

## Effects of Eicosapentaenoic Acid Supplementation on Lipid and Lipoprotein Profile in Hypertriglyceridemic Subjects with Different Proliferator-activated Receptor Alpha Genotypes

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### ABSTRACT

**Background:** We determined the blood lipid-lowering effects of eicosapentaenoic acid (EPA) on hypertriglyceridemic subjects with Leu162/Val in exon 5 and G/C in intron7 polymorphism of peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ) genotypes that, to our knowledge, have not been previously studied.

**Methods:** A total of 170 hypertriglyceridemic subjects were enrolled and genotyped for Ala54Thr, Leu162Val, and intron7 polymorphism by the use of a polymerase chain reaction–restriction fragment length polymorphism method. After determination of their genotypes, the first 23 eligible subjects who were found as Ala54 carriers and the first 23 eligible Thr54 carriers were enrolled in the study and stratified for PPAR $\alpha$  genotypes. Participants took 2 g of pure EPA daily for 8 weeks. Fasting blood lipid and lipoprotein profiles were determined and changes from baseline were measured.

**Results:** We observed significant difference between EPA supplementation and Leu162 and Val162, Intron 7 (GG and GC) carriers ( $P < 0.001$ ). We did not observe significant associations between the PPAR $\alpha$  L162V single nucleotide polymorphism and multiple lipid and lipoprotein measures. Although EPA consumption lowered lipid and lipoprotein concentrations in Leu162 and Val162 carriers and Intron 7 CC and GC carriers, these differences between the studied groups were not statistically significant.

**Conclusions:** EPA consumption has a lipid-lowering effect in hypertriglyceridemic subjects in both Leu162 and Val162 carriers. But there was no significant interaction between EPA supplementation and PPAR $\alpha$  genotypes. Thus, genetic variation within the PPAR $\alpha$  Leu162/Val cannot modulate the association of EPA intakes with lipid and lipoprotein profile. However, we must note that the sample size in this study was small.

**Keywords:** Eicosapentaenoic acid, hypertriglyceridemic, lipid profile, peroxisome proliferator-activated receptor alpha, polymorphism

## INTRODUCTION

Cardiovascular diseases (CVD) are the major cause of death in adults in several countries.<sup>[1]</sup> The development of CVD is multifactorial, with risk factors that are modifiable, such as diet, exercise, and smoking, and others that are not, such as gender, age, and genetics. The behavioral factors might modify a number of biochemical markers involved in dyslipidemia, hypertension, inflammation, and insulin resistance.<sup>[2,3]</sup>

Alterations in lipid and lipoprotein metabolisms have been investigated as important CVD risk factors. High triglycerides (TG) and low high-density lipoprotein (HDL) concentration are strong predictable factors of cardiac events.<sup>[1,4]</sup> Either environmental or genetic factors can affect the lipoproteins concentration, which may affect the risk of developing CVD. Thus, key metabolism regulators such as the peroxisome proliferator-activated receptor (PPAR) family are the candidate genes to investigate genetic predisposition of this complex disease.<sup>[1]</sup>

PPAR family members are nuclear receptors involved in lipid and carbohydrate metabolisms, adipogenesis, and insulin sensitivity.<sup>[5]</sup> PPAR $\alpha$  is highly expressed in tissues with high fatty acid catabolism and integrates several metabolic pathways, including fatty acid  $\beta$ -oxidation, lipoprotein synthesis, and amino acid catabolism.<sup>[5]</sup> PPAR $\alpha$  is located on 22q13.3, and it consists of eight exons. Single nucleotide polymorphisms (SNPs) were described in this gene and were found to be associated with dyslipidemia, insulin resistance, diabetes, and CVD.<sup>[1,5,6]</sup> Hypertriglyceridemia (HTG) is a common and heterogeneous metabolic disorder that represents a risk factor for premature coronary heart disease. HTG can be caused by various interactions between environmental and genetic factors.<sup>[6]</sup>

In humans, several variants in PPAR $\alpha$  have been identified. One variant in exon 5, Leu162Val, has been studied extensively and has been associated with body mass index (BMI), fasting concentration of total cholesterol (TC), HDL-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), apolipoprotein B (ApoB), apolipoprotein A-I (ApoA-I), and the progression of atherosclerosis.<sup>[7-12]</sup> Furthermore, this variant has been shown to have an effect on transactivation efficiency *in vitro*.<sup>[8-13]</sup> In addition to its effect on

fasting lipid parameters, PPAR $\alpha$  variants may exert their effect on atherosclerosis progression via the modulation of postprandial lipid metabolism.<sup>[12]</sup>

L162V polymorphism represents a C to G substitution leading to a leucine to valine amino acid exchange in codon 162.<sup>[8]</sup> Studies evaluating the association between this polymorphism and lipid profile reported controversial results.<sup>[5]</sup> The intron7 G/C polymorphism leads to a G to C substitution on intron7. An association between this SNP and lower TG levels was observed in diabetic patients. Conversely, the C allele of intron7 G/C was associated with atherosclerosis progression<sup>[14]</sup> and left ventricular growth induced by exercise.<sup>[15]</sup> The V162 allele was also associated with higher levels of TC,<sup>[8,11]</sup> LDL-C,<sup>[11]</sup> and ApoB levels<sup>[11,16]</sup> in other studies. Previous studies demonstrated that although the V162 allele was associated with lipid abnormalities, carriers of the less common allele were not at increased risk of developing cardiovascular disease or diabetes.<sup>[8,11,12,17]</sup>

Activation of PPAR $\alpha$  by agonists leads to reduced adiposity and lowered triglyceride levels by reduced food intake.<sup>[18]</sup>

Prospective cohort studies and secondary prevention trials indicate that *n*-3 polyunsaturated fatty acids (PUFAs) from fish or plant sources lower the risk of CVD.<sup>[19-24]</sup> Furthermore, since fatty acids are ligands for PPAR $\alpha$ , we conducted this study to determine the lipid lowering effects of eicosapentaenoic acid (EPA) supplementation in PPAR $\alpha$  polymorphism.

## METHODS

### Subjects

The participants were selected from the hypertriglyceridemic subjects referred from the Tehran Central Laboratories to the Endocrinology and Metabolism Research Center (EMRC). The inclusion criteria were serum TG level higher than 200 mg/dL (>2.3 mmol/L), and fasting blood glucose of less than 110 mg/dL (<6.2 mmol/L). Those who received lipid lowering agents, oral contraceptive pills, diuretics, sex hormones, thyroid medications, omega-3 supplement, patients with a history of gastrointestinal diseases, and smokers were excluded from the study.

In total, 170 hypertriglyceridemic subjects were selected and genotyped for Ala54Thr and

PPAR $\alpha$  genotypes, using a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method.<sup>[25]</sup> After determination of their genotypes, the first 23 eligible subjects who were found to be Ala54 carriers and the first 23 eligible Thr54 carriers were enrolled in the study, and stratified for PPAR $\alpha$  genotypes.

Participants took two grams per day of pure EPA for 8 weeks (four gel caps, each containing 500 mg ethyl ester EPA 90%, a gift from Minami Nutrition, Edegem, Belgium). Two capsules were taken in the morning and two in the evening. The participants were followed-up weekly at the EMRC; a checklist for weekly consumption of capsules was filled and capsules for the next week were given to them.

A blood sample was drawn from each participant following a 14 h overnight fasting at the baseline and after 8 weeks of EPA supplementation.

To determine the plasma fatty acid composition, fatty acid was extracted and methylated by Folsh's method,<sup>[26]</sup> then the fatty acids methyl esters were measured by gas chromatography.

The study was approved by the Ethics Committee of EMRC, Tehran University of Medical Sciences (TUMS). All the participants were informed of the nature of the study and gave a written consent. The biochemical analyses were carried out at the EMRC Laboratory, TUMS, Tehran, Iran. Genetic studies were conducted at the Department of Medical Genetics, TUMS, Tehran, Iran. The determination of composition of plasma fatty acids was carried out at the Department of Medicinal Chemistry and Pharmaceutical Sciences Laboratory, TUMS, Tehran, Iran.

### Laboratory analyses

Plasma and sera were separated from the blood samples by centrifuging at 4°C and 1800g for 15 min and were stored in 1-ml aliquots in sterile tubes at –80°C until used. Serum and plasma lipid and lipoprotein levels were measured as described previously.<sup>[25]</sup>

### Genotyping

#### *Leu162Val (gene ID55465)*

The Leu162Val mutation of the PPAR $\alpha$  gene is caused by a C to G transversion at nucleotide 484 in exon 5. The PCR–RFLP method was performed as described previously.<sup>[27]</sup>

### Intron7

The PCR–RFLP method was performed to determine intron7 polymorphism (mutation) as described previously.<sup>[27]</sup>

### Statistical analyses

The normality of distribution of continuous variables was tested by one-sample Kolmogorov–Smirnov test. To normalize the continuous variables not normally distributed, a log transformation was applied. Differences between serum lipid levels and fatty acids concentration between the two study groups with different PPAR $\alpha$  genotypes were tested separately by analysis of covariance, and baseline levels of lipids, gender, BMI, and age were considered covariates. Because only a few subjects with Val/Val were found among the participants, they were pooled with Leu162/Val subjects and analyses were carried out on the pooled data. Results are presented as mean  $\pm$  standard deviation unless otherwise noted. Analyses were performed by SPSS for Windows (SPSS Inc., Chicago, IL, USA, Version 11.5).  $P < 0.05$  was considered statistically significant.

## RESULTS

The baseline characteristics of subjects were described previously.<sup>[25,27]</sup> For comparison of groups with different PPAR $\alpha$  genotypes, the data obtained from Val162/Val and Leu162/Val subjects were combined. EPA supplementation decreased the levels of serum lipid and lipoprotein in the two study groups. No interaction was observed between PPAR $\alpha$  genotypes and the degree of changes in plasma lipids and lipoproteins after EPA consumption [Table 1]. EPA supplementation decreased levels of serum lipid and lipoprotein in the two study groups. No interaction was observed between CC and GC carriers and the degree of changes in plasma lipids and lipoproteins after EPA consumption [Table 2]. Although EPA consumption lowered the lipid and lipoprotein concentrations in Leu162 and Val162 carriers and CC and GC carriers, the observed difference between the studied groups was not statistically significant [Tables 1 and 2]. EPA supplementation increased the plasma EPA in both Leu162 and Val162 carriers [Table 3] ( $P = 0.01$  and  $P = 0.05$ , respectively), but it was observed that it has been

**Table 1:** Changes in serum lipids and lipoproteins of hypertriglyceridemic subjects at baseline and after 8-weeks eicosapentaenoic acid supplementation in the studied groups

Parameter (mg/dl)	Genotype								
	Leu162 (n=38)		P <sup>§</sup>	Val162 (n=8)		P <sup>§</sup>	Leu162 (n=38)	Val162 (n=8)	P <sup>§</sup>
	Before intervention	After intervention		Before intervention	After intervention		After versus before	After versus before	
TG	417.7±146.8	251.8±98.9 <sup>D</sup>	<0.001	385.1±82.2	267.9±107.8 <sup>D</sup>	<0.01	170.9±126.6	117.2± 97.4	NS
TC	222.4±45.6	199.7±38.0 <sup>D</sup>	<0.001	219.5±46.9	189.3±57.9 <sup>D</sup>	<0.05	22.7±24.5	30.2±26.8	NS
LDL-C	119.8±34.9	180.3±96.6 <sup>b</sup>	<0.005	120.7±30.0	108.6±25.9 <sup>D</sup>	<0.05	60.5±23.7	12.1±13.8	NS
VLDL	83.5±29.4	50.4±19.7 <sup>D</sup>	<0.001	77.0±29.2	53.6±21.6 <sup>D</sup>	<0.01	33.2±24.9	23.4±19.5	NS
HDL-C	33.8±12.5	49.9±11.8 <sup>b</sup>	<0.001	31.5±4.7	39.1±4.2 <sup>b</sup>	<0.001	9.1±6.2	7.6±1.9	NS
ApoB	122.8±27.6	106.7±30.8 <sup>D</sup>	<0.001	124.0±113.3	113.0±25.9 <sup>D</sup>	<0.05	16.0±25.8	11.0±11.1	NS
ApoCIII	16.7±14.8	11.9±4.9 <sup>D</sup>	<0.001	16.0±6.1	11.4±4.6 <sup>D</sup>	<0.01	4.8±3.4	4.7±3.6	NS

ApoB=Apolipoprotein B, ApoC3=Apolipoprotein CIII, HDL-C=High-density lipoprotein-cholesterol, LDL-C=Low-density lipoprotein-cholesterol, TC=Total cholesterol, TG=Triacylglycerol, VLDL=Very low-density lipoprotein. \*Values are as means±SDs, <sup>§</sup>Analysis of covariance (considering baseline levels, gender, BMI, and age as covariates) was performed on data from the combined Leu162/Val, Val162/Val group and Leu162 groups, <sup>§</sup>Paired *t* test, NS=Not significant, <sup>D</sup>Decreased, <sup>b</sup>Increased

**Table 2:** Changes in serum lipids and lipoproteins of hypertriglyceridemic subjects at baseline and after 8-weeks eicosapentaenoic acid supplementation in the studied groups

Parameter (mg/dl)	Genotype								
	GG (n=24)		P <sup>§</sup>	GC (n=22)		P <sup>§</sup>	GG (n=24)	GC (n=22)	P <sup>§</sup>
	Before intervention	After intervention		Before intervention	After intervention		After versus before	After versus before	
TG	376.2±145.9	87.16±24.5 <sup>D</sup>	<0.001	447.8±139.1	265.7±110.1	<0.001	289.04±120.4	182.1±120.3	NS
TC	213.4±35.9	192.5±28.9 <sup>D</sup>	<0.001	230.3±52.5	203.3±52.5	<0.001	20.9±24.6	27.3±25.2	NS
LDL-C	115.8±27.9	103.6±24.4 <sup>D</sup>	<0.01	124.1±38.9	113.1±28.2	<0.04	12.3±20.8	10.1±23.8	NS
VLDL	75.3±29.2	47.7±17.4	<0.001	89.6±27.8	53.1±22.2	<0.001	26.6±24.1	36.4±23.8	NS
HDL-C	33.5±11.1	41.8±11.4 <sup>b</sup>	<0.001	33.±12.19	42.7±10.6 <sup>b</sup>	<0.001	8.3±6.0	8.7±5.8	NS
ApoB	120.4±23.1	104.5±25.2	<0.01	125.5±29.8	111.2±31.4 <sup>D</sup>	<0.002	15.9±27.9	14.4±19.5	NS
ApoCIII	16.1±5.2	11.9±3.4	<0.001	17.1±4.8	11.7±4.0 <sup>D</sup>	<0.001	4.1±3.7	5.4±2.9	NS

ApoB=Apolipoprotein B, ApoC3=Apolipoprotein CIII, HDL-C=High-density lipoprotein-cholesterol, LDL-C=Low-density lipoprotein-cholesterol, TC=Total cholesterol, TG=Triacylglycerol, VLDL=Very low-density lipoprotein. \*Values are as means±SDs, <sup>§</sup>Analysis of covariance (considering baseline levels, gender, BMI, and age as covariates) was performed on data from the combined GC, CC group and GG groups, <sup>§</sup>Paired *t* test, NS=Not significant, <sup>D</sup>Decreased, <sup>b</sup>Increased

**Table 3:** Changes in plasma eicosapentaenoic acid (EPA) levels in hypertriglyceridemic subjects after 8-week EPA supplementation stratified by Leu162 and Val162 peroxisome proliferator-activated receptor alpha genotypes\*

Fatty acids	Genotype								
	Leu162 (n=38)		P <sup>§</sup>	Val162 (n=8)		P <sup>§</sup>	Leu162 (n=38)	Val162 (n=8)	P <sup>§</sup>
	Before	After		Before	After		After versus before	After versus before	
EPA	3.0±0.7 <sup>D</sup>	21.9±3.9	<0.001	3.5±1.2	104.4±28.9 <sup>b</sup>	0.05	18.9±3.5	100.9±28.5 <sup>§</sup>	<0.001

EPA=Eicosapentaenoic acid. \*Values are as means±SDs, <sup>§</sup>Analysis of covariance (considering baseline levels, gender, BMI, and age as covariates) was performed on data from the combined Leu162/Val, Val162/Val group and Val162/Val groups, <sup>§</sup>Paired *t* test, <sup>§</sup>Significant difference between Leu162 and Leu162/Val, and Val162/Val groups, <sup>D</sup>Decreased, <sup>b</sup>Increased

significantly increased more in Val162 carriers than Leu162 (*P* = 0.001) [Table 3].

Supplementation increased plasma EPA in GG and GC carriers (*P* = 0.001 and *P* =0.001) [Table 4],



**Table 4:** Changes in plasma eicosapentaenoic acid levels in hypertriglyceridemic subjects after 8-weeks EPA supplementation stratified by GG and GC polymorphism of peroxisome proliferator-activated receptor alpha genotypes\*

Fatty acids	Genotype								
	GG (n=24)		P	GC (n=22)		P	GG (n=24)	GC (n=22)	P
	Before	After		Before	After		After versus before	After versus before	
EPA	1.83±0.6	15.4±5.2 <sup>b</sup>	<0.001	4.5±0.9	60.2±13.4 <sup>b</sup>	<0.001	13.5±4.9	55.7±13.1 <sup>s</sup>	<0.001

EPA=Eicosapentaenoic acid. \*Values are as means±SDs, <sup>§</sup>Analysis of covariance (considering baseline levels, gender, BMI, and age as covariates) was performed on data from the combined GC, CC group and GG groups, <sup>§</sup>Paired *t* test, <sup>§</sup>Significant difference between Leu162 and GG/GC CC/CC groups, <sup>b</sup>Decreased, <sup>b</sup>Increased

but it was observed that it has been significantly increased more in GC carriers than in GG carriers ( $P = 0.001$ ) [Table 4].

## DISCUSSION

As PPAR $\alpha$  has a role in lipid homeostasis, we were therefore interested in their variable plasma lipid levels that often account for the independent increased risk of atherosclerosis<sup>[28,16]</sup> and cardiovascular risk factors.<sup>[29]</sup>

The major aim of this study was to identify the role that PPAR $\alpha$  Leu162Val polymorphism may play in the variability of serum lipid levels, and changes in lipids and lipoproteins level in response to EPA supplementation.

Among the reported polymorphisms for the PPAR $\alpha$  gene, Leu162Val polymorphism is the most prevalent with a frequency of 10.45% in diabetic and non-diabetic patients.<sup>[13]</sup> It is also associated with alterations in lipoprotein concentrations in both diabetic and non-diabetic subjects.<sup>[16]</sup> We found a prevalence of 21.8% for the Leu162/Val polymorphism in these Iranian hypertriglyceridemic subjects.<sup>[27]</sup> There was no difference in the frequency of Leu162/Val between men and women, and there were no significant differences between lipid and lipoprotein in Leu162 and Val162 carriers except for TC. In our study, a comparison between Val162 carries and Leu carriers has indicated a significant association between PPAR $\alpha$  Leu162Val polymorphism and blood lipids, where the Val162 carriers showed higher concentrations of TC.<sup>[27]</sup>

This polymorphism has been previously found to be associated with greater blood TC,<sup>[16]</sup> LDL-C,<sup>[8,11,12]</sup> TG levels,<sup>[9,30]</sup> and lower HDL-C concentration<sup>[8]</sup> depending upon the population studied. Most studies that have found an association between the Leu162Val polymorphism and greater

blood lipid concentrations were conducted in diabetic subjects; few studies have found a positive association in healthy subjects.<sup>[11,31]</sup>

The intron7 allele has been shown to be associated with increased progression of atherosclerosis. The Val162 allele and intron7 allele are in strong allelic association, such that 78% of Val162 carriers are found in combination with the intron7.<sup>[14]</sup> In our study the frequency of the intron7 polymorphism was 55.3%, which is much greater than the frequency of Leu162Val polymorphism in the studied subjects, and all of the subjects who were Leu162/Val carriers also had an intron7 allele. Except for blood TC level, there were no significant associations between intron7 allele and blood lipid, ApoB, or apolipoprotein CIII (ApoCIII) concentrations. Similar results have been observed in Foucher's study in which intron7 polymorphism was only associated with blood TC levels.<sup>[32]</sup>

Frequency of intron7 polymorphism is greater than Leu162Val and all hypertriglyceridemic subjects with Leu162 polymorphism might be carriers of the intron7 polymorphism.<sup>[31]</sup>

The expected changes in post-supplementation plasma lipids and lipoproteins were observed with EPA intervention. Results of the present study clearly show that two carriers of PPAR $\alpha$  genotypes can influence the lipid-lowering effects of EPA supplementation in hypertriglyceridemic subjects.

Although EPA supplementation decreased serum lipids and lipoproteins and increased serum HDL-C in Leu162, Val162 carriers and GG, GC carriers, there was no significant interaction between the two carriers after EPA consumption.

We believe that these data provide some insight into the pathways that could be involved in generating the association between LDL cholesterol concentration and the Leu162Val polymorphism at the PPAR $\alpha$  locus.<sup>[11]</sup> There was no association

between the Leu162Val polymorphism at the PPAR $\alpha$  locus and TG, HDL-C, very low-density lipoprotein, ApoB, and ApoCIII concentrations. Several possible reasons exist for these results. Intra-individual variation in the plasma lipid and lipoprotein. This phenomenon will reduce the chance of finding an association between a genetic variant and plasma lipid and lipoprotein concentrations, especially when the changes are small, as in this case. Alternatively, additional gene–gene or gene–diet interactions may exist that modulates the effect of the L162V polymorphism, and this will need to be addressed in future studies. Potential gene–environment interactions may be particularly relevant to this polymorphism. The level of exposure to known endogenous (such as non-esterified fatty acids) or exogenous ligands (such as dietary PUFAs) may therefore be important in determining the biochemical phenotype observed with this polymorphism.<sup>[11]</sup>

Previous studies have shown the PPAR $\alpha$  L162V SNP to be associated with multiple lipid and lipoprotein measures, with one study finding the effect of the L162V SNP on triacylglycerol and ApoCIII concentrations to be dependent on PUFA intake.<sup>[9-12,33]</sup>

In the current study, we did not observe significant associations between the PPAR $\alpha$  L162V SNP and multiple lipid and lipoprotein measures. However, we did not observe a significant interaction between the Leu162, Val162 and EPA consumption with regards to lipid and lipoprotein concentrations in hypertriglyceridemic subjects. But we could observe a significant difference between EPA supplementation and Leu162 and Val162, GG and GC polymorphism. This finding suggests that genetic variation within the PPAR $\alpha$  Leu162/Val cannot modulate the association of EPA intakes with lipid and lipoprotein profiles. It is possible that the sample of study is not high enough to affect the association between PPAR $\alpha$  genotype and lipid after EPA consumption. However, previous studies of genetic variation in the PPAR $\alpha$  gene have found gene-by-diet interactions specific to different polymorphisms in different racial-ethnic groups (i.e., L162V in whites and V227A in Asians).<sup>[33]</sup>

The role of dietary fatty acids in regulating plasma lipoprotein and lipid concentrations is well documented.<sup>[34]</sup> Compared with SFA intake,

PUFA intake has been shown to lower LDL-C and HDL-C concentrations. However, the great inter-individual lipoprotein and lipid response to such dietary changes is not well understood. Genetic factors are likely to play a significant role, because polymorphisms in several genes seem to modulate lipoprotein and lipid responses to dietary modifications.<sup>[35]</sup> This study is the first in hypertriglyceridemic subjects to examine whether plasma lipid and lipoprotein responsiveness to EPA supplementation are influenced by the PPAR $\alpha$  Leu162Val gene polymorphism or not.

*In vitro* studies have shown that the Val162 allele has a greater transactivation activity when treated with a PPAR $\alpha$  agonist.<sup>[8]</sup> Previous studies showed the increasing effect of the Leu162Val polymorphism on TC, LDL-C, HDL-C,<sup>[8]</sup> ApoB,<sup>[11,12,16]</sup> apo A-1,<sup>[16]</sup> and ApoCIII.<sup>[11]</sup> This polymorphism was also found to be associated with decreased concentrations of fasting serum triacylglycerol among white subjects with normal glucose tolerance.<sup>[36,37]</sup> Their investigation of the gene-by-EPA consumption interaction effects on the metabolic profile led us to several interesting observations. They found that EPA consumption interacts with the Leu162/Leu and Leu162Val polymorphism to modulate the TC concentrations. These gene-by-EPA supplementation interaction effects may then modulate plasma lipid concentrations and need further replication in different intervention studies and later in a meta-analysis.<sup>[37]</sup> Although we cannot interaction between EPA consumption and PPAR $\alpha$  genotypes. One study of the gene-by-diet interaction effects on the metabolic profile led to several interesting observations. They found that diet interacts with the Leu162Val polymorphism to modulate TC concentrations, plasma ApoA-1 concentrations, and cholesterol in small LDL particles. This gene-by diet interaction effects may then modulate plasma lipid concentrations and need further replication in different intervention studies and later in a meta-analysis. In addition, independent of the genotype, diet had an effect on HDL.<sup>[36,38]</sup>

## CONCLUSIONS

EPA consumption has lipid-lowering effect in hypertriglyceridemic subjects in both Leu162

and Val162, GG, and GC carriers. But there was no significant interaction between EPA supplementation and PPAR $\alpha$  genotypes. Thus, genetic variation within the PPAR $\alpha$  Leu162/Val cannot modulate the association of EPA intakes with lipid and lipoprotein profiles. However, we must note the sample size in this study.

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