Healthy PNPLA3 Risk Allele Carriers Present with Unexpected Body Fat Composition. A Study of One Thousand Subjects

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ABSTRACT

Introduction: The common PNPLA3 (adiponutrin) variant p.I148M represents a major genetic driver of progression in non-alcoholic fatty liver disease (NAFLD). NAFLD is commonly associated with traits of the metabolic syndrome, therefore it is mostly suspected in obese individuals. Here, we investigate the association between the PNPLA3 variant and anthropometric traits in a cohort of healthy individuals.

Patients and methods: We recruited 1,000 (500 females; age 18 - 66 years) healthy blood donors. The PNPLA3 variant was genotyped using TaqMan assays. All individuals were phenotyped with respect to anthropometric characteristics. We also determined the percentage of total fat (F%) and active tissue (TA%) of body weight.

Results: Healthy carriers of the PNPLA3 [IM] and [MM] genotypes, although not differing in height from individuals with the genotype [II], displayed significantly lower body weight and lower BMI (both P = 0.005), higher TA% (P = 0.03) but lower F% (P = 0.03) and smaller waist, chest and shin circumferences (all P < 0.05). Separate analysis for males and females demonstrated an association between the [IM] and [MM] genotypes and higher TA% but lower F% (P = 0.04) in females. In males, BMI and total weight were significantly (P = 0.04) lower among carriers of the [M] allele.

Discussion: Healthy individuals carrying the prosteatotic PNPLA3 allele p.I48M may be leaner as compared to the carriers of the common allele. Hence in clinical practice they might be overlooked since they do not necessarily present with the anthropometric characteristics commonly associated with severe hepatic steatosis.

Key words: autotaxin – body mass index – obesity – single nucleotide polymorphism.

INTRODUCTION

The common PNPLA3 (adiponutrin) variant p.I148M has been identified in recent years as the major genetic determinant of non-alcoholic fatty liver disease (NAFLD) [1]. Indeed, a genome-wide association study (GWAS) published in 2008 [2] demonstrated that the amino acid substitution [I] > [M] at position 148 of the PNPLA3 protein is associated with higher liver fat contents [2]. This association, replicated subsequently in pediatric [3, 4] and additional adult NAFLD cohorts [1], was also extended to patients with alcoholic [5, 6] and viral liver diseases [7, 8]. Carriers of the PNPLA3 p.I48M allele are characterized by increased hepatic fat content and are prone to progressive liver fibrosis [7, 9], cirrhosis [6, 7, 9], and hepatocellular carcinoma [10], which all render this variant the first common genetic risk factor for severe forms of chronic liver diseases [11]. Although several functional studies have been performed, the functional mechanisms underlying this association have not been fully elucidated. Increased intracellular synthesis of triglycerides in the liver might be one of the mechanisms triggering the PNPLA3-related phenotypes. As demonstrated by Kumari et al [12], PNPLA3 metabolizes lysophosphatidic acid (LPA) into...
phosphatidic acid (PA), which can be used in the synthesis of triglycerides in the liver. The p.148M PNPLA3 variant, in turn, might be a ‘gain-of-function’ mutation [12], resulting in enhanced synthesis of hepatic lipids. Others reported that the PNPLA3 variant leads to decreased hepatic secretion of very low-density lipoprotein (VLDL) and decreased lipolysis which might contribute to increased hepatic lipid accumulation [13, 14]. Interestingly, a significant association between lipid and glucose metabolism and the PNPLA3 p.1148M variant was demonstrated in selected cohorts only [15-17].

Increased hepatic fat accumulation in patients who do not consume excessive quantities of alcohol is often detected during routine abdominal ultrasound in asymptomatic individuals without any apparent liver disease [18]. In general, fatty liver is regarded to be a benign condition. However, in patients with non-alcoholic steatohepatitis (NASH) it may progress to liver cirrhosis and deterioration of liver function. Indeed, NASH is claimed to be one of the leading causes for cryptogenic cirrhosis [19]. Fatty liver disease is commonly associated with traits that fall into the spectrum of the so-called metabolic syndrome, in particular central obesity and diabetes [20]. Hence, fatty liver is mostly suspected in obese individuals, whereas its presence in lean patients might easily be overlooked.

Previous reports focused on investigating the PNPLA3 polymorphism in individuals with liver diseases. In the current study we investigate the effects of the PNPLA3 variant on several traits of body composition, in particular the amount of fat tissue and body mass index (BMI), in a large cohort (n = 1,000) of healthy individuals.

**PATIENTS AND METHODS**

**Patients**
A cohort of 1,000 Caucasian (median age 24 years; mean 27 years; range 18 - 66 years) blood donors from the Regional Blood Donor Center in Szczecin (Poland) was investigated. In total, 500 females (median age 22.5 years; mean 27.1±10.2 years; range 18 - 66 years) and 500 males (median age 26 years; mean 27.8±8.3 years; range 18 - 62 years) were included. All subjects had a medical checkup, and a good state of health was a prerequisite to qualify for blood donation. Informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Ethics Committee of Pomeranian Medical University.

**Anthropometrical examination**
Anthropometric measurements, taken with a medical scale, an anthropometer and an anthropometrical centimeter (body mass, height, hip, chest, shin, and forearm measurements), served as a basis for calculating BMI, the total fat of body weight in percentage (F%) and kilograms (Fkg), and the active tissue of body weight in percentage (TA%) and kilograms (TAkg). The following formula was applied: BMI = body mass (kg) / (height)2 (m). The BMI values were stratified according to the WHO (1995) classification. Adipose tissue distribution was assessed using the waist-to-hip ratio (WHR). In accordance with the anatomical classification of obesity, two types of obesity were defined: android and gynoid.

The distribution of genotype and allele frequencies of the PNPLA3 variant p.1148M is presented in Table I. We achieved a 100% genotyping success. In the whole cohort, a total of 61.0% subjects had the genotype [II], while others were heterozygous [IM] (33.3%) or homozygous [MM] (5.7%). The analysis of allelic and genotype frequencies of the PNPLA3 polymorphism demonstrated that [I] represented the major allele. The genotype and allele frequencies in the studied cohort were in line with the genotype and allele frequencies deposited with the Entrez database and with frequencies presented in previous reports. As presented in Table I, the minor allele [M] was significantly (P < 0.05) more prevalent among females as compared to males. We did not identify any departure from the HWE either in the whole cohort or in the separate analyses performed in males and females (all P > 0.05) proving a robust genotyping.

Overall, 37.6% of studied individuals were overweight, i.e. presented with BMI ≥ 25 kg/m2. As demonstrated in Fig. 1, we detected an increased prevalence of overweight individuals (i.e. BMI 25 ≥ kg/m2) among carriers of the genotype [II] as compared to individuals with the genotypes [IM] or [MM] (females: 60.3% vs. 39.7%; males: 65.3% vs. 34.7%). Overall,
obesity was more prevalent among males as compared to females (49.6% and 25.6%, respectively). The anthropometric analyses showed that 44.4% of females and only 10.4% of males were at a higher risk of android obesity, while the others were at a risk of or suffered from gynoid obesity. As demonstrated in Table II, individuals with genotypes [IM] and [MM], although they did not differ in height from the carriers of the [II] genotype, had lower weight and BMI, higher TA%, lower F% and smaller chest, waist, hip shin and forearm circumferences. These differences were significant for weight and BMI (both $P = 0.005$), chest circumference ($P = 0.01$), waist circumference ($P = 0.03$), shin circumference ($P = 0.002$), TA% ($P = 0.03$), and F% ($P = 0.03$). Thus, carriers of the PNPLA3 allele [M], which is associated with liver steatosis, were overall leaner and demonstrated lower whole body fat content as compared to carriers of the major allele. The strength of this correlation differed, when women and men were examined separately. Results of this analysis are presented in Table III. Fisher’s correlation coefficient values demonstrated significant differences between carriers of the genotype [II] and individuals with genotypes [IM] + [MM] for TA% and F% (both $P = 0.04$) in females. In males, only BMI and total weight were significantly lower among carriers of the [M] allele. Moreover, the shin circumference differed significantly ($P = 0.02$).

**Table I.** Distribution of the PNPLA3 alleles and genotypes

<table>
<thead>
<tr>
<th>Allele</th>
<th>Whole cohort (n = 1000)</th>
<th>Females (n = 500)</th>
<th>Males (n = 500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[I]</td>
<td>1553 (77.7)</td>
<td>753 (75.3)*</td>
<td>800 (80.0)</td>
</tr>
<tr>
<td>[M]</td>
<td>447 (22.3)</td>
<td>247 (24.7)*</td>
<td>200 (20.0)</td>
</tr>
<tr>
<td>[II]</td>
<td>610 (61.0)</td>
<td>289 (57.8)*</td>
<td>321 (64.2)</td>
</tr>
<tr>
<td>[IM]</td>
<td>333 (33.3)</td>
<td>175 (35.0)</td>
<td>158 (31.6)</td>
</tr>
<tr>
<td>[MM]</td>
<td>57 (5.7)</td>
<td>36 (7.2)</td>
<td>21 (4.2)</td>
</tr>
</tbody>
</table>

*Abbreviations: I, isoleucine; M, methionine (prosteatotic allele); PNPLA3 - adiponutrin. * $P < 0.05$ between females and males.

Since the PNPLA3 p.148M variant represents a major risk factor for liver steatosis, one would expect that carriers of the risk variant present with increased whole body fat content as well. Here, we demonstrate that healthy individuals carrying

**Table II.** Association of the PNPLA3 polymorphism with anthropometric variables

<table>
<thead>
<tr>
<th>PNPLA3</th>
<th>[II]</th>
<th>[IM] + [MM]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.7±0.1</td>
<td>1.7±0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.4±16.1</td>
<td>72.5±14.9</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9±4.2</td>
<td>24.2±3.8</td>
<td>0.005</td>
</tr>
<tr>
<td>WHR</td>
<td>0.8±0.4</td>
<td>0.83±0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Active tissue (kg)</td>
<td>50.3±7.9</td>
<td>49.0±7.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>25.1±9.3</td>
<td>23.5±8.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Active tissue (%)</td>
<td>67.6±5.5</td>
<td>68.4±6.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>32.4±5.5</td>
<td>31.6±6.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>86.5±11.8</td>
<td>84.6±10.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>85.4±13.4</td>
<td>83.6±12.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>117.8±15.6</td>
<td>101.5±8.12</td>
<td>0.4</td>
</tr>
<tr>
<td>Shin circumference (cm)</td>
<td>37.1±3.7</td>
<td>36.4±3.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Forearm circumference (cm)</td>
<td>25.9±3.6</td>
<td>25.5±4.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28±10</td>
<td>27±9</td>
<td>0.3</td>
</tr>
<tr>
<td>Female / male (n)</td>
<td>289/321</td>
<td>211/179</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are shown as means ± standard deviation. [M] represents the risk allele for hepatic steatosis. Abbreviations: BMI, body mass index; I, isoleucine; M, methionine; PNPLA3, adiponutrin; WHR, waist-hip ratio.

**DISCUSSION**

Since the PNPLA3 p.148M variant represents a major risk factor for liver steatosis, one would expect that carriers of the risk variant present with increased whole body fat content as well. Here, we demonstrate that healthy individuals carrying
the prostatic PNPLA3 allele p.I148M might be leaner and have lower amounts of whole body fat as compared to the carriers of the common allele. Therefore, they may lack the anthropometric characteristics that are commonly associated with hepatic steatosis.

Several reports have previously investigated the effects of the PNPLA3 variant on metabolic phenotypes. For example, Palmer et al [17] demonstrated that severely obese individuals carrying the [M] allele are prone to diabetes mellitus and insulin resistance but present with lower serum triglyceride levels. In our previous study, we also detected an association between the PNPLA3 polymorphism and serum fasting glucose concentrations [16]. However, other studies in extensive cohorts did not underline any link between the PNPLA3 variant and serum glucose levels, BMI or insulin resistance in the general population or in patients with NAFLD [2, 22-24]. On the other hand, a large meta-analysis demonstrated the link between the PNPLA3 p.I148M mutation and serum cholesterol but not lipid levels [15]. Although we do not posses data on metabolic profiles of individuals included in the current study, our results emphasize the notion that healthy individuals carrying the prostatic PNPLA3 variant might be overall leaner as compared to carriers of the common variant. To date, the function of adiponutrin and the effects of the p.I148M mutation remain controversial [12-14]. One explanation of the association between the PNPLA3 mutation and whole body fat composition might be based on studies indicating that LPA, a potential substrate of PNPLA3 and whole body fat composition might be based on studies explaining the association between the PNPLA3 I148M mutation [12] and serum cholesterol but not lipid levels [15]. In addition, previous studies in mouse models and cell lines show that LPA signaling modulates adipogenesis and the expansion of adipose tissue [25]. However, further studies in carriers of specific PNPLA3 genotypes are required to delineate the pathobiological mechanisms.

In our current study we investigated a cohort of blood donors, who per se are in good health and present without any viral or non-viral liver diseases or elevated serum liver enzymes. Since obese carriers of the adiponutrin polymorphism would most likely develop hepatic phenotypes, including elevated serum liver enzyme activities, that excluded them from the pool of blood donors, our cohort might be biased towards lean individuals in comparison to the general population. This could also explain the increased number of female carriers of the risk allele, since women, due to overall healthier life style [26], are more likely to be accepted as blood donors as compared to males. In contrast, our findings indicate that the combination of the PNPLA3 risk variant and protective environmental factors can prevent or delay NAFLD manifestation. Given the current epidemics of obesity, the prevalence of fatty liver is predicted to rise dramatically. Since liver disease often progresses insidiously in patients with NASH an early detection of individuals who are at-risk of severe lipid accumulation in the liver is crucial to prevent the progression of liver disease. As pointed out previously [11], patients with the adiponutrin variant are predisposed to rapid progression of chronic liver diseases, but may present without any additional traits that indicate the presence of this critical genetic risk factor.

CONCLUSION

Our study indicates that a subgroup of the carriers of the PNPLA3 mutation might actually be leaner than individuals carrying the common allele. Hence, they might easily be overlooked in clinical practice, since they do not necessarily present with the anthropometric characteristics that are commonly associated with severe hepatic steatosis.

Conflicts of interest: We declare that we have no conflict of interest.

Financial disclosure: We have no financial support to disclose.

REFERENCES


