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 enzymes2 but also a risk factor for NASH and fibrosis.8,9 PNPLA3 is regulated by the lipogenic pro-
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

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

<table>
<thead>
<tr>
<th></th>
<th>I/I n=6 (M=5)</th>
<th>I/M n=34 (M=20)</th>
<th>M/M n=20 (M=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P50</td>
<td>10</td>
<td>7</td>
<td>12</td>
<td>.41</td>
</tr>
<tr>
<td>IQR</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>7</td>
<td>1</td>
<td>12</td>
<td>.41</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5</td>
<td>4.2</td>
<td>26.7</td>
<td>.28</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>2.1</td>
<td>1.2</td>
<td>1.9</td>
<td>.34</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>82</td>
<td>7</td>
<td>88</td>
<td>.58</td>
</tr>
<tr>
<td>Insulin (mg/dL)</td>
<td>17</td>
<td>6</td>
<td>13</td>
<td>.06</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>85</td>
<td>48</td>
<td>92</td>
<td>.14</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>44</td>
<td>43</td>
<td>72</td>
<td>.16</td>
</tr>
</tbody>
</table>

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

FIG. 1. Number of subjects with liver steatosis before and after treatment with docosahexaenoic acid (DHA) or placebo.
quantify these effects, these changes are in keeping with the known effects of DHA on serum lipids.

**DISCUSSION**

In this secondary analysis of an RCT, 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. 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.

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**

All authors declare that no financial assistance was received to support the analysis and writing of this study.

**REFERENCES**

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