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SHORT COMMUNICATION

The I148M Variant of PNPLA3 Reduces the Response to Docosahexaenoic Acid in Children with Non-Alcoholic Fatty Liver Disease

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ABSTRACT The aim of this secondary analysis of a randomized controlled trial was to test whether the I148M variant of Patatin-like phospholipase domain-containing protein-3 (PNPLA3) is associated with the response to docosahexaenoic acid (DHA) in children with non-alcoholic fatty liver disease (NAFLD). Sixty children with NAFLD were randomized in equal numbers to DHA 250 mg/day, DHA 500 mg/day or placebo. Coherently with the primary analysis, the probability of more severe steatosis after 24 months of DHA supplementation was 50% lower [95% confidence interval (CI) −59% to −42%)] in the combined DHA 250 and 500 mg/day groups versus placebo. The present secondary analysis revealed an independent effect of PNPLA3 status on the response to DHA. In fact, the probability of more severe steatosis was higher (37%, 95% CI 26–48%) for the PNPLA3 M/M versus I/M genotype and lower (−12%, 95% CI −21% to −3%) for the I/I versus I/M genotype (Somers' D for repeated measures). We conclude that the 148M allele of PNPLA3 is associated with lower response, and the 148I allele with greater response, to DHA supplementation in children with NAFLD.

KEY WORDS: • ***children*** • ***docosahexaenoic acid*** • ***non-alcoholic fatty liver disease*** • ***randomized controlled trial***

INTRODUCTION

Following the trail of the obesity epidemics, non-alcoholic fatty liver disease (NAFLD) has become the major cause of pediatric chronic liver disease in industrialized countries, as it affects 3–10% of children, reaching approximately 70–80% in the presence of obesity.[1] NAFLD encompasses a spectrum of liver damage ranging from simple fat accumulation to non-alcoholic steatohepatitis (NASH), which may evolve to cirrhosis later in life.[2]

Although no therapy has yet demonstrated efficacy with regard to NASH and fibrosis,[1] docosahexaenoic acid (DHA), an omega-3 poly-unsaturated fatty acid (N3-PUFA) with anti-inflammatory and insulin-sensitizing properties, has shown promising results in the reduction of liver fat content.[3,4]

NAFLD has a major heritable component, especially in children.[5,6] In particular, the patatin-like phospholipase domain-containing protein-3 (PNPLA3) rs738409 C > G polymorphism, encoding for the I148M protein variant, is not only the major genetic determinant of hepatic fat content and increased liver enzymes[7] but also a risk factor for NASH and fibrosis.[8,9] PNPLA3 is regulated by the lipogenic program and is involved in lipid metabolism in hepatocytes, and the I148M polymorphism alters the activity of this enzyme.[10,11] The I148M polymorphism influences liver damage and susceptibility to NASH early in life,[5,9,12–15] synergizing with abdominal fat and carbohydrate intake.[16,17] Interestingly, there is some evidence that the I148M-NAFLD association is affected by the dietary N-6-PUFA/N-3-PUFA ratio, and it has been hypothesized that dietary supplementation with N-3-PUFA may reverse steatosis in carriers of the I148M polymorphism.[18]

The aim of this secondary analysis from a randomized controlled trial (RCT)[3,4] was to test whether the I148M PNPLA3 polymorphism is associated with the response to DHA supplementation in children with NAFLD.

MATERIALS AND METHODS

The study protocol and the primary analysis of this RCT are described in detail elsewhere[3,4] (trial identifier: NCT00885313 www.clinicaltrials.gov). Briefly, 60 children with NAFLD were randomized in equal numbers ($n = 20$) to DHA 250 mg/day, DHA 500 mg/day, or placebo using a parallel-arm design. Written informed consent was obtained from the parents or legal guardians of the children, and the study protocol was approved by the ethics committee of the Bambino Gesù Hospital. As reported in detail elsewhere,[3,4] the primary analysis showed that DHA supplementation

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Table 1. Baseline Measurements of the 60 Study Children Stratified According to the I148 PNPLA3 Polymorphism

	I/I n = *6* (M = *5*)		*I/M* n = *34* (M = *20*)		*M/M* n = *20* (M = *10*)		
	P50	*IQR*	*P50*	*IQR*	*P50*	*IQR*	P *value*
Age (years)	10	7	11	3	12	3	.41
BMI (kg/m^2)	25.5	4.2	26.0	4.4	26.7	2.3	.28
BMI (SDS)	2.1	1.2	1.8	0.7	1.9	0.6	.34
Glucose (mg/dL)	82	7	85	10	88	12	.58
Insulin (mg/dL)	17	6	10	6	13	11	.06
Triglycerides (mg/dL)	85	48	85	33	92	27	.14
ALT (U/L)	44	43	68	30	72	27	.16

P50, median; IQR, interquartile range; M, males; BMI, body mass index; SDS, standard deviation score; ALT, alanine transaminase.

decreased liver steatosis and that doses of 250 and 500 mg/day of DHA were equally effective.

PNPLA3 I148 variants were assessed at the end of the RCT by means of 5′ nuclease Taqman assays.[9]

The present analysis evaluated the changes in liver fat, triglycerides, and alanine transaminase (ALT) using Somers' D association measure.[19] Somers' D models employed for this secondary analysis had ultrasonographically determined liver fat (discrete, 0 = none, 1 = mild, 2 = moderate, and 3 = severe), triglycerides (continuous, mg/dL), or ALT (continuous, U/L) as outcomes and time (discrete, 0 = baseline, 1 = 24 months), DHA (discrete, 0 = placebo, 1 = 250 or 500 mg/day), a DHA × time interaction (discrete), and PNPLA3 I148 variants (discrete, 0 = I/M, 1 = M/M, 2 = I/I) as predictors. Repeated measures were taken into account by specifying cluster confidence intervals for each patient. The analysis was intention to treat, and there were no missing data. Statistical analysis was performed using Stata 12.1 along with the somersd package.[20]

RESULTS

The main features of the patients stratified on the basis of the PNPLA3 I148 variants are reported in Table 1.

Subjects with I/I, I/M, and M/M variants were similar for all characteristics. The number of I/M, M/M, and I/I polymorphisms in the DHA and placebo groups were 24 (71%), 13 (65%), 3 (50%) and 10 (29%), 7 (35%), 3 (50%).

Figure 1 depicts the number of subjects with liver steatosis before and after treatment with DHA or placebo.

Table 2 reports the independent contribution of PNPLA3 I148 to the effect of DHA on liver steatosis (Somers' D).

Coherently with the primary analysis, the probability of more severe steatosis after 24 months of DHA supplementation was 50% lower [95% confidence interval (CI −59 to −42%, $P < .001$) in the combined DHA 250 and 500 mg/day groups versus placebo. The secondary analysis, however, revealed an independent effect of PNPLA3 on the response to DHA. In fact, the probability of more severe steatosis was higher [37%, 95% CI 26–48%, $P < .001$) for the PNPLA3 M/M versus I/M allele and lower (−12%, 95% CI −21 to −3%, $P < .05$) for the I/I versus I/M allele. Expectedly, the availability on only six subjects with the I/I allele was responsible for large confidence intervals of the corresponding effect sizes. Interestingly, the higher probability of more severe steatosis at the end of the trial in the M/M group was accompanied by a higher probability of increased triglycerides (14%, 95% CI 1–27%) and ALT (14%, 3–25%). Even if larger numbers of subjects are needed to precisely

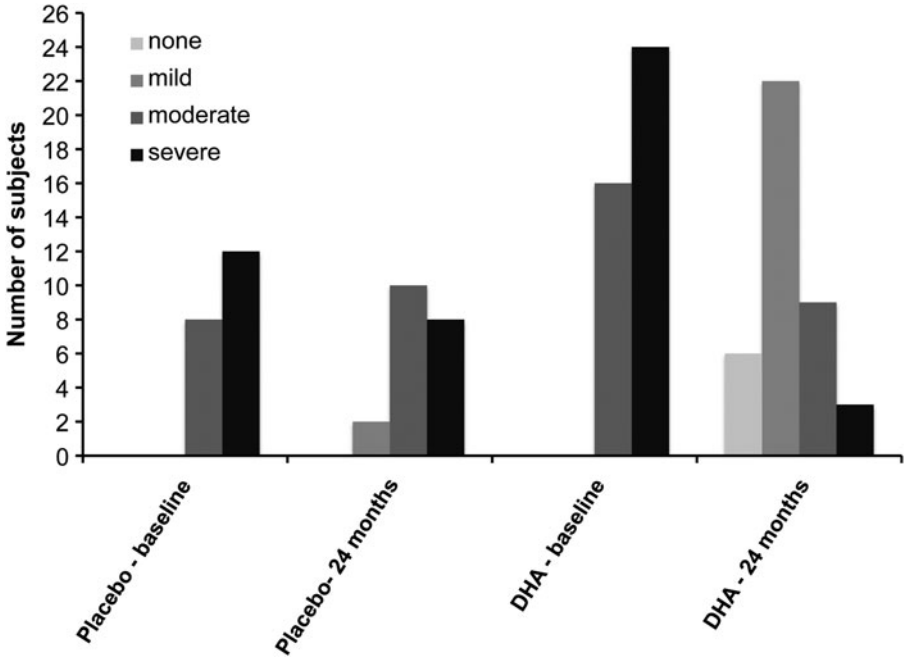

FIG. 1. Number of subjects with liver steatosis before and after treatment with docosahexaenoic acid (DHA) or placebo.

Table 2. Independent Effect of the I148M PNPLA3 Genotype on the Response to Docosahexaenoic Acid

	Prob. (more severe FL)	*Prob. (higher TG)*	*Prob. (higher ALT)*
DHA versus placebo	−.50*** [−0.59 to −0.42]	−.05 [−0.12 to 0.02]	−.29*** [−0.38 to −0.20]
M/M versus I/M	.37*** [0.26 to 0.48]	.14* [0.01 to 0.27]	.14* [0.03 to 0.25]
I/I versus M/M	−.12* [−0.21 to −0.03]	.01 [−0.07 to 0.09]	−.05 [−0.13 to 0.03]

Values are probabilities with 95% confidence intervals. Effect sizes were calculated using Somers' D (see statistical analysis for details).
*$P < .05$, ***$P < .001$.
Prob., probability; FL, fatty liver; TG, triglycerides; ALT, alanine aminotransferase.

quantify these effects,[3,4] these changes are in keeping with the known effects of DHA on serum lipids.

DISCUSSION

In this secondary analysis of an RCT,[3,4] we tested whether the PNPLA3 I148M variant is associated with the response to DHA supplementation in children with NAFLD. We found that the response to DHA was lower in children who were homozygous for the 148M and higher in those who were homozygous for the 148I allele as compared with heterozygotes.

The 148M allele was associated with 50% higher probability of more severe steatosis at the end of the trial, even if the effect of DHA on steatosis was independent from PNPLA3. The frequency of the 148M variant in our children was expectedly high, owing to NAFLD severity and persistently elevated ALT.[5,8,14,15] To our knowledge, this is the first demonstration of a different response of NAFLD to treatment on the basis of a genetic factor. More effective treatments are needed for the subgroup of patients with NAFLD homozygous for the 148M PNPLA3 variant, because the spectrum of liver disease associated with this genetic risk factor may extend to liver cirrhosis and hepatocellular carcinoma.[21]

In a recent study, the dietary N-6/N-3 PUFA ratio was associated with hepatic fat content and ALT in a multiethnic sample of obese children from the United States.[18] Such association was evident only in subjects who were homozygous for the 148M PNPLA3 allele, leading us to hypothesize that the 148M allele does not allow effective hepatic lipid remodeling in the presence of an altered dietary N-6/N-3 PUFA ratio. These data suggest that the possibility that the position of the last unsaturated bond of fatty acids may be differentially affected by the 148I and 148M PNPLA3 variants. This hypothesis needs to be addressed in further studies, as insufficient data are available on PNPLA3 affinity for N-3 PUFA.[11]

A reduction of dietary N-6 PUFA or alternatively, an increase of dietary N3-PUFA may be, therefore, needed to correct steatosis in subjects with the 148M variant. Our data are not consistent with the latter hypothesis, because DHA, a N-3 PUFA, was less effective in 148M/M subjects. Our data are more consistent with (but, of course, do not prove) the alternative hypothesis that excessive dietary N-6 PUFA favor lipogenesis[10,22] or reduce PNPLA3 hydrolytic activity or PNPLA3 contribution to lipoprotein export[11,23,24] in 148M subjects.

The higher levels of triglycerides in 148M/M subjects after treatment are at variance with previous results, supporting an association between the 148M allele and decreased levels of serum triglycerides,[25,26] possibly related to impaired export of very low density lipoproteins.[24] This finding may be, however, explained by the higher severity of steatosis, which is a major determinant of very low-density lipoprotein secretion, in 148M/M subjects at the end of treatment, associated with the lower response to the effect of DHA on hepatic lipid metabolism.

Since our findings rely on an unplanned secondary analysis of an RCT, they need confirmation in specifically designed RCT, where the subjects are stratified on the basis of PNPLA3 status. However, points of strengths of the present secondary analysis are that (1) the genetic background is inherited and not susceptible to modification; (2) all patients were tested; and (3) the PNPLA3 genotypes were equally distributed among treatment arms.

In conclusion, homozygosity for the 148M PNPLA3 allele is associated with lower regression of steatosis after DHA supplementation in pediatric NAFLD. Further studies are needed to confirm our findings and to determine the effects of PNPLA3 on the changes of ''hard'' hepatological outcomes such as NASH and liver fibrosis.

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AUTHOR DISCLOSURE STATEMENT

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