.2) adjusted for age, sex and body mass index was used to test for the effects of genotype on the expression of transcripts; SNP = Single-Nucleotide Polymorphism; * Heterozygotes merged with rare homozygotes (dominant model).
| SNP       | Effect | Gene | Chromosome | Position | p-Value |
|-----------|--------|------|------------|----------|---------|
| rs2595260 | c      |      | CHR 3, 158962761 | 0.0001 * |
| rs9827242 | c,d    |      | CHR 3, 158955027 | 0.0077   |
| rs1449009 | c,e    |      | CHR 3, 158962761 | 0.0001 * |
| rs2621309 | c,e    |      | CHR 3, 158962761 | 0.0001 * |
| rs6133235 | c,d,e  |      | CHR 3, 158962761 | 0.0002 * |
| NXP1      | rs6954210 | cg06328127 | CHR 7, 8469546 | 0.0009   |
| rs2107779 | c      |      | CHR 7, 8480779 | 0.0048   |
| rs9827242 | c      |      | CHR 7, 8477029 | 0.0109   |
| rs12216689| c      |      | CHR 7, 8473457 | 0.0120   |
| rs696344  | c      |      | CHR 7, 8483569 | 0.0388   |
| rs1013868 | c      |      | CHR 7, 8483710 | 0.0404   |
| rs4318981 | c      |      | CHR 7, 8481460 | 0.0051   |
| rs7801099 | c      |      | CHR 7, 8634300 | 0.0254   |
| rs1012960 | c      |      | CHR 7, 8666902 | 0.0259   |
| rs4725120 | c      |      | CHR 7, 8477156 | 0.0452   |
| rs1859275 | c      |      | CHR 7, 8481306 | 0.0211   |
| rs1012960 | c      |      | CHR 7, 8483569 | 0.0474   |
| rs1176429 | c      |      | CHR 7, 8481036 | 0.0261   |
| rs7805772 | c      |      | CHR 7, 8482235 | 0.0445   |
| rs2349780 | c      |      | CHR 7, 8476128 | 0.0017   |
The GLM procedure (SAS v9.2) adjusted for age, sex and BMI was used to test for the associations between tagged SNPs and methylation levels; *CpG site positions according to genome build 37; *p Values are derived from log10-transformed data; *Heterozygotes were merged with rare homozygotes (dominant model); *GWAS hit identified in the FAS study; *SNP showed genotype or
gene-diet interaction effects on plasma triglyceride levels. * p Value remained significant after controlling for false discovery rate (MULTTEST procedure, SAS v9.2).

Spearman correlation coefficients between gene expression and CpG site methylation levels are presented in Table 5. Significant correlations between gene expression and methylation levels were obtained with two CpG sites of IQCJ (cg15736726 and cg23982461), one of NXPH1 (cg06328127), three of PHF17 (cg04482257, cg27628849 and cg17233452) and two of MYB (cg01369646 and cg02127509).

Table 5. Marginal and significant correlations between transcript expression and DNA methylation levels (n = 29 individuals).

| Gene  | Transcript       | CpG Site       | p Value |
|-------|------------------|----------------|---------|
| IQCJ  | NM_001042706.1   | cg034455716    | 0.0936  |
|       | NM_001042705.1   | cg15736726     | 0.0230  |
|       |                  | cg23982461     | 0.0141  |
| NXPH1 | NM_152745.2      | cg0212438      | 0.0696  |
|       |                  | cg06328127     | 0.0446  |
| PHF17 | NM_024900.3      | cg26766900     | 0.0937  |
|       |                  | cg04482257     | 0.0050  |
|       |                  | cg00296291     | 0.0953  |
|       |                  | cg12676803     | 0.0806  |
|       |                  | cg26347359     | 0.0842  |
|       |                  | cg27628849     | 0.0038  |
|       |                  | cg17233452     | 0.0417  |
| MYB   | NM_005375.2      | cg13400176     | 0.0567  |
|       |                  | cg01369646     | 0.0240  |
|       |                  | cg02127509     | 0.0496  |

The CORR procedure (SAS v9.2) adjusted for age, sex and BMI was used to test for the correlations between transcript expression and CpG site methylation levels.

Spearman correlation coefficients between TG levels and CpG site methylation levels are presented in Table 6. Significant correlations between gene expression and methylation levels were obtained with one CpG site of IQCJ (cg09784347), three of NXPH1 (cg00852549, cg00399951 and cg20378002), four of PHF17 (cg04482257, cg09863040, cg12832492 and cg01223512) and two of MYB (cg18253802 and cg07363239). As to correlations between TG levels and expression levels, the NM_001042705.1 transcript in IQCJ was significantly correlated with pre-supplementation TG levels (p = 0.0163).

Table 6. Marginal and significant correlations between pre-supplementation triglyceride levels and DNA methylation levels (n = 35 individuals).

| Gene  | CpG Site       | p Value |
|-------|----------------|---------|
| IQCJ  | cg09784347     | 0.0399  |
|       | cg034455716    | 0.0610  |
| NXPH1 | cg00852549     | 0.0459  |
|       | cg00399951     | 0.0404  |
|       | cg20378002     | 0.0368  |
| PHF17 | cg15389440     | 0.0070  |
|       | cg09863040     | 0.0317  |
|       | cg12832492     | 0.0050  |
|       | cg22922695     | 0.0686  |
|       | cg09884389     | 0.0629  |
|       | cg12204423     | 0.0943  |
|       | cg01223512     | 0.0424  |
| MYB   | cg18253802     | 0.0134  |
|       | cg07363239     | 0.0407  |
The CORR procedure (SAS v9.2) adjusted for age, sex and BMI was used to test for the correlations between transcript expression and CpG site methylation levels.

3. Discussion

Our research group recently revealed, in a GWAS, potential SNPs associated with the plasma TG response to ω-3 FA supplementation [6]. We increased the density of markers around GWAS-associated SNPs in the IQCJ, NXPH1, PHF17 and MYB genes to further verify whether they are associated with plasma TG levels following ω-3 FA supplementation [11]. We found several effects of genotype and gene-diet interactions on plasma TG levels [11]. In the present study, we analysed gene expression and methylation levels, and conducted in silico analyses in order to better understand the potential underlying mechanisms by which IQCJ, NXPH1, PHF17 and MYB interact with plasma ω-3 FA supplementation to modulate plasma TG levels.

We first observed that two SNPs of IQCJ, rs2595260 and rs9827242, had different affinities with splice sites depending on alleles. In our previous study, these SNPs were also found to exert significant gene-diet interactions modulating plasma TG levels [11]. These results therefore indicate that an individual’s genotype may possibly affect gene expression of IQCJ through the alteration of RNA splicing. Likewise, there was a marginal association between one SNP of IQCJ, rs17782879, and its expression levels. We did not find any other substantial results in RNA splicing analyses, nor did we find specific affinities of tagged SNPs with transcription factors. This can be attributable to the location of tagged SNPs. Accordingly, the vast majority of these SNPs are located within introns, whereas regulatory regions are located in splice sites, promoter regions, 3′ UTR, near gene regions (3′ or 5′), and transcribed DNA sequences are located in exons. We therefore searched for other relevant SNPs in LD with tagged SNPs. However, none of these tagged SNPs were in LD with any other SNPs located in coding or promoter regions that have previously been associated with lipid metabolism or with SNPs previously identified in a lipid traits-related GWAS.

Additionally, according to in silico analyses, all candidate genes were poorly expressed in tissues related to lipid metabolism, such as adipose tissue, liver or pancreas. These observations are coherent with known functions of these genes. In fact, very few links between IQCJ, NXPH1, PHF17 and MYB and lipid metabolism have been made in the past. IQCJ is bound to the schwannomin-interacting protein 1 (SCHIP1) to form another transcriptional unit (IQCJ-SCHIP1) [12]. IQCJ-SCHIP1 plays roles in the function of initial axon segments and nodes of Ranvier, which are essential structures to saltatory conduction in the central and peripheral nervous systems [13]. According to the results of a GWAS conducted on participants of the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study, which aimed to study the effect of lipid-lowering therapy to fenofibrate on the response of various lipid traits, IQCJ-SCHIP1 may actually be associated with TG levels as well as very-low-density lipoprotein (VLDL) particle clearance [20]. VLDL particle clearance is an important determinant of blood TG levels [21]. Dysfunctional VLDL hydrolysis can increase TG-rich lipoprotein remnant particles synthesis, which is associated with an increased risk of atherosclerosis [21-23]. In the same GWAS, several SNPs of NXPH1 were associated with type 2 diabetes, blood pressure and plasma LDL-cholesterol, HDL-cholesterol, TG as well as C-reactive protein levels [20]. In another study, NXPH1 was associated with type 2 diabetes traits among a population of obese Hispanic children [24]. NXPH1 is known to code for a neuronal glycoprotein that binds to α-neurexin to regulate neuronal function, neurotransmission, signalisation and various cell interactions [14,15,25]. PHF17 codes for the Jade-1 protein. This protein interacts with histone acetyltransferase HBO1 and promotes histone acetylation in chromatin to regulate DNA replication and gene transcription [16,26,27]. MYB encodes the c-Myb transcription factor, which regulates the expression of a wide variety of genes involved in cell function [18]. MYB has been reported to be associated with the development of CVD, intracellular accumulation of lipid, differentiation of mesenchymal stem cells from adipose tissue, intestinal absorption of nutrients and possibly BMI [28-31]. The c-Myb protein is expressed in the vascular smooth muscle and it is induced by homocysteine, while high levels of homocysteine are recognized as an independent CVD risk factor [28,32].
Moreover, we tested whether tagged SNPs are associated with pre-supplementation methylation levels and observed several significant associations. Many tagged SNPs that were marginally or significantly associated with plasma TG levels and the plasma TG response to ω-3 FA supplementation in our previous study appear now to be significantly associated with methylation levels as well. This is particularly true for IQCJ and NXPH1. Mostly all SNPs of IQCJ showing significant gene-diet interactions are associated with one particular mutual CpG site (cg16975599). This CpG site is close to rs548649590, which was not tested for associations with plasma TG levels in our previous study. This SNP is not in LD with any SNP previously associated with plasma TG levels either. Furthermore, we accounted for multiple testing with a false discovery rate and found out that the strongest associations were in IQCJ. None of the SNP of MYB that was previously found to exert effects of gene-diet interactions on plasma TG levels was associated with methylation levels at CpG sites. None was in LD with any SNPs associated with CpG sites either. These outcomes suggest that the modulation of plasma TG levels through genes revealed by GWAS, especially IQCJ and NXPH1, may be regulated by DNA methylation, which is here influenced by genotype. As DNA methylation is strongly related to gene expression, individuals’ genotype could directly upregulate or downregulate gene expression [33,34]. Gene expression analyses also support this rationale. Indeed, gene expression of IQCJ, NXPH1 and MYB appeared to be affected by DNA variations within these genes. It was also shown to be influenced by TG levels and methylation levels in IQCJ, NXPH1, PHF17 and MYB.

4. Materials and Methods

4.1. Study Population

A total of 254 participants were recruited to participate in the FAS Study between September 2009 and December 2011 in the Quebec City metropolitan area through advertisements in local newspapers and electronic messages sent to university students and employees. Participants had to be between 18 and 50 years of age, to be non-smokers and to have a BMI between 25 and 40 kg/m². Candidates were excluded if they had taken fish oil supplements for at least six months prior to the beginning of the study or if they were suffering from any thyroid or metabolic disorders such as diabetes, hypertension, dyslipidemia or coronary heart disease. Statistical analyses were conducted on 210 subjects who completed the supplementation period. Data on plasma TG levels were missing for two of them, leaving 208 individuals in the final study sample.

4.2. Study Design and Diets

The complete study design and diets have been reported in previous papers [6,11]. Briefly, participants followed a run-in period of two weeks, wherein a trained registered dietitian gave dietary instructions for them to achieve the recommendations from Canada’s Food Guide, to ensure constant dietary intake and to keep a stable body weight throughout the protocol. Subsequently, they received ω-3 FA capsules (Ocean Nutrition, Dartmouth, NS, Canada) in a sufficient amount to cover the six-week supplementation protocol. Each capsule contained 1 g of fish oil concentrate. They had to take five capsules a day, providing 3 g of ω-3 FA, including 1.9–2.2 g of EPA and 1.1 g of DHA. They had to report any deviation from the protocol as well as experienced side effects. They also had to note their alcohol and fish consumption if any. Finally, oral and written dietary instructions were given to participants before each phase.

4.3. Anthropometric Measurements

Height and body weight were measured before the run-in period, as well as before and after the intervention following the recommendations of the Airlie Conference. BMI (kg/m²) was obtained by dividing weight (kg) by the squared height (m²).

4.4. Laboratory Methods
4.4.1. Plasma Lipids

Methods to measure plasma lipids have previously been detailed [6,11]. Briefly, blood samples were collected after a 12 h overnight fast and 48 h alcohol abstinence. Blood samples were taken before the run-in period to verify whether individuals were presenting any metabolic disorders. Blood samples of remaining participants were taken before and after the ω-3 FA supplementation period. Enzymatic assays were used to measure plasma total cholesterol and TG concentrations [35,36].

4.4.2. SNP Selection and Genotyping

SNPs were identified using 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. The Gene Tagger procedure in haploview v4.2 was used to identify tagging SNPs with a minor allele frequency (MAF) >5% and pairwise tagging ($r^2 \geq 0.80$) located in gene regions and surrounding regions (2 kb upstream and downstream gene). The mean LD ($r^2$) between SNPs was 0.96 for IQCJ, 0.96 for NXP1, 0.97 for PHF17 and 0.95 for MYB. SNPs were selected in a way to cover ≥85% of all common variations (MAF > 5%). The GenElute Gel Extraction Kit (Sigma-Aldrich Co., St. Louis, MO, USA) was used to extract genomic DNA (gDNA) from blood samples. Tagged SNPs were genotyped using TaqMan technology: 2.5 µL of each gDNA (40 ng/µL) and 2.5 µL of OpenArray Genotyper Master Mix (Life Technologies, Carlsbad, CA, USA) were mixed in a 384-well plate with validated primers and loaded onto genotyping plates using the QuantStudio™ 12K Flex OpenArray® AccuFill™ System (Life Technologies). The QuantStudio™ 12K Flex Real-Time PCR System (Life Technologies) was used for genotyping. Finally, TaqMan Genotyper v1.3 (Life Technologies) was used to call genotypes and export data.

4.4.3. In Silico Analyses

RNA splicing analyses were performed for each tagged SNP using ESEfinder and Berkeley Drosophila Genome Project splice site prediction tools [37–39]. LD between tagged and other non-tagged SNPs was assessed using SNP Annotation and Proxy Search (SNAP) with an $r^2$ threshold of 0.8 and a distance limit of 500 kb [40]. LD was calculated with the Northern Europeans from Utah (CEU) population. We also used the Transcription factor Affinity Prediction (TRAP) web tools for single sequences to measure the possible transcription factor binding affinities to tagged SNP regions in the presence or absence of SNPs [41-43]. Furthermore, we used the TiGER database as well as the Expression Atlas to evaluate expression levels of our genes of interest in different tissues [44-47].

4.4.4. Transcriptomic Gene Expression Analyses

The expression of transcripts associated with the four genes of interest was measured in peripheral blood mononuclear cells of the first 30 participants of the FAS study to complete the intervention via the Human-6 v3 Expression BeadChips (Illumina, San Diego, CA, USA). Transcriptomic analyses have previously been detailed [5].

4.4.5. DNA Methylation Analyses

DNA methylation analyses were conducted on blood cells of 35 participants of the FAS study. Bisulfite conversion was made on 1 µg of DNA. Quantitative DNA methylation analyses were conducted at the Génome Québec Innovation Centre and McGill University (Montreal, QC, Canada). The Infinium HumanMethylation450 BeadChip (Illumina) was used for methylation sites coverage following the manufacturer’s instructions. CpG site positions were located according to genome build 37. The GenomeStudio software version 2011.1 (Illumina) as well as the methylation module were used to visualise and analyse methylation data. The ratio of signal intensity of the methylated alleles to the sum of methylated and unmethylated intensity signals of the alleles ($\beta$ value = C/(T + C)) was used to estimate methylation levels ($\beta$ value). Internal control probe pairs was applied for
data correction. CpG sites were extracted using the GenomeStudio Methylation Module. A total of 10 CpG sites in IQCJ, 43 in NXPH1, 34 in PHF17 and 24 in MYB were used.
4.4.6. Statistical Analyses

The ALLELE procedure in SAS Genetics Statistical Software v9.3 (SAS Institute, Cary, NC, USA) was used to assess genotype distribution for any deviation from the HWE and to calculate the MAF. All other statistical analyses were performed in SAS v9.2. Normal distribution was evaluated with the box-plot, skewness and kurtosis ranges. Abnormally distributed variables were log10-transformed. Common genotype homozygotes, heterozygotes and rare genotype homozygotes were analysed separately in an additive model. However, rare genotype homozygotes showing a genotype frequency <5% were merged with heterozygotes for statistical analyses in a dominant model. The GLM procedure adjusted for age, sex and BMI was used to test for associations between pre-supplementation methylation levels and tagged SNPs for each gene independently. It was also used to test for the effects of genotype on gene expression levels using again age, sex and BMI as co-variates in the model. The MULTTEST procedure was then used to account for multiple testing controlling for false discovery rate. The CORR procedure adjusted for age, sex and BMI was used to correlate gene expression and methylation levels on 29 subjects using Spearman rank correlation. It was also used to correlate TG levels with gene expression and methylation levels on respectively 30 and 35 subjects using Spearman rank correlation. Statistical significance was fixed at $p \leq 0.05$.

4.5. Consent

The study was approved by the Université Laval and Centre hospitalier universitaire (CHU) de Québec ethics committees and was performed in accordance with the principles of the Declaration of Helsinki. All participants provided written and informed consent. This study is derived from a registered clinical trial (NCT01343342).

5. Conclusions

Results of the present study demonstrate that the response of plasma TG to an ω-3 FA supplementation may be modulated through the effect of DNA methylation on expression levels of genes previously identified in a GWAS by our group (IQCJ, NXPH1, PHF17 and MYB). Further research is needed to provide a better understanding of the relationship between these genes and TG metabolism.

Acknowledgments: Bastien Vallée Marcotte received a Ph.D. studentship from Chaire de recherche en nutrition de l’Université Laval. Hubert Cormier is the recipient of the Frederick Banting and Charles Best Canada Graduate Scholarships Doctoral Awards from the Canadian Institutes of Health Research (CIHR). Iwona Rudkowska and Patrick Couture are recipients of a scholarship from the Fonds de recherche en santé du Québec (FRSQ). Marie-Claude Vohl is Tier I Canada Research Chair in Genomics Applied to Nutrition and Health. We thank Ann-Marie Paradis, Bénédicte L. Tremblay, Véronique Garneau, Élisabeth Thifault, Karelle Dugas-Bourdage, Catherine Ouellette and Annie Bouchard-Mercier, who met study participants and contributed to the success of this study. We would also like to express our gratitude to Catherine Raymond for contributing to the laboratory work.

Author Contributions: Iwona Rudkowska, Simone Lemieux and Marie-Claude Vohl conceived and designed the experiments; Patrick Couture was responsible for the medical follow-up; Bastien Vallée Marcotte performed the experiments; Bastien Vallée Marcotte analyzed the data; Bastien Vallée Marcotte, Frédéric Guénard and Hubert Cormier contributed reagents/materials/analysis tools; B.V.M. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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